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Circadian rhythms and metabolic syndrome: from experimental genetics to human disease

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Abstract

The incidence of the metabolic syndrome represents a spectrum of disorders that continue to increase across the industrialized world. Both genetic and environmental factors contribute to metabolic syndrome and recent evidence has emerged to suggest that alterations in circadian systems and sleep participate in the pathogenesis of the disease. In this review, we highlight studies at the intersection of clinical medicine and experimental genetics that pinpoint how perturbations of the internal clock system, and sleep, constitute risk factors for disorders including obesity, diabetes mellitus, cardiovascular disease, thrombosis and even inflammation. An exciting aspect of the field has been the integration of behavioural and physiological approaches, and the emerging insight into both neural and peripheral tissues in disease pathogenesis. Consideration of the cell and molecular links between disorders of circadian rhythms and sleep with metabolic syndrome has begun to open new opportunities for mechanism-based therapeutics.

Keywords

Clock; Circadian; Metabolic syndrome

I) Introduction: metabolic syndrome and obesity

The metabolic syndrome (MS) is comprised of several metabolic abnormalities, including central (intra-abdominal) obesity, dyslipidaemia, hyperglycaemia, and hypertension. This syndrome has become a major public-health challenge worldwide; an estimated 25–40% of individuals between the ages of 25 and 64 years of age have MS (San Antonio Heart Study) 1–4. MS is further defined by the presence of other components, including elevated circulating levels of triglycerides, reduced levels of HDL-cholesterol, high blood pressure and impaired fasting glycaemia. Elevated circulating inflammatory and/or thrombotic markers [C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and plasminogen activator inhibitor type 1 (PAI-1)] or reduced levels of anti-inflammatory molecules such as adiponectin are further markers of MS2, 4.

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Excess food intake and physical inactivity underlie the growing worldwide epidemic of obesity and MS, not only in industrialized nations but also in developing countries. In addition, mounting evidence from clinical epidemiological studies has led to the hypothesis that one of the major changes in the industrialized world that contributes to the pathogenesis of the MS involves the introduction of artificial light and work into the night-time, in addition to the pervasive rise in voluntary sleep curtailment [5]. Indeed these common disorders of circadian behaviour and sleep are associated with increased hunger, decreased glucose and lipid metabolism, and broad changes in the hormonal signals involved in satiety [6]. Recently, Sheer et al. demonstrated adverse cardiometabolic endpoints in human subjects who underwent forced misalignment of behavioural and circadian cycles, simulating the conditions of jet lag and shift work within a controlled clinical setting [7]. Against this backdrop of human studies, advances in the field of experimental genetics have uncovered the fundamental molecular mechanism governing these 24 h circadian rhythms of physiology, revealing that all circadian processes are programmed by a conserved transcription-translation feedback loop that oscillates with a periodicity of 24 h [8]. Remarkably, obesity and high-fat feeding also reciprocally affect the circadian system in mice, indicating that metabolism, circadian rhythms, and possibly sleep, are interconnected through complex behavioral and molecular pathways [9]. Thus alterations in energy homeostasis associated with obesity may set in motion a “vicious cycle” of circadian disruption, in turn leading to exacerbation of the original metabolic disturbance.

II) Adverse effects of alterations in circadian rhythms: clinical evidence

The decrease in sleep duration in the US has occurred over the same time period as the increase in the prevalence of metabolic disease (for review, see 5). Numerous cross-sectional, as well as prospective clinical studies, have demonstrated that short-duration and poor-quality sleep predicts the development of type 2 diabetes and obesity after age, Body Mass Index (BMI) and various other confounding variables are taken into account 10–13. For instance, reduced sleep duration in children is associated with increased risk of being overweight 14. The gradual decline in the amount of time spent asleep and also the routine extension of normal activity during the night may disrupt synchrony between the periods of sleep/activity with alternating periods of feeding/fasting and energy storage/utilization. Indeed, the relationship between sleep restriction, weight gain and diabetes risk may involve at least in part alterations in glucose metabolism, stimulation of appetite, and decreased energy expenditure (for review, see 5). For example, healthy subjects who underwent six consecutive nights of sleep restricted to 4 h exhibited impaired insulin sensitivity following a glucose challenge 15, 16. Moreover, the induction of hunger may be partially related to reduced circulating levels of leptin (an adipose tissue-specific hormone which promotes satiety) and increased levels of the orexigenic hormone ghrelin (a peptide released primarily from the stomach) induced by sleep deprivation 17. Both hormones may also impact energy expenditure (for review 18). Curiously, individuals diagnosed with night eating syndrome appear to have greater propensity towards obesity 19. Diseases related to changes in time and/or quality-sleep duration are also associated with metabolic disorders. For example, sleep apnoea syndrome, a sleep disorder that is highly prevalent in metabolic disorders 20, was proposed to cause clock gene dysfunction 21, and effective treatments of sleep apnoea have been found to improve glucose metabolism and energy balance [11]. In addition, the circadian oscillation of leptin was found to be disrupted in narcoleptic patients, which may predispose them to weight gain [22]. Understanding the molecular pathophysiology of metabolic disorders in states of disrupted sleep remains a major challenge.

It has also long been recognized that serious adverse cardiovascular events, including myocardial infarction, sudden cardiac death, pulmonary embolism, limb ischemia, and aortic aneurysm rupture, all have pronounced circadian rhythmicity, reaching a peak during the

morning [23]. More recent evidence has accumulated to suggest that chronic circadian disruption may also increase susceptibility to such disorders. For example, shift work is associated with a 1.6 and 3.0-fold increased risk of cardiovascular disease for 45–55 years old men and women, respectively [24]. Cardiovascular disease and hypertension are also associated with sleep loss: the risk of a fatal heart attack increases 45% in individuals who chronically sleep 5 h per night or less [25]. Interestingly, the incidence of acute myocardial infarction was also significantly increased for the first 3 weekdays after the transition to daylight saving time in the spring [26]. This observation underscores the deleterious effects of transitions involved in daylight saving time on the disruption of chronobiologic rhythms. Another adverse aspect of sleep perturbation is its impact on the human immune system [27–29]. For instance, sleep deprivation dysregulates monocyte production of several pro-inflammatory cytokines, including IL-6 and TNF-α 30, 31. This point is of interest since obesity is recognized to involve a low-grade inflammatory state (for review, see 32). Conversely, the inflammatory process can induce sleep disturbances 27. Other metabolic disorders may be induced by a phase shift, such as altered postprandial lipid excursion, thereby providing a partial explanation for the increased occurrence of cardiovascular disease reported in shift workers 33.

Recently, Sheer et al. investigated the causal link between circadian misalignment and metabolic homeostasis using a controlled simulation of ‘shift-work’ in the clinical laboratory [7]. In this study, 10 subjects underwent a progressive misalignment of behavioural and circadian cycles. Their behavioural cycle was extended to a 28h-day, under dim light, with 14h rest and fasting alternated with 14h of wakefulness, interspersed with four evenly spaced and isocaloric meals. When subjects ate and slept approximately 12 h out of phase from their habitual times, circadian desynchrony decreased leptin levels and resulted in hyperglycemia and hyperinsulinemia. In addition, their daily cortisol rhythm was reversed, arterial pressure elevated and sleep efficiency decreased. Interestingly, some of the subjects also exhibited postprandial glucose responses comparable to those of a prediabetic state [7]. Thus, this study suggests that synchrony between behavioural and physiological rhythms is advantageous to maintain normal glucose metabolism in otherwise healthy persons [34]. An important question for future clinical studies will be to determine the impact of short sleep and/or circadian misalignment on molecular clock function, especially within metabolic tissues.

In addition to environmental sleep disruption (e.g. shift work disorders), genetic polymorphisms in several clock genes have also been linked to sleep disorders [35–37]. For instance, genetic variation in circadian clock genes has been associated with psychiatric diseases, such as bipolar disorders and schizophrenia 35, while many depressed patients, particularly bipolar patients, show delayed sleep phase 38, and depression is also a co-morbidity of obesity [39]. Interestingly, polymorphisms in *Clock* and *Bmal1*, whose proteins form the core mammalian clock, have been linked to some features of the metabolic syndrome. In small sample populations, polymorphisms in the *Clock* gene have been correlated with predisposition to obesity [40, 41], and two *Bmal1* haplotypes are associated with type 2 diabetes and hypertension [42]. Polymorphisms within other clock core genes (i.e *Per2* and *Npas2*) have also been associated with hypertension and high fasting blood glucose in studies of similar sample size [43]. Interestingly, a rare variant in *Nampt* (*Visfatin/Pbef1*), which is involved in a negative clock feedback loop [44, 45], is associated with protection from obesity [46].

Recently, several genome-wide association studies led to the unexpected discovery that melatonin, a hormone implicated in seasonal and circadian rhythms, may be important in the regulation of mammalian glucose levels [47, 48]. Indeed genetic variants of the melatonin 1B receptor gene (*mtnr1b*) increase type 2 diabetes risk [47, 48]. In agreement, *mtnr1b* is expressed in pancreatic β-cells, and melatonin modulates glucose-stimulated insulin secretion [49]. Interestingly, melatonin secretion is reported to be impaired in type 2 diabetic patients [50], and the melatonin profile relative to the feeding/ fasting cycle is reversed when individuals are

subjected to forced desynchrony [7]. Taken together, these recent findings raise the possibility that disruption of circadian systems, either directly at the level of altered clock gene expression, or indirectly through effects on melatonin, may contribute to human metabolic syndrome and cardiovascular complications.

III) Molecular and hierarchical organisation of the clock

A) The core molecular clock network

Forward genetics and positional cloning enabled identification of the first mammalian circadian clock gene and provided an entrée point into a molecular understanding of the clock mechanism [51, 52]. The core molecular clock is composed of transcription/translation feedback loop that oscillates with 24 hr rhythmicity (Fig 1). The driving force is the positive limb of the clock comprised of the basic helix-loop-helix (*bHLH*)-PAS (*Period-Arnt-Single-minded*) transcription factors CLOCK (*Circadian locomotor output cycles kaput*), and its paralogue NPAS2 (*neuronal PAS domain protein 2*), and BMAL1/ARNTL (*aryl-hydrocarbon receptor nuclear translocator-like*). CLOCK or NPAS2 and BMAL1 heterodimerize and activate the rhythmic transcription of downstream target genes that contain E-box cis-regulatory enhancer sequences, including the *Period* (*Per1*, *Per2*, and *Per3*) and *Cryptochrome* (*Cry1* and *Cry2*) genes [51, 53–56]. Following translation, PER/CRY dimerize and translocate back to the nucleus where they directly inhibit the CLOCK/BMAL1 complex, effectively repressing their own transcription [57–59].

Additional regulatory loops are interconnected with the core loop described above, providing multiple layers of control of the core circadian clock [60, 61]. In addition to the *Per* and *Cry* genes, CLOCK/BMAL1 also activate transcription of the retinoic acid-related orphan nuclear receptors *Rev-erbα*and *Rorα*.62–65. REV-ERBα binds to the retinoic acid-related orphan receptor response element (RORE) in the *Bmal1* promoter resulting in inhibition of transcription and this action is opposed by RORα which activates the RORE 62–64, [66]. In addition to the nuclear hormone receptor feedback loop, PAR-domain basic leucine zipper transcription factors (PAR bZIP), including DBP, TEF, HLF, and the cyclic adenosine 3',5'-monophosphate (cAMP) pathway (CREB-ATF-CREM) also feedback on the clock, acting through cognate D box and CREB elements respectively [60, 61, 67–70. Post-translational modification, including phosphorylation and ubiquitination, provide further regulation of the clock network. *Casein kinase 1 epsilon* and *delta* (*CK1ε* and *CK1δ*) phosphorylates PER and CRY, tagging them for polyubiquitylation by the E3 ubiquitin ligase complexesβTrCP1 and FBXL3, respectively, ultimately leading to their degradation by the 26S proteosome 71–78. In addition to phosphorylation mediated by the caseine kinase family, in Drosophila and mammalian cells, a role for GSK3-β signalling has been established [68, 79]. As a result of this degradation, CLOCK/BMAL1 is released from repression, activating the forward limb of the 24 hr cycle. Lastly, epigenetic regulation has emerged as an additional node in circadian systems (discussed further below), including the possibility that CLOCK participates directly in protein acetylation and chromatin modification [80].

Genetic mouse models have revealed key roles for each of the core clock genes in the generation and maintenance of circadian rhythms. *Bmal1* knockout mice display a complete loss of circadian rhythmicity in constant darkness [53]. Mice with a dominant-negative *Clock* mutation have an approximately 4 hr lengthening of their free-running period and become arrhythmic in constant darkness [52]. Of note, *Clock* knockout mice have normal locomotor activity rhythms, due to developmental compensation by NPAS2 [81, 82]. Compensation can also be demonstrated within the negative limb of the clock, as both *Per1*/*Per2* and *Cry1*/*Cry2* knockout mice display a much more pronounced loss of circadian rhythmicity compared to their single mutant counterparts [56, 83–86]. While the aforementioned studies have focused on overt locomotor activity rhythms, it remains uncertain as to whether compensation extends to functions of the

clock within peripheral tissues. It is likely that future genetic studies will continue to identify additional regulators and modifying loops of the core clock mechanism.

B) Central clock organisation

Many metabolic functions occur at specific times of the day. Indeed, understanding the effects of molecular clock gene disruption on organismal physiology can be advanced through consideration of the molecular and hierarchical organization of the clock. The location of the master neural clock in mammals was originally discovered through classical lesioning studies within pacemaker neurons of the brain: the suprachiasmatic nucleus (SCN) of the hypothalamus (for more complete review, see 87, 88). The SCN controls physiological and behavioural circadian rhythms and coordinates peripheral clocks through hormonal and neural signals 87. The master role played by SCN was demonstrated by transplantation experiments in hamster. Neural grafts from the suprachiasmatic region restored circadian locomotor and feeding activity to arrhythmic animals whose own SCN had been ablated 89. The restored rhythms of the host always matched the rhythms of the donor, demonstrating the strong impact of this nucleus in circadian activity. However, despite the restoration of locomotor rhythmicity, melatonin and glucocorticoids remained arrhythmic, suggesting that neural connections must be critical for the generation of certain circadian rhythms [90]. Interestingly, this master pacemaker has anatomic connections with several regions of the CNS involved in the control of appetite, energy expenditure regulation and behavioural activity, namely with the subparaventricular area, the arcuate nucleus and the lateral hypothalamic area [91].

The SCN clock is entrained by light through the retinohypothalamic tract (Fig. 2). Photic input provides a dominant time-keeping signal (zeitgeiber), orienting the animal each day to geophysical time. Endogenous period length is not precisely 24 hr (humans are longer while mice are shorter), thus the daily entrainment to light is a critical mechanism to maintain organismal synchrony with the external environment (Fig 2). Perception of light occurs through activation of a population of directly light-sensitive ganglion cells within the eye, the melanopsin cells; these regulate both circadian rhythms and melatonin synthesis [92, 93]. Direct output of the SCN, and the entrainment of the SCN axis to light, plays a key role in synchronizing endogenous hormonal rhythms including the glucocorticoid rhythm [94]. In turn, light-induced entrainment of the glucocorticoid rhythm may maintain phase coherence of multiple cellular oscillators, such as those in fibroblasts and liver [95, 96]

In addition to photic entrainment, food also entrains circadian processes in neural and peripheral cells (Fig. 2). Food restriction to the normal rest period in rodents also induces a burst of anticipatory activity, an effect that is altered in some experimental systems following lesioning of the dorsomedial nucleus 97–99. However there remains controversy concerning the involvement of circadian oscillators in food anticipatory activity (FAA) since the behavior persists in *Bmal1* nullizygous mice [97, 100, 101]. Interestingly, FAA appears to involve the melanocortin signalling pathway, since restriction failed to increase wakefulness before food presentation in melanocortin-3 receptor null mice [102]. Rather than localization to a single nucleus of hypothalamus, the food entrainable oscillator may in fact involve a more dispersed network of cell groups [103]. Further, the FAA may constitute a metabolic oscillator responsive to peripheral neural or circulating signals elicited by food ingestion 104, 105. An interesting question remains concerning whether macronutrient flux in the postprandial state may participate in establishing FAA (reviewed in 104, 105). A related question is whether nutrient signalling *per se* may affect core properties of the SCN pacemaker.

C) Peripheral clocks

Molecular analyses have revealed that the clock network is also widely expressed throughout nearly every tissue/ cell type in vertebrates [106, 107]. Original studies by Schibler and colleagues

demonstrated cell autonomous clock gene oscillation within fibroblasts *ex vivo* 108. Following this discovery, in addition to the master clock in the SCN, independent circadian oscillators have been found in a number of peripheral tissues in mammals. Gene expression profiling has shown that 3% to 20% of genes display a 24h rhythmic expression, and a large proportion of these genes have a role in metabolic processes (for review, see 8). The circadian rhythms of peripheral organs are also self-sustained, as demonstrated using a mouse line in which luciferase expression is driven from the endogenous *Per1* or *Per2* loci [106, 107]. Variation in temporal gene expression was reported to play an important role in tissues implicated in glucose and lipid metabolism, such as fat, liver, cardiac and skeletal muscle [109–117]. Many nuclear receptors expressed in liver and white and brown adipose tissues also display rhythmic patterns of expression 118–120. Therefore, the nuclear receptors may link clock genes to metabolism by integrating energy flux with varying physiological demands across the light-dark cycle. In this way, circadian patterns of metabolic gene expression may optimize the switch between daily anabolic and catabolic states corresponding with periods of feeding and fasting. For example, the cyclic expression of gastrointestinal tract enzymes may ensure that factors involved in nutrient absorption are expressed in anticipation of daily episodes of food ingestion 105. In addition, adipose enzymes involved in fatty acid storage peak coincident with feeding (for review, 121). Moreover, components of gluconeogenesis, glycolysis, and fatty acid metabolism cycle with a peak during the subjective night in mouse liver 112. Coordinating gene expression patterns according to the varying metabolic demands across the active and rest period is also important in muscle, where elaboration of aerobic and anaerobic enzymes varies during the sleep-wake cycle 111, 122. Thus, peripheral oscillators are self-sustained, cell autonomous and tissue-specific, yet a major question is: What are the mechanisms involved in maintaining synchrony within and between peripheral tissue clocks? A related question is whether misalignment of local circadian oscillation within and between peripheral tissues contributes to cardiovascular and metabolic pathologies?

To discern whether the rhythmic expression of genes in peripheral organs is driven by local (cell autonomous) oscillators or by circadian systemic signals, Schibler and colleagues have recently exploited the tetracycline-inducible system of Bujard, enabling conditional *Rev-erbα* overexpression within liver [123]. In this model, REV-ERBα represses the transcription of the essential core clock gene *Bmal1* in a doxycycline-dependent manner. Among 351 genes with rhythmic expression revealed in the doxycycline-fed mice, only 31 genes, including the core clock gene *mPer2*, oscillated robustly irrespective of whether the liver clock was running or not. These studies suggest that the rhythmicity of metabolic liver genes is driven by both cell-autonomous and non-autonomous signals [123].

Multiple signals related to feeding, and even fasting, may entrain peripheral clocks. Indeed, in *in vitro* experiments, a bewildering variety of stimuli can induce or reset circadian gene expression. These factors include chemical activators of protein kinase A (forskolin, butyryl cAMP), protein kinase C and/or MAP kinase (phorbol esters, FGF, endothelin) and glucocorticoids receptors (dexamethasone), and even glucose; dissecting how these signalling pathways converge on the clock remains an area of intensive investigation (reviewed in 108).

IV) Evidence for a molecular link between circadian and metabolism systems

The availability of genetic models of circadian disruption has provided new opportunities to dissect the interrelationship of circadian and metabolic systems. Early studies indicated the cellular redox status, represented by the nicotinamide adenine dinucleotide cofactors NAD(H) and NADP(H), regulate the transcriptional activity of CLOCK/BMAL1 and NPAS2/BMAL1 124. The reduced forms of these cofactors increase DNA binding, while the oxidized forms decrease binding, thus coupling activity of these core clock components with the metabolic state of the cell. Two recent studies have further linked the biology of NAD production with

the core molecular clock 44, 125. The gene encoding the rate-limiting enzyme in NAD biosynthesis, nicotinamide phosphoribosyltransferase (NAMPT), displays circadian rhythmicity in peripheral tissues, including liver and WAT, and is under the direct control of CLOCK/BMAL1. Such rhythmicity translates to daily oscillations in NAD levels in liver. Both *Nampt* RNA and NAD levels are reduced in liver from $Clock^{\Delta 19/\Delta 19}$ and $Bmal1^{-/-}$ mice, while increased in liver from mice deficient for both CRY1 and CRY2, suggesting that *Nampt*, and therefore NAD production, is a downstream target of CLOCK/BMAL1. Not only is NAD important in cellular redox reactions, but it also serves as a substrate for SIRT1, an NAD-dependent and nutrient responsive deacetylase, which has also recently been described as a novel regulator of circadian clock function 126, [127]. Of note, the timing of the peak in NAMPT and NAD levels corresponds with the peak in SIRT1 activity. SIRT1 then physically associates with components of the positive limb of the core clock machinery (CLOCK and BMAL1) and is recruited to clock target genes. Genetic and pharmacologic manipulation of SIRT1 and the NAD biosynthesis pathway reveal that SIRT1 negatively regulates CLOCK and BMAL1. Together, these studies demonstrate the existence of a negative feedback loop whereby CLOCK/BMAL1 positively regulate both NAD production and SIRT1 activity, while in turn, SIRT1 negatively regulates the activity of CLOCK/BMAL1.

The existence of this pathway is particularly intriguing in light of the fact that NAMPT and SIRT1 are regulated not only by the clock, but also by the nutritional status of the organism. For example, *Nampt* is upregulated in response to glucose restriction in skeletal muscle in an AMPK-dependent manner [128, 129], and SIRT1 has been demonstrated in numerous tissues to be increased during fasting or caloric restriction [130–133]. Thus, regulation of the clock by NAD and SIRT1 allows for coordination and fine-tuning of the core clock machinery with the daily cycles of fasting/feeding and rest/activity. Furthermore, NAD and SIRT1 are also involved in the regulation of a myriad of metabolic processes, including regulation of glucose-stimulated insulin secretion, adipocyte differentiation, and gluconeogenesis 134. Regulation of NAD and SIRT1 by the clock likely has a cascade of effects on downstream metabolic pathways, and it is tempting to speculate that the reduction in NAD and SIRT1 activity in the circadian mutant mice contributes to some of their metabolic phenotypes. It has also recently been demonstrated that NAMPT is secreted and is present in the circulation 135, though it is not yet known whether extracellular NAMPT is regulated in a circadian manner, thereby influencing downstream processes on a systemic level [136]. Recent evidence has also implicated the other NAD-dependent-sirtuin family members (SIRT2–7) in a variety of metabolic processes [137]; it will therefore be of great interest to determine whether any of these other sirtuins are also involved in the crosstalk between the core circadian clock and metabolism.

Additional key nutrient sensors that have been implicated in the cross-talk between circadian rhythms and metabolism are the nuclear receptor peroxisome proliferator-activated receptor-γ (PPARγ) and the co-activator PGC1α (PPARγ coactivator 1-α). PPARγ is rhythmically expressed and directly regulates *Bmal1* transcription, and mice lacking PPARγ exhibit reduced rhythmicity of clock gene expression, blood pressure, and heart rate [138]. It is interesting to note that SIRT1 promotes fat mobilization during fasting by binding to and repressing PPARγ [139]. PGC1α also displays circadian oscillations in liver and skeletal muscle and upregulates the transcription of *Bmal1* and *Rev-erbα*. Mice lacking PGC1α have abnormal diurnal locomotor activity rhythms, body temperature, and metabolic rate, along with altered expression of clock and metabolic genes [140]. PGC1α levels are elevated in response to cold exposure, starvation, and physical activity, and hence may also help coordinate the circadian clock with the nutritional status of the organism. Of note, SIRT1 also deacetylates and activates PGC1 [133], indicating an additional mechanism linking molecular clock function and energy utilization. A more detailed understanding of the molecular links between the core molecular clock machinery and metabolism will be necessary in order to develop therapies targeting disease states involving disruption of both rhythms and metabolism, such as type 2 diabetes.

V) From circadian disruption to metabolic disease

A) What have we learned from the experimental models?

How might circadian misalignment impact the metabolic co-morbidities of obesity, diabetes, and cardiovascular disease? Several lines of evidence suggest that circadian dysregulation may exert a broad impact not only on glucose control, but also on inflammation, fibrinolysis, fluid balance, and vascular reactivity. A central node linking metabolic and circadian pathways involves the nuclear receptor superfamily, including those downstream of REV-ERBα and the RORs that modulate the core clock and diverse metabolic processes ranging from adipogenesis to inflammation and thrombosis (reviewed in 141). Experimental models have helped to demonstrate the impact of the clock network in metabolic gene expression and provide evidence that this clock disruption leads to metabolic abnormalities. Homozygous *Clock*$^{\Delta 19}$ mutant mice, which express a loss of function mutation in *Clock*, have yielded new insight in this field [142]. In addition to disruptions in sleep and circadian behaviour, these mice also develop hyperphagia early in life, with subsequent development of hyperlipidemia, hyperleptinemia, and hypoinsulinemic hyperglycemia, indicating that this animal exhibits features of the metabolic syndrome [142]. The feeding rhythm in these mice is damped, with increased food intake during the day, and, in addition, these mice have significantly increased food intake overall. High-fat feeding studies revealed exaggerated weight gain of *Clock*$^{\Delta 19}$ mutant mice, and DEXA scanning and fat pad weight both demonstrated significant increases in fat and lean mass relative to controls following high fat feeding [142]. It is likely that the obese phenotype results, at least in part, from altered rhythms of neuropeptides in the hypothalamus, as ghrelin, CART and orexin are all expressed at constitutively low levels in the *Clock*$^{\Delta 19}$ mutant mice [142]. In addition, the anorectic neuropeptide *POMC* was decreased throughout the entire LD cycle in hypothalami of young *Clock*$^{\Delta 19}$ mutant prior to the onset of weight gain and overt diabetes and is consistent with a deficit in the central homeostatic regulation of weight constancy. Since the original *Clock*$^{\Delta 19}$ mutant was developed in a melatonin-deficient strain (C57BL/6J), Kennaway et al. evaluated the contribution of melatonin deficiency on glucose metabolism by crossing *Clock*$^{\Delta 19}$ mutant mouse with the melatonin-producing CBA strain to produce the "*Clock*$^{\Delta 19}$ + *MEL*" mouse [143]. Interestingly, in this model, the restoration of melatonin did not rescue gene expression rhythms in liver or muscle [144]. Such studies underscore the importance of strain background in the evaluation of metabolic phenotype. For example, when introgressed onto the ICR strain, the *Clock*$^{\Delta 19}$ mutation results in malabsorption of lipid and thus resistance to diet-induced obesity, thus primary effects of the *Clock* mutation on energy balance and fuel homeostasis cannot be evaluated in the ICR strain [145]. Disruption of other circadian clock genes also leads to metabolic alterations. For example, gene disruption in *Bmal1* induces an abnormal metabolic phenotype characterised by impaired gluconeogenesis, hyperleptinemia, glucose intolerance and dyslipidemia [146–148]. In addition, *Per2* knock-out mice develop increased weight gain on high-fat diet (HFD) [149]. Conversely, mice deficient in the circadian deadenylase nocturin remain lean and resistant to hepatic steatosis when fed a HFD despite equivalent caloric intake, similar metabolic rates, and reduced activity compared with control mice 150.

Although clock genes impact metabolic homeostasis, a reciprocal effect of metabolic disruption on circadian rhythms also exists, since diet-induced obesity *per se* alters circadian behavioral and molecular rhythms in C57BL/6J mice 9. Indeed, HFD also attenuates the amplitude of diurnal rhythms of feeding and locomotor activity, as high fat fed mice increase their food intake during their normal rest (light) period [9]. Interestingly, genetically obese animals are resistant to weight gain when feeding is restricted to the active (dark) phase 151. In agreement with these observations, recent evidence demonstrated that circadian timing of food intake contributes to weight gain 152. Indeed, mice fed a HFD only during the 12-h light phase gain significantly more weight compared to isocalorically-fed mice provided food only

during the 12-h dark phase 152. Further studies are necessary in order to understand how the timing of food intake impacts energy constancy. Interestingly, a recent study demonstrated that treatment with an antagonist of T-type calcium channel, which is involved in sleep-wake regulation, improved HFD-induced behavioural alterations, including both a decrease in inactive phase activity, core body temperature, feeding and adiposity 153. Taken together, these observations largely based upon animal studies, raise important questions concerning the impact of circadian misalignment and clock gene disruption on obesity and its metabolic complications, and suggest avenues for future investigation in human subjects.

B) Clock disruption in adipose tissue

Excess adipose tissue and altered body fat distribution, rather than adiposity *per s*e, is an important risk factor for obesity-related disorders. Excess intra-abdominal fat rather than subcutaneous fat (central *vs.* peripheral obesity) is associated with MS and cardiovascular disease [3]. However, the mechanisms responsible for this association, and its causality, remain uncertain. Emerging evidence from both cell-based and human studies suggests that expression of the circadian clock transcription network within adipose tissue may influence both adipogenesis and the relative distribution of subcutaneous versus visceral depots [121, 154, 155].

In adipose tissue, the clock machinery controls the expression of a large array of enzymes involved in lipid metabolism. Indeed, adenovirus-mediated expression of BMAL1 in 3T3-L1 adipocytes resulted in induction of several factors involved in lipogenesis, while BMAL1 deletion in adipose cell lines resulted in impaired adipogenesis [148]. Furthermore, heme, the REV-ERBα/β natural ligand, has long been known to enhance adipocyte differentiation *in vitro* [156]. Activation of SIRT1, which regulates the clock network, may increase insulin sensitivity and reduce the inflammatory response in adipocytes [157, 158], however it is unclear whether the effect is direct or not.

Experiments in mice have revealed that temporally restricted feeding causes a coordinated phase-shift in circadian expression of core clock genes and their downstream targets in adipose tissues [117]. In addition, high fat diet also alters the cyclic expression and function of core clock genes and clock-controlled genes in adipose tissue, resulting in disrupted fuel utilization [9, 159]. Of further interest, clock gene disruption targeted to the fat body in flies is sufficient to induce increased food consumption, decreased glycogen levels, and increased sensitivity to starvation [160]. At least in flies, these findings suggest involvement of a peripheral tissue clock in neural energy homeostasis [160].

Several teams have recently started to examine the potential relationship between clock gene expression and metabolic syndrome parameters in humans. Expression levels of the core molecular clock genes in cultured visceral and subcutaneous fat explants obtained from morbidly obese subjects correlated with certain metabolic syndrome parameters, such as waist circumference, sagittal diameter and BMI [154, 155, 161]. However, biopsies from human fat likely represent a heterogenous mixture of adipose cells in addition to macrophages, thus conclusions must be viewed with caution regarding the contribution of adipose tissue to the observed circadian patterns of gene expression. Interestingly, circadian rhythms of gene expression are sustained *ex vivo* in human fat explants [161, 162], including the rhythmic oscillation of genes involved in glucocorticoid turnover [162]. In agreement, human adipose biopsies removed at different zeitgeber times reflect different levels of gene expression consistent with the observed circadian rhythmicity found in cell-culture studies [163]. Further studies are necessary in order to gain more detailed insight into the relationship between temporal patterns of gene expression in adipose tissue and development of MS.

In addition to effects of circadian transcription on intracellular metabolic pathways, clock dysregulation in AT and/or misalignement with meal times may lead to inappropriate

expression patterns of enzymes involved in lipid metabolism such as lipoprotein lipase [121]. For example, misalignment between the fasting/feeding cycle and lipogenic and/or lipid catabolic gene expression pathways may perturb fatty acid flux and contribute to lipotoxicity. Indeed, circadian synchrony may play a distinct role not only within different tissue types (liver *vs* muscle), but also within distinct adipose depots (visceral *vs* subcutaneous) [161]. It is further possible that differences of circadian gene expression patterns within visceral AT and subcutaneous AT depots may contribute to cell-autonomous differences in inflammatory, lipogenic and/or lipolytic pathways within these locales 164, 165. The limited storage-capacity of fat and/or increased lipolysis results in an overflow of fatty acids to ectopic sites such as liver, muscle, and islets (for review 166, 167). Interestingly, both have been proposed to be involved in the etiology of the MS 3, 168.

Another important function of adipose tissue is its secretion of numerous bioactive peptides or proteins, collectively named "adipokines". These may play a central role in energy and vascular homeostasis, as well as immunity, and are fundamental to the pathogenesis of the MS (for review 169). As obesity-related inflammation is receiving increased attention for its potential role in the pathogenesis of MS, steatosis and cardiovascular disease, it may be opportune to consider the impact of circadian systems at the level of adipokine regulation. In mice, leptin exhibits rhythmic production across the light-dark cycle that appears to be dependent of the feeding rhythm 170. Interestingly, in obese humans, disruption of the 24 hr profiles of leptin and adiponectin was observed compared to healthy lean subjects 171, 172. These adipokines play major roles in fuel partitioning and insulin sensitivity but also regulate immunity [169, 173]. Indeed, leptin was the first adipokine found to control energy balance [174]. The metabolic effects of leptin are thought to primarily involve its actions within brain [175–177], while adiponectin function primarily within peripheral target tissues. Many of the metabolic effects of leptin and adiponectin involve activation of AMPK signaling in muscle/liver [178, 179]. Following the discovery of leptin, a growing list of adipokines has been identified, some of which also exert pro-inflammatory roles. Since many adipokines are expressed in a circadian fashion in humans [163], it is tempting to speculate that regulation of adipokine oscillation may be important in metabolic homeostasis. In addition, certain adipokines may also interact with or modulate sleep. For instance, leptin is involved in sleep regulation as demonstrated by EEG monitoring of sleep in leptin deficient and leptin resistant mice [180, 181]. IL-6 and TNF-α plasma levels, which are increased in obesity [182, 183] may also impair circadian clock gene oscillations and promote sleep [184, 185].

Circadian regulation may extend to effects within adipose tissue on endoplasmic reticulum (ER) stress, an important component of the inflammatory response in this tissue [186]. Obesity results in conditions that increase demand on the ER in metabolic tissues including liver, adipocytes and pancreas, resulting in a persistent inflammatory state 187. For example, accumulation of reactive oxygen species, which are abundantly produced by both the ER and the mitochondria during conditions of stress are increased in metabolic organs in MS 186, 187. In adipose tissue, ER stress is involved in adipogenesis and adipokine oversecretion [188, 189]. Interestingly, the endoplasmic reticulum chaperone protein BiP, a key protein involved in the ER stress response, is expressed in a circadian manner in flies [190]. It has also been reported that clock genes may influence the production of reactive oxygen species [8, 191, 192]. Thus disrupted synchrony of stress response gene expression may alter adipose function and thereby directly contribute to insulin resistance. ER stress may also be induced in brain following high fat feeding, thereby contributing to leptin resistance 193 and perhaps circadian and sleep disturbances (for review, 194).

In addition to the impact of white AT excess in MS pathogenesis, several independent groups recently demonstrated that brown adipose tissue is present and active in adult humans, and its presence and activity are inversely associated with adiposity and indexes of the metabolic

syndrome [195–197]. As numerous genes including nuclear receptors exert circadian expression profiles in BAT [116, 117], alterations of circadian oscillator genes in fat may have significant metabolic implications.

C) Clock disruption and impaired glucose tolerance

Disruption in the normal cyclic pattern of glucose tolerance is a hallmark of type 2 diabetes [198], and as such, understanding the circadian control of glucose metabolism is critical for delivering the best clinical diabetes management. Strong evidence from human studies demonstrates rhythmic variation in glucose tolerance and insulin action across the day [199–203]. For example, oral glucose tolerance is impaired in the evening compared to the morning [204, 205], an effect which is believed to be due to a combination of both decreased insulin secretion and altered insulin sensitivity in the evening [201, 206–211]. The 'dawn phenomenon' is also a well-described phenomenon where glucose levels are known to peak prior to the onset of the active period [212, 213]. Furthermore, studies in rats have revealed that the SCN is critical for the maintenance of diurnal variations in glucose metabolism [208].

While these studies indicate a role for circadian systems in the control of glucose metabolism, the molecular mechanisms underlying these phenomena are not well understood. Recently, human genome-wide association studies and experimental mouse model systems have begun to provide clues as to the nature of the molecular links between rhythms and glucose metabolism. As described above, data from several independent groups has now demonstrated that genetic variants of the melatonin receptor may be involved in abnormal glucose homeostasis [47, 48] and that melatonin treatment of pancreatic β-cells inhibits glucose-induced insulin release [49]. Further, mice that have a mutation in the *Clock* gene develop hypoinsulinemic hyperglycemia, and mice nullizygous for *Bmal1* have impaired glucose tolerance [142, 146, 147], suggesting that a functional clock network is required for the maintenance of glucose homeostasis. The cellular etiology of impaired glucose tolerance in circadian mutant animals remains an important yet unresolved area of research.

Finally, it is interesting to speculate that the NAD biosynthetic enzyme NAMPT and SIRT1 may play an important role in the circadian control of glucose metabolism. As described above, NAMPT and SIRT1 are regulated by CLOCK/BMAL1 and constitute a negative feedback loop within the core circadian network. NAMPT and SIRT1 have been demonstrated to be involved in a myriad of metabolic functions, including regulation of gluconeogenesis in liver and glucose-stimulated insulin secretion in islets [44, 125]. Indeed *Nampt*-deficient (*Nampt* +/−) mice showed impaired glucose tolerance due to a defect in glucose-stimulated insulin secretion, which was corrected by intraperitoneal administration of NMN [135], and mice overexpressing SIRT1 specifically in their β-cells displayed improved glucose tolerance and increased glucose-stimulated insulin secretion [214]. As NAMPT and SIRT1 function are impaired in circadian mutant mice, these data suggest that circadian rhythms of NAMPT and SIRT1 may act in β-cells to regulate the daily cycles of insulin secretion and that NAD^+ might function as an oscillating metabolite linking circadian and metabolic cycles.

D) Impact of circadian systems on cardiovascular function

Circadian variation in endogenous factors such as autonomic nervous system function, blood catecholamine concentrations, coagulability, heart rate, blood pressure regulation, and platelet aggregability have been suggested to explain the morning onset of myocardial infarction 23. Conversely, pressure overload-induced hypertrophy and diabetes mellitus result in alterations in the circadian clock within the heart 215, 216. In this regard, it was suggested that alterations in circadian control of cardiac fuel handling may contribute to myocardial contractile dysfunction (for review, see 217). The identification of genes that exhibit circadian regulation within large vessels of the mouse 218–221 has provided clues as to the impact of the circadian

system within vasculature. Indeed, clock gene expression within vasculature has been shown to impact blood pressure and thrombo-occlusive response [220, 222, 223]. Clock genes may influence the temporal incidence of clinical cardiovascular events by regulating the magnitude of the early morning rise in blood pressure [220]. Indeed, the circadian variation in blood pressure and heart rate is disrupted in mice with deleted or mutated core clock genes, a phenomenon that may be partially explained by the altered diurnal variation in epinephrine and norepinephrine in these mice [220]. Interestingly, the mutated mice also showed a reduced response to immobilization stress compared to wild-type mice. Thus, expression of the core clock within peripheral vasculature may modulate the capacity to respond to environmental stressors at different times of day [220]. Effects of the clock system on blood pressure may also involve the modulation of aldosterone biosynthesis by *Per1* [224]. Further, Anea and colleagues demonstrated that *Bmal1*-knockout and *Clock* mutant mice present a loss of vascular adaptation and predisposition to thrombosis [223], both hallmarks of endothelial dysfunction [225]. The endothelial dysfunction in *Bmal1*-knockout mice has been related to defects in Akt and nitric oxide signaling [223]. Interestingly, the defects in endothelium-dependent arterial relaxation of *Clock* mutant mice were normalized by entrainment to light, indicating that the vascular phenotype is not simply a consequence of *Clock* mutation or *Bmal1* deficiency, but rather the result of behavioral disruption in these animals [223].

As noted above, NAD^+ regulation has recently emerged as a major factor coupling circadian rhythms and metabolic signalling pathways. Since NAMPT-mediated NAD biosynthesis has also been shown to impact cardiomyocyte survival pathways, it will be important to ascertain whether dysregulation of NAD contributes to the adverse cardiovascular consequences of circadian disruption [226].

Cardiomyocytes must adapt rapidly to changes in circulating fatty acid, the primary fuel source for contraction. In this regard, PPAR signalling is important in the control of cardiac energy metabolism [227]. Of note, it was also demonstrated that the circadian clock within the cardiomyocyte is essential for responsiveness of the heart to fatty acids [228–230]. To address the circadian function of vascular tissue and the role of PPARγ in the vascular clock, conditional deletion of PPARγ targeted to this tissue was performed [231]. These mice developed abnormalities in blood pressure and heart rate in parallel with a reduction of diurnal variation in the sympathetic nerve activity [231]. Furthermore, vascular PPARγ exhibits a robust cyclic expression, whose rhythmic phase may be reset by changes in feeding time as well as changes in the photoperiod [231]. Thus, the temporal environment may be integrated within the heart by PPARγ [231]. In agreement, PPARγ agonists were found to shift the circadian fluctuation of blood pressure in patients with type 2 diabetes, indicating that vasculoprotective actions of thiazolidinediones may in part involve effects on the clock transcription network [232].

Emerging clinical evidence has uncovered unique actions of the PPARα agonist fenofibrate in the circadian control of blood pressure and heart rate in diabetic subjects. In particular, fenofibrate exerted its most marked anti-hypertensive effects at night [233]. In contrast, only modest decrease anti-hypertensive effects were detected in studies involving a single measurement during the day [234, 235]. In addition, fenofibrate lowered heart rate throughout the 24-h period [233]. Taking together, these data suggest an interaction between PPARα, blood pressure control and circadian rhythms in diabetes.

A comprehensive temporal map of the nuclear receptor transcriptome provides additional clues concerning the circadian control of cardiovascular physiology [120]. For instance, RARα and RXRα interact with CLOCK and MOP4 resulting in repression of CLOCK/MOP4: BMAL1 activity in vascular cells [219]. Indeed, the ligation of retinoic acid, the oxidized form of Vitamin A, to its receptors can phase shift *Per2* mRNA rhythm *in vivo* and in smooth muscle cells *in*

vitro [219]. Whether additional nuclear hormone receptor agonists impact circadian regulation of vascular tone remains a question for future investigation.

Finally, the role of circadian rhythms in the time of onset of thrombotic events has been recognized for many years. Numerous coagulation/ fibrinolytic factors, such as protein C, anti-thrombin, Factor VII, protein S and fibrinogen, have been demonstrated to fluctuate in a circadian manner in humans. Among these factors, PAI-1, the most important physiological inhibitor of plasminogen activation, peaks in the early morning, explaining at least in part the occurrence of hypofibrinolysis and of pro-thrombotic state 236. Circadian control of PAI-1 gene expression by the REV-ERBα in liver may contribute to the circadian variation in fibrinolysis [237], an effect that may also involve interactions between the cycle-like factor (CLIF) and CLOCK [238]. Thus further studies on the circadian gene control of fibrinolysis may shed new light on factors contributing to the prothrombotic state.

E) Circadian rhythms and hepatic function

Liver also plays a key role in the development of metabolic syndrome (for review, see 239). BMAL1 and CLOCK control gene expression of enzymes critical in liver and influence both glucose and lipid homeostasis (for review, see 240). A recent study reported that mice with a liver-specific deletion of *Bmal1* exhibited hypoglycemia during fasting, indicating a role for the liver clock in maintaining euglycemia during rest [147]. Some of the effects of BMAL1 and CLOCK in liver may involve direct regulation of *phosphoenolpyruvate carboxykinase* (*Pepck*) [146]. In addition, circadian gene expression in hepatocytes is altered in mouse models of type 2 diabetes [241] and by high fat feeding [9, 242]. Moreover, HFD induced a phase delay of components of the adiponectin signaling pathway [242]. Alterations in circadian control of adiponectin signalling may reduce its protective effects [242] and thereby increase susceptibility to steatosis, a major risk factor in cardiovascular disease [243].

Hepatic clock gene expression also modulates both bile acid and apolipoprotein biosynthesis, raising the possibility that clock disruption may impact multiple components of hepatic lipid homeostasis [244]. For example, several proteins involved in lipid metabolism (such as hepatic cytochrome P450 cholesterol 7 α-hydroxylase, HMG CoA reductase, or apolipoprotein AIV) show diurnal variation in both humans and rodents [105]. Interestingly, *Rev-erbα* was recently found to play an important role in the control of bile acid metabolism via the regulation of the neutral bile acid synthesis pathway [245]. In mouse liver, *Rev-erbα* expression levels are high during the late light phase, leading to the repression of both small heterodimer partner (SHP) and E4 promoter binding protein 4 (E4BP4) hepatic expression. Reduced levels of SHP and E4BP4 may counter the suppressive effects of bile acids on the cholesterol 7α-hydroxylase (CYP7A1) gene transcription, thereby contributing to the circadian regulation of bile acid and cholesterol homeostasis [245]. In addition, *Rev-erbα* also controls the daily expression of genes involved in cholesterol and lipid homeostasis through circadian modulation of SREBP signalling [246].

F) Clock dysfunction in the immune system

In addition to effects on lipogenesis, lipid catabolism and thrombosis, the circadian system may also promote inflammatory pathways that contribute to the development of cardiovascular disease. At the molecular level, the circadian transcription factor *Rev-erbα*, which is expressed in cells from the immune system such as macrophages and other cell types, may impact the inflammatory response 141 (also see for review, see 247). Intriguingly, REV-ERBα increases the TNF-α-induced NF-κB response, whereas RORα impedes it 141. As rhythmic mRNA expression of the clock genes is dampened in peripheral leucocytes of patients with type 2 diabetes, this impairment might be involved in its pathogenesis 248.

VI) Conclusion

Both inter- and intra-organ desynchrony may be involved in the pathogenesis of cardiometabolic disease due to effects in brain and multiple metabolic tissues including heart, liver, fat, muscle, pancreas and gut. In this context, strategies to improve alignment between the cycles of sleep/wakefulness and feeding/fasting may ameliorate physiological processes including appetitive behavior, carbohydrate and lipid metabolism, inflammation, thrombosis and sodium handling. Efforts to dissect the molecular mediators that coordinate circadian, metabolic and cardiovascular systems may ultimately lead to both improved therapeutics and preventive interventions.

Glossary

Non-standard Abbreviations and Acronyms

ATF	Activating transcription factor
ARNTL	Aryl-hydrocarbon receptor nuclear translocator-like
BMAL1	Brain and muscle aryl-hydrocarbon receptor nuclear translocator-like1
BMI	Body mass index
bHLH-PAS	basic helix-loop-helix-Period-Arnt-Single-minded
bZIP	basic leucine zipper transcription factor
cAMP	cyclic adenosine 3',5'-monophosphate
CART	Cocaine- and amphetamine-regulated transcript
CK1	Casein kinase 1
CLIF	Cycle-like factor
CLOCK	Circadian locomotor output cycles kaput
CREB	cAMP response element binding protein
CREM	cAMP-responsive element modulator
CRP	C-reactive protein
Cry	Cryptochrome
DBP	D-site binding protein
ER	Endoplasmic reticulum
FAA	Food anticipatory activity
FBXL3	F-box and leucine-rich repeat protein 3
HLF	Hepatic leukemia factor
IL-6	Interleukin-6
LD	Light Dark cycle
MS	Metabolic syndrome
NAD	Nicotinamide adenine dinucleotide
NAMPT	Nicotinamide phosphoribosyltransferase
NF-κB	Nuclear factor-κB
NPAS2	Neuronal PAS domain protein 2

PAI-1	Plasminogen activator inhibitor type 1
Pbef	Pre-B-cell colony enhancing factor
Pepck	Phosphoenolpyruvate carboxykinase
Per	Period
PGC1	PPARγ coactivator 1
PPARγ	Peroxisome proliferator-activated receptor-γ
ROR	Retinoic acid-related orphan receptor
SCN	Suprachiasmatic nucleus
SIRT1	Sirtuin 1
SHP	Small heterodimer partner
SREBP	Sterol regulatory element-binding protein
TEF	Thyrotroph embryonic factor
TNF-α	Tumor necrosis factor-α
β̃TrCP	β-transducin repeat containing protein

Glossary

Core Molecular Clock	molecular machinery of the clock within all cells. The circadian gene network in mammals is controlled by a network of autoregulatory transcription-translation feedback loops.
Circadian rhythm	biochemical, physiological or behavioral processes that persist under constant conditions with a period length of ~24 hours
Clock	a central mechanism controlling circadian rhythms
Clock-controlled gene	a gene whose expression is rhythmically regulated by a clock
Entraining agent or external cues or zeitgeber or inputs	extrinsic stimuli able to reset the rhythms (i.e. daylight or food).
Oscillator	a system of components that produces a circadian rhythm
Outputs	circadian rhythmicity of most physiological and behavioral functions, such as feeding, sleep-wakefulness, hormone secretion, and metabolic homeostasis.
Period	duration of one complete cycle in a rhythmic variation

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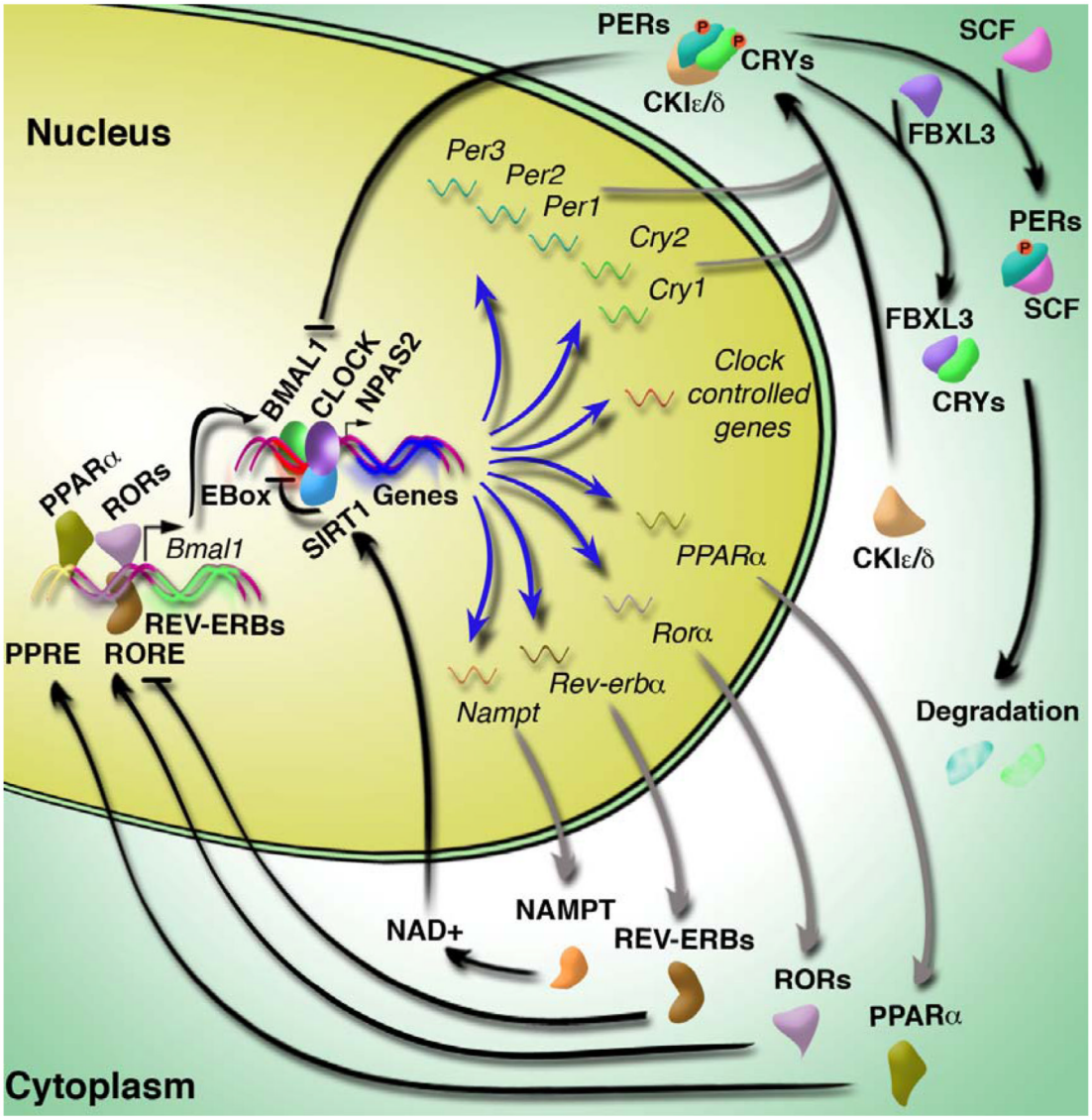

Fig 1. The core molecular clock components

The mammalian circadian clock consists of a series of interlocking transcription/translation feedback loops. The positive limb of the clock is composed of the transcription factors CLOCK/NPAS2 and BMAL1, which heterodimerize and activate transcription of downstream clock target genes, including the *period* (*Per1*, *2*, and *3*) and *cryptochrome* (*Cry1* and *2*) genes, *Rev-erba*, *Rora*, and other clock-controlled genes. Upon translation, the PERs and CRYs heterodimerize, translocate back to the nucleus, and inhibit CLOCK/BMAL1. Multiple additional interlocking loops are shown and are described within the text.

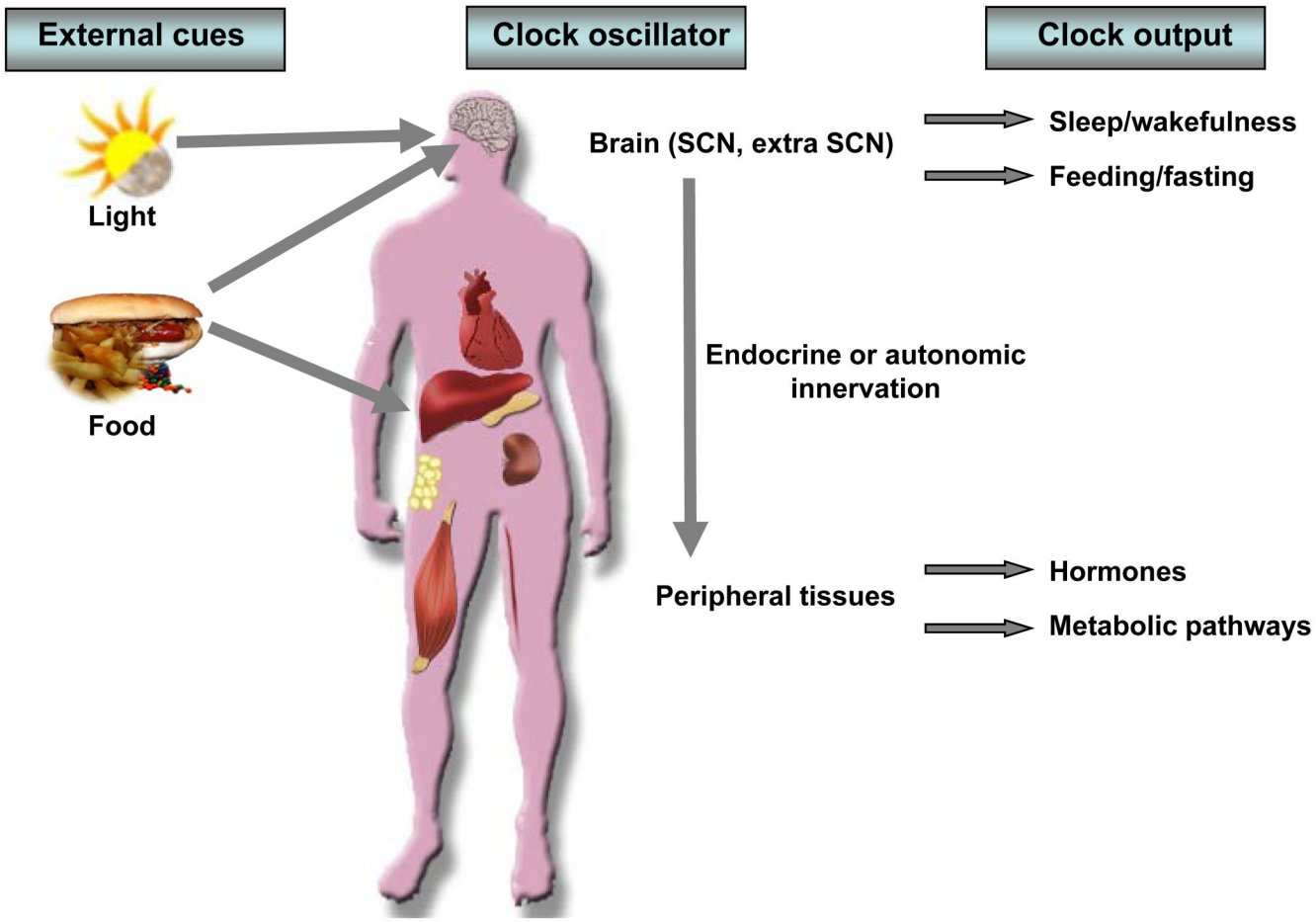

Fig. 2. Synchronization of internal biological rhythms by external cues

Light is the predominant environmental cue that is received by the SCN; photic input is transmitted via the retinohypothalamic tract. In turn, the SCN maintains circadian synchrony of peripheral clocks, a process that involves transmission via both autonomic innervation and/or humoral signals. Circadian oscillators may also be entrained by food and hormones. Circadian synchrony and the entrainment process are reflected in the robustly rhythmic behavioral and physiological outputs such as feeding, sleep-wakefulness, hormone secretion, and metabolic homeostasis.

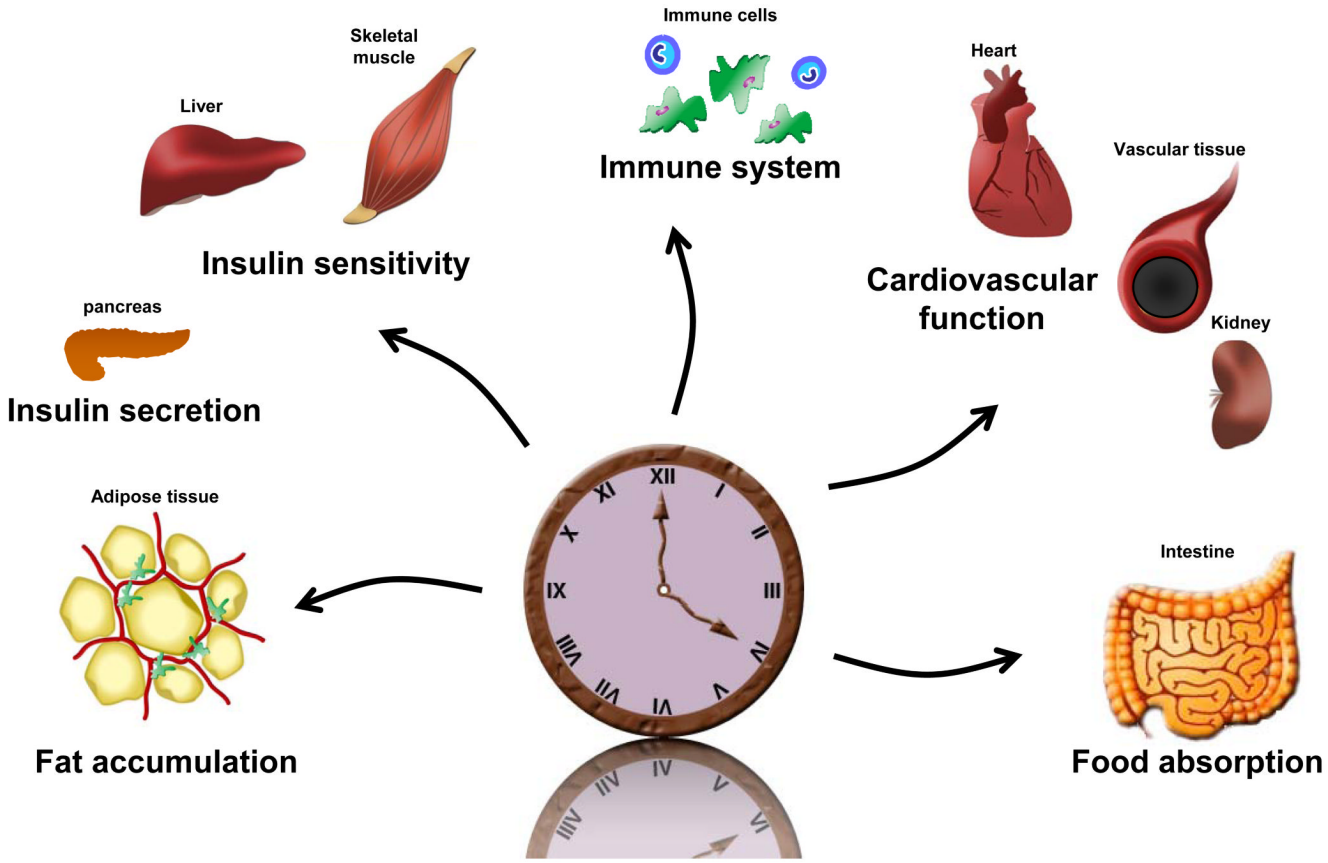

Fig. 3. Peripheral clock output

The core clock machinery has been identified in most peripheral tissues. In addition, rhythmic gene expression appears to be regulated in a tissue-specific manner, enabling each tissue to appropriately calibrate local physiological processes within the appropriate overall temporal schedule. However, circadian disruption either within or amongst individual tissues may lead to organ dysfunction. Indeed, recent studies suggest that peripheral clock alteration is involved in body weight gain as well as abnormalities in glucose homeostasis and blood pressure regulation, thereby contributing to the development of the metabolic syndrome. These alterations may be initiated by disruptions in circadian behavioral and/or environmental factors such as high-fat diet. Circadian and physiological systems are interconnected through reciprocal feedback loops within each tissue locale.