REVIEW ARTICLE

# Circadian Clock and The Cardiometabolic Risk

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## Abstract

**BACKGROUND:** Epidemiological data reveal parallel trends of decreasing sleep duration and increases in metabolic disorders such as obesity, diabetes and hypertension. There is growing evidence that these trends are mechanistically related.

**CONTENT:** The circadian system orchestrates the temporal organization of many aspects of physiology, including metabolism, in synchrony with the 24 hr rotation of the Earth. The circadian system is a complex feedback network that involves interactions between the central nervous system and peripheral tissues. Circadian regulation is intimately linked to metabolic homeostasis and that dysregulation of circadian rhythms can contribute to disease. Conversely, metabolic signals also feed back into the circadian system, modulating circadian gene expression and behavior.

**SUMMARY:** Both inter- and intraorgan desynchrony may be involved in the pathogenesis of cardiometabolic disease attributable to effects in brain and multiple metabolic tissues including heart, liver, fat, muscle, pancreas, and gut. Efforts to dissect the molecular mediators that coordinate circadian, metabolic, and cardiovascular systems may ultimately lead to both improved therapeutics and preventive interventions.

**KEYWORDS:** Circadian rhythms, clock genes, nuclear receptor, sleep, obesity, cardiometabolic risk.

## Introduction

Daily behaviours that are asynchronous with the natural light/dark cycle, such as shift work, jet lag and sleep deprivation, have been strongly and consistently associated with a number of chronic diseases, including diabetes, heart disease and obesity. We now live in a 24-h mechanized world in which one can shop, eat, exercise, drink, work and play around the clock, and the consequences in terms of health may ultimately be considerable. With artificial lighting, we no longer experience a 10–12-h period of true darkness at night, and our sleep, eating and activity periods are often disconnected from the earth's rotational rhythm. Modern air travel forces our bodies to abruptly acclimate to new time zones and altered light/dark cycles, often inducing 'jet lag'. People experience increasing circadian stresses as globalization forces factories, retail commerce and computer-based jobs towards a '24/7' operating schedule. However, the impacts of these stresses on health are not fully appreciated and require comprehensive integration of research information from the fields of circadian biology, sleep, metabolism, nutrition and human physiology (1).

The prevalence of diabetes and obesity is increasing at an alarming rate worldwide, and the causes of this pandemic are not fully understood. Chronic sleep curtailment is a behavior that has developed over the past 2-3 decades. Laboratory and epidemiological studies suggest that sleep loss may play a role in the increased prevalence of diabetes and/or obesity (2).

Today, more than 30% of adult men and women between the ages of 30 and 64 years report sleeping less than 6 hours per night. The decrease in average sleep duration has occurred over the same time period as the

increase in the prevalence of obesity and diabetes (3).

Sleep restriction may affect energy balance and result in weight gain because of an upregulation of appetite, more time to eat and a decrease in energy expenditure. Significant weight gain may in turn result in insulin resistance, a condition that increases the risk of developing diabetes and may promote further adiposity. This cascade of negative events is likely to be accelerated in many overweight and obese individuals by sleep-disordered breathing (SDB), a reported independent risk factor for insulin resistance (4,5).

Circadian and metabolic regulatory systems evolved to be functional and adaptive during a time when the lifestyles and the environmental exposures of our hunter-gatherer ancestors were quite different from today. The circadian rhythms of our early ancestors were more closely synchronized to the rising and setting of the sun, so they were likely to have obtained more sleep. Today we have artificial lights to extend our active phases and distractions, such as longer work days and commuting times, increases in shift work and an increase in the use of television, personal computers and the internet, which curtail our sleep. Hunter-gatherers ate foods that were available seasonally whereas today we can eat virtually any food year-round (6).

Evidence from epidemiological studies is consistent with hypothesized mechanistic links between circadian biology, sleep and metabolism. Modern lifestyles are incompatible in many ways with the intrinsic attributes we inherited. Imbalances can result from this process to dys-regulate our circadian and metabolic systems, resulting in maladaptive and dysfunctional health outcomes such as obesity, diabetes and hypertension. Given the wide disparities between our current environments and lifestyles and those of our hunter-gatherer ancestors, it is not surprising that a large proportion of us are suffering from these illnesses (6).

If metabolic changes resulting from sleep restriction function to increase body weight, insulin resistance and blood pressure then interventions designed to increase the amount and improve the quality of sleep could serve as treatments and as primary preventative measures for metabolic disorders (6).

## Metabolic Consequences of Sleep Deprivation

Poor sleep has increasingly gained attention as a potential contributor to the recent obesity epidemic. The increased prevalence of obesity in Western nations over the past half-century has been paralleled by a severe reduction in sleep duration. Physiological studies suggest reduced sleep may impact hormonal regulation of appetite. Prospective studies suggest reduced habitual sleep duration as assessed by self-report is an independent risk factor for an increased rate of weight gain and incident obesity (7).

Results from experimental studies suggest a mechanistic link between inadequate sleep and increasing body weight. Short sleep duration has also been theorized to decrease energy expenditure (8) by impacting non-exercise activity thermogenesis (9) and by dropping core body temperature (10). Sleep deprivation has been shown to decrease glucose tolerance and compromise insulin sensitivity (11). Short sleep duration could also lead to weight gain and obesity by increasing the time available to eat and by making maintenance of a healthy lifestyle more difficult.

Individuals who engage in shift work have been shown to be at increased risk for the metabolic syndrome, implicating both the timing of sleep and sleep duration in the association (12). One of the primary effects of circadian desynchrony from shift work is a reduction in total sleep time, so the most parsimonious explanation for the apparent impact of shift work on metabolism-related outcomes is the effect of circadian desynchrony on sleep duration rather than direct effects from perturbations in the circadian system.

A number of cross-sectional, as well as prospective, epidemiologic studies (13,14) have provided evidence of an association between self-reported short-duration and/or poor-quality sleep and the prevalence or incidence of diabetes mellitus, after age, BMI and various other confounding variables are taken into account. short sleep duration was found to predict an increased incidence of diabetes in four of the six studies, whereas poor-quality sleep was associated with an increased risk of diabetes mellitus in five of the six studies (15).

In a randomized, crossover study that involved two nights of 4 h in bed versus two nights of 10 h in bed, the daytime profiles of the satiety hormone, leptin, and the appetite-stimulating hormone, ghrelin, were measured and the participants completed validated scales for hunger and appetite for various food categories. Overall leptin levels decreased by an average of 18%, while ghrelin levels increased by 28%; the ghrelin:leptin ratio increased by more than 70%. Hunger increased by 23%, and appetite for nutrients with a high carbohydrate content was increased by more than 30% when sleep was restricted. If this increase in hunger during sleep restriction were to translate into a commensurate increase in food intake, weight gain would

be expected to occur over time (17).

The effect of sleep restriction on appetite regulation seems to be similar in the short term (2–6 days) (16,18) and in chronic conditions. Indeed, two epidemiologic studies have shown reduced leptin levels after controlling for BMI or adiposity in habitual short-duration sleepers (19,20). High ghrelin levels were also associated with short-duration sleep. This body of epidemiologic evidence supports the hypothesis that sleep curtailment may be a plausible 'nontraditional' lifestyle factor that contributes to the epidemic of obesity (21). Increasing the duration of sleep for those who regularly curtail it has been suggested as a means to improve the health of the population as a whole (22).

The existence of a 'vicious circle', in which short-duration sleep may initially promote weight gain; the resultant excess adiposity would then induce sleep disturbances and psychological stress, with a net further decrease in total sleep time. The prevalence of Obstructive Sleep Apnea (OSA) in metabolic and endocrine disorders is very high. In patients with diabetes mellitus, the prevalence of OSA is between 17% (23) and 48% (24). In a preliminary report, which involved only obese patients with diabetes mellitus, undiagnosed OSA was found in 97% of the participants (25). Polycystic-ovary syndrome (PCOS), the most common endocrine disorder of premenopausal women, involves obesity, insulin resistance and a substantially elevated risk of early-onset, impaired glucose tolerance and diabetes mellitus. whereas the risk of OSA in healthy young women, even if they are overweight or obese, is less than 10%, in women with PCOS, recent reports have found a prevalence of OSA of 44–70% (26,27).

OSA involves respiratory disturbances, hypoxic stress, poor-quality sleep (owing to sleep fragmentation and low levels of slow-wave sleep) and reduced total sleep time. The alterations in glucose regulation and/or appetite regulation observed with experimentally reduced sleep duration and quality (11,16) suggest that poor-quality and short-duration sleep, in addition to hypoxia, could contribute to altered glucose homeostasis and weight gain in patients with OSA.

In conclusion, chronic sleep loss, behavioral or sleep disorder related, may represent a novel risk factor for weight gain, insulin resistance, type 2 diabetes, hypertension and cardiovascular disease (28).

Restorative sleep is essential for well-being, but sleep curtailment has become a common behavior in modern society. In addition, sleep disorders, particularly OSA, are very common in individuals with metabolic and endocrine disorders, but often remain undiagnosed. The accumulated evidence for a deleterious effect of short-duration or poor quality sleep on metabolic and endocrine function supports the hypothesis that chronic, voluntary sleep curtailment and sleep disorders such as OSA may adversely affect the course of disease in patients with metabolic and endocrine disorders (17).

## Sleep is Necessary for Health

'Why do we sleep?' Nonetheless, we are beginning to understand the contribution of sleep to fundamental brain processes (29,30). Mammalian sleep is an active process that consists of alternating periods of non-rapid eye movement (NERM) and rapid eye movement (REM) sleep (31). The length of NREM – REM cycle in human is approcimately 90 min and is repeated four to six times per night. NREM sleep is divided into stages I, II, III and IV of progressively deeper sleep. The synchronization of cortical activity during stages III and IV results in high – amplitude low – frequency electroencephalograph waveforms known as "slow – wave sleep" (SWS). Genetic and environmental factors such as sex, race/ethnicity, chronological age, socioeconomic status and others, contribute to the considerable interindividual variability in the quantity and architecture of human sleep (32-35).

Although the brain gives us signals that indicate when we have had insufficient sleep, data show that more and more of us are ignoring these signals and reducing the amount of sleep we obtain each night. The percentage of adults who sleep less than 6 h per night is now greater than at any other time on record (36).

It is clear that our current practice of sleeping less is largely driven by societal changes, including increased reliance on longer work hours and shift work, the trend for longer commute times, and increased accessibility to media of all sorts. Sleep loss is associated with increased obesity (19,37) and with reduced levels of leptin and increased levels of ghrelin (16,19), the combination of which increases appetite. Sleep loss is also associated with diabetes and impaired glucose tolerance in a dose-related manner: individuals that report sleeping less than 6 h per night are ~1.7 times as likely, and those that report sleeping less than 5 h per night are ~2.5 times as likely, to have diabetes than individuals that obtain 7 h of sleep (38). 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 (39). Collectively, these examples demonstrate

wide-ranging consequences of sleep loss on physical health. Obesity, diabetes and cardiovascular disease are pathologies that are characterized, in part, by inflammatory processes.

Feeding and sleep are two of the most time – consuming but mutually exclusive behaviours in terrestrial mammals. Converging data now indicate that the control of mammalian sleep in closely integrated with the mechanisms that support the overall energy balance and metabolic survival of the species (31,40-42).

It has been hypothesized that, similar to the process of seasonal hibernation, one of the functions of sleep may be to conserve energy in the course of the day. Sleep is generally the time when mammalian core body temperature and physical activity are reduced the most. Apart from its well – recognized potential for energy savings through enforced immobility, however, human sleep is only accompanied by a modest decline in resting energy expenditure (43).

A recent understanding of neural mechanisms that control sleep and wakefulness show their close integration with pathways that regulate energy homeostasis, feeding, activity, reward and motivation (31,41). The identification of a new class of excitatory neurons in the lateral hypothalamus, which contains peptides known as hypocretins or orexins, has revealed a complex system of central and peripheral pathways that support the activity and vigilance of the organism in the presence of environmental or metabolic threats to its survival (41). Orexin neurons respond to circulating factors such as glucose, insulin, leptin, and ghrelin, and project to various regions of the brain to control neuroendocrine and autonomic activity, and promote feeding and arousal. Not surprisingly, changes in this system have been hypothesized to contribute to the pathogenesis of metabolic disorders associated with the human short sleep (44).

Many laboratories have developed what is now overwhelming evidence linking sleep deprivation-enhanced inter-leukin-1 beta (IL1), and the related cytokine tumor necrosis factor alpha (TNF), to symptoms associated with sleep deprivation, such as sensitivity to kindling and pain stimuli, cognitive, memory, and performance impairments, depression, sleepiness, and fatigue. Further, chronic sleep loss is associated with pathologies such as metabolic syndrome, chronic inflammation, and cardiovascular disease (45).

Many of the symptoms induced by sleep loss, e.g. sleepiness, fatigue, poor cognition, enhanced sensitivity to pain, can be elicited by injection of exogenous IL1 or TNF, that ATP, released during neurotransmission, acting via purine P2 receptors on glia releases IL1 and TNF. This mechanism may provide the means by which the brain keeps track of prior usage history. IL1 and TNF in turn act on neurons to change their intrinsic properties and thereby change input-output properties (i.e. state shift) of the local network involved (45).

Sleep, like any behaviour, is regulated in the brain by multiple overlapping neuroanatomic circuits and related neurochemical systems. IL-1 and TNF interact with several of these systems, including the serotonin (also known as 5-hydroxytryptamine (5-HT)) system (46). The 5-HT system is one of the most investigated transmitter systems with respect to the regulation of sleep.

The importance of the 5-HT system's role in sleep regulation is supported by both experimental data and clinical observations: pharmacological manipulations that affect the 5-HT system by altering neurotransmitter synthesis, release, binding or re-uptake and metabolism result in profound alterations in sleep (47). Sleep is also altered during clinical conditions such as depression, in which the functionality of the 5-HT system is thought to be chronically altered (48).

Thus, sleep is a local use-dependent process influenced by cytokines and their effector molecules such as nitric oxide, prostaglandins and adenosine (45). Good sleep is necessary for physical and mental health. For example, sleep loss impairs immune function, and sleep is altered during infection. Immune signalling molecules are present in the healthy brain, where they interact with neurochemical systems to contribute to the regulation of normal sleep (49).

Many people currently sleep only 5 – 6 h per night. Epidemiological studies have demonstrated that self – reported short sleep is associated with increased incidence of obesity and diabetes, highlighting the importance of this trend for public health. This finding has triggered renewed research trip into the mechanisms that link the regulation of mammalian sleep and metabolism (43).

## Central and Peripheral Circadian Clock

With increasing economic and social demands, we are rapidly evolving into a 24-h society. In any urban economy, about 20% of the population are required to work outside the regular 08.00–17.00 h working day and this figure is likely to increase. Although the increase in shiftwork has led to greater flexibility in work schedules, the ability to provide goods and services throughout the day and night, and possibly greater employment opportunities, the

negative effects of shiftwork and chronic sleep loss on health and productivity are now being appreciated (50).

The 24-h society is an environmental challenge that outstrips our biological adaptation to the natural 24-h cycle of light and darkness. One of the major influences on time-of-day variations in physiology and behaviour is the activity of internal rhythm generating systems. Circadian (about 24 h) rhythms, are controlled by a master biological clock. In mammals, the master biological clock is located in the suprachiasmatic nuclei of the hypothalamus. At the subcellular level of organisation, circadian rhythms are generated by transcriptional and translational feedback loops involving multiple clock genes. The precise periodicity (or cycle length) of the biological clock is known to be genetically determined, and variation in clock genes is thought to be related to individual differences in natural wake and sleep times (50).

These network features act in concert as a genetic buffering system to maintain clock function in the face of genetic and environmental perturbation (51). A key characteristic of the biological clock is its ability to re-adjust (either by phase advancing or delaying) to changes in the environment, for example after transmeridian travel. Symptoms of jetlag are thought to be caused by desynchronisation of circadian rhythms from the external environment, the transient change in the phase relationship of individual rhythms, and perhaps changes in the amplitude of rhythms.

Symptoms of jet-lag include daytime tiredness, difficulty initiating sleep at night (after eastward flight) or early awakening (after westward flight), disturbed night-time sleep, impaired daytime alertness and performance, gastrointestinal problems, loss of appetite, and inappropriate timing of defecation and urination. Such symptoms can seriously impair a person's performance and ability to function, in part because of the reduction in sleep quality and quantity, and because performance and alertness rhythms will take several days to resynchronize (50).

Biological rhythms are an integral component of essentially all aspects of life. These rhythms are controlled

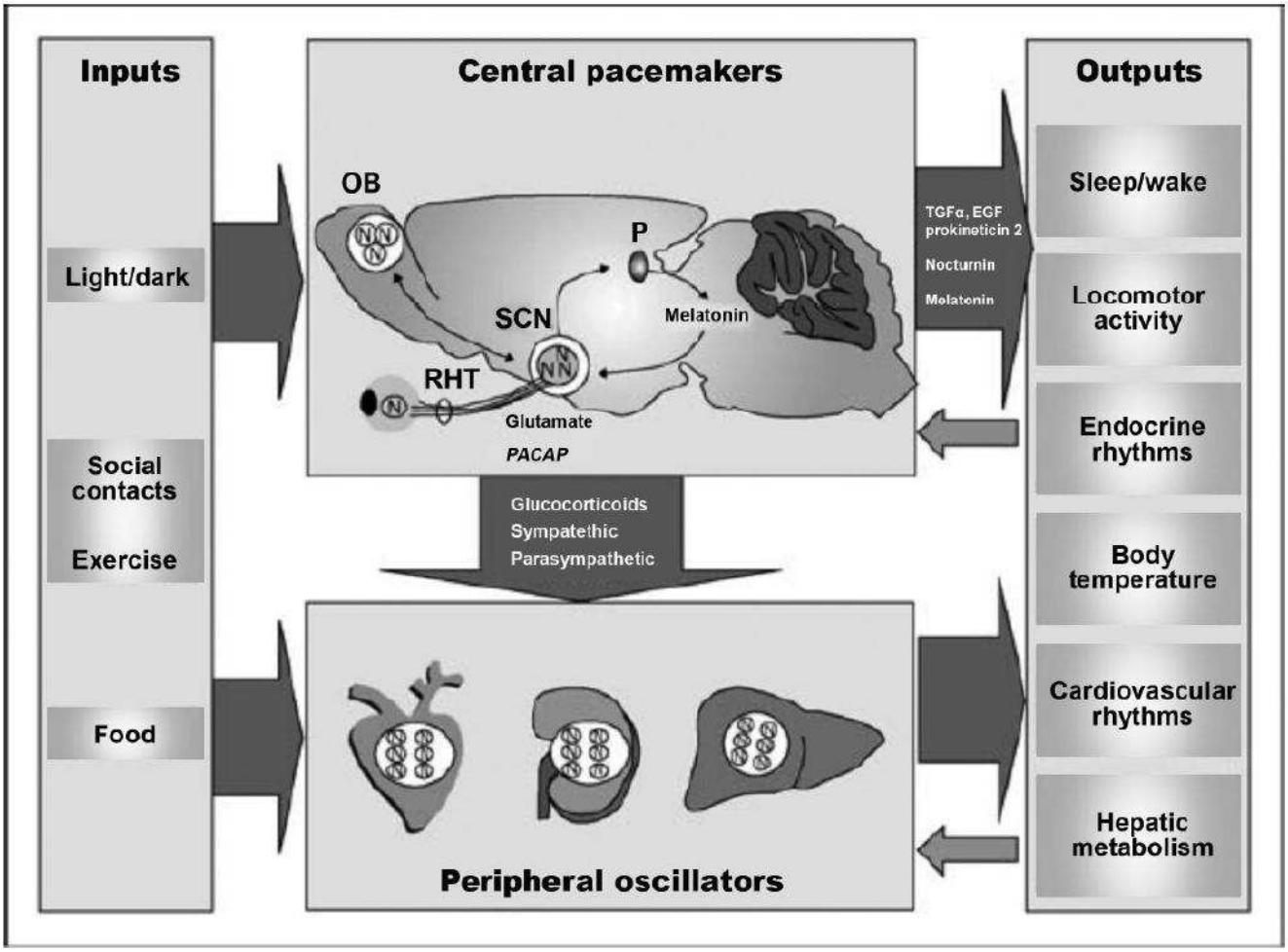


**Figure 1**. Organization of the circadian system in mammals (Adapted with permission from Lippincott Williams & Wilkins) (161).

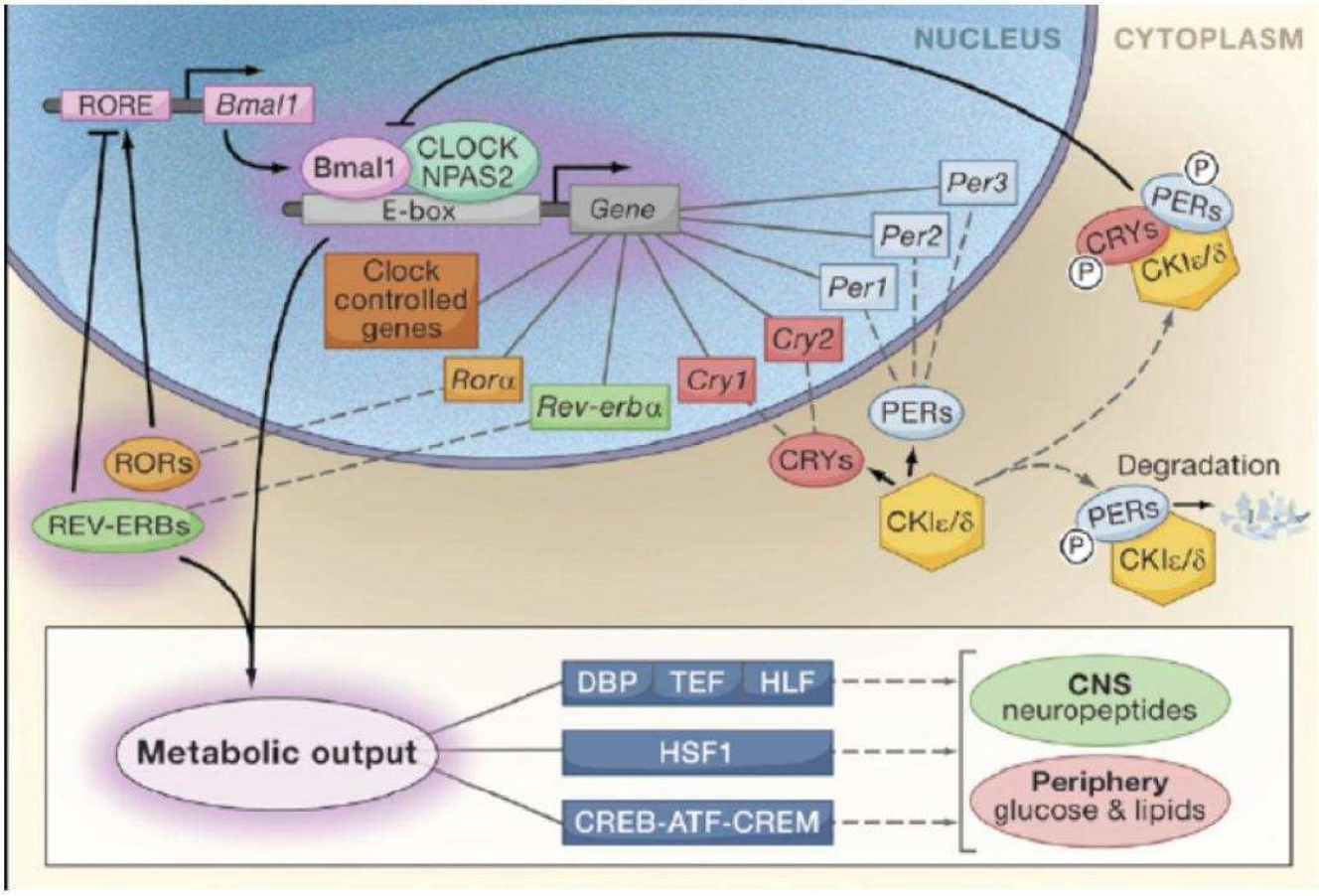


**Figure 2**. Building blocks of the molecular clock (Adapted with permission from Elsevier Inc.) (74).

in part by circadian clocks, transcriptionally based mechanisms that synchronize the organism to its changing environment. The central circadian clock is located within the suprachiasmatic nucleus of the brain, while peripheral clocks are located within virtually all cells outside of the suprachiasmatic nucleus.

Both central and peripheral circadian clocks likely regulate many physiological functions, including insulin sensitivity, endocrine regulation, energy homeostasis, satiety signalling, cellular proliferation and cardiovascular function (52). Clock genes and their protein products are involved in negative and positive regulatory feedback loops. In mammals, the transcriptional activators CLOCK (circadian locomoter output cycles kaput) and BMAL1 (brain and muscle ARNT-like protein 1) heterodimerize and activate the expression of the clock genes *Period (Per) 1, 2* and *3* and *Cryptochrome (Cry) 1* and *2* (53-55). PER and CRY proteins together enter the nucleus and CRYs repress CLOCK-BMAL1-driven transcription, and thus, the expression of the *Per* and *Cry* genes (56-58). The outcome of this feedback loop is the oscillation of Per and Cry transcripts and protein products. Other feedback loops, involving transcription factors such as orphan nuclear receptors (59-62) and DEC1/2 proteins (63,64) add up onto the basic loop to fine tune circadian periodicity (59,61) and mediate outputs pathways (65,66). These output pathways are the links between the molecular clock mechanism and physiological rhythms.

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* (59,61,62,67) REV-ERBα binds to the retinoic acid–related orphan receptor (ROR) response element (RORE) in the *Bmal1* promoter resulting in inhibition of transcription and this action is opposed by RORα, which activates the RORE (59,60-62). In addition to the nuclear hormone receptor feedback loop, PAR domain basic leucine zipper transcription factors (PAR bZIP), including DBP (D-site binding protein), TEF (thyrotroph embryonic factor), HLF (hepatic leukemia factor), and the cAMP pathway (CREB-ATF-CREM) also feedback on the clock, acting through cognate D box and CREB elements respectively (51,67,69-72). Posttranslational modification, including phosphorylation and ubiquitination, provide further regulation of the clock network. Casein kinase 1ε and -δ (CK1ε and CK1δ) phosphorylates PER and CRY, tagging them for polyubiquitylation by the E3 ubiquitin ligase complexes β-TrCP1 (β-transducin repeat containing protein

1) and FBXL3 (F-box and leucine-rich repeat protein 3), respectively, ultimately leading to their degradation by the 26S proteosome (73).

These clocks coordinate biological processes to maintain synchrony with the environmental cycles of light and nutrients. It has been known for many years that numerous aspects of metabolism exhibit daily rhythmicity, including many types of circulating and intracellular metabolites, feeding-related hormones, and ingestive behaviors (74).

Metabolism and circadian clocks are tightly interlocked: clocks drive metabolic processes, and various metabolic parameters affect clocks, producing complex feedback relationships.

The numbers of genes judged to be rhythmic ranged from 3% to 20% in the different microarray studies of gene expression profiles, suggesting that a large proportion of the transcriptomes in these tissues are under circadian control. Among the rhythmic genes identified, many have roles in biosynthetic and metabolic processes, including cholesterol and lipid metabolism, glycolysis and gluconeogenesis, oxidative phosphorylation, and detoxification pathways. Importantly, the rate-limiting enzymes in many of these pathways are under circadian control, suggesting that the clock's influence on these processes may be even broader than indicated by the numbers of rhythmic genes (74,75). Thus, multiple signals may be involved in the entrainment of clocks, including nutrients (sterols, lipids, and/or carbohydrates), humoral signals (insulin, glucocorticoid, and perhaps incretin), and possibly even signals from vagal efferents that travel from autonomic centers to the liver (74,76-78).

In a screen for differentially displayed retinal transcripts selectively increased at night in *Xenopus*, Green and Besharse identified a novel RNA deadenylase, which they termed "nocturnin." *Nocturnin* expression has subsequently been identified in a wide range of mammalian tissues, including liver, oocytes, and brown fat, in addition to retina Intriguingly, expression of *Nocturnin* increases ~100-fold in the early evening hours in the liver, and mice that have mutations in the core circadian clock gene, *Clock*, have reduced levels of Nocturnin (79).

It is important to note that the key role of the liver is to maintain glucose levels through hepatic gluconeogenesis during sleep; thus, Nocturnin may normally play an important role in the metabolic transition from wakefulness to sleep (i.e., the fed to the fasted condition). Transcription of *Nocturnin*, a well characterized deadenylase, is highly rhythmic in several peripheral tissues and has now been identified as an important factor modulating the response to diet-induced obesity (79).

Accumulating evidence reveals intriguing links between the circadian clock and cellular metabolism (74,80,81), which, at least in part, rely on epigenetic control and chromatin remodeling (82). The circadian regulator CLOCK has an intrinsic acetyltransferase activity, which enables circadian chromatin remodeling by acetylating histones (83) and nonhistone proteins, including its own partner BMAL1 (84). The histone deacetylase (HDAC) that counterbalances the histone acetyltransferase function of CLOCK is SIRT1 (85,86), an enzyme whose activity is dependent on intracellular NAD+ levels (87). Although NAD+ is SIRT1's natural cosubstrate, the reduced form of NAD+ (NADH) and the by-product of NAD+ consumption, nicotinamide (NAM), repress the activity of SIRT1 (87) and generate an enzymatic feedback loop on the HDAC function of this enzyme. Two main systems determine NAD+ levels in the cell, the de novo biosynthesis from tryptophan and the NAD+ salvage pathway (88). A critical step of the latter pathway is controlled by the enzyme nicotinamide phosphoribosyltransferase (NAMPT) (89), also known as visfatin or pre–B cell colony-enhancing factor (PBEF), which catalyzes the first step in the biosynthesis of NAD+ from NAM. NAMPT is implicated in cellular metabolism, senescence, and survival in response to genotoxic stress (90-92). Because of the role of SIRT1 in modulating clock function, so that circadian tuning may be achieved by NAD+ oscillating levels (93).

Daily remodelling of histone proteins underlies interactions between circadian clock genes and metabolic genes. This regulatory mechanism could be widespread, affecting other physiological processes. Particular attention was paid to how the molecular circadian clock affects processes related to energy homeostasis. There were two main reasons for this. First, mutation of a key circadian gene, *Clock*, leads to obesity and metabolic syndrome – a combination of disorders that increases the risk of diabetes and heart disease. Second, several genes involved in metabolism, including those mediating the formation of fatty tissue and carbohydrate metabolism (such as *Rev-erbα*, *Rorα* and *Pparα*), show reciprocal regulation with core circadian clock genes.

Alenghat and colleagues' results (94) link these 'chronometabolic' molecular interactions to the cyclic regulation of chromatin through histone acetylation. They show that a specific genetic disruption of the interaction between the nuclear receptor co-repressor 1 (Ncor1) and the chromatin-modifying enzyme histone deacetylase 3 (Hdac3), which is activated by Ncor1, leads to aberrant regulation of clock genes and abnormal circadian behaviour. In turn, the oscillatory expression pattern of several metabolic genes is disrupted, leading to alterations

in energy balance. They find that mice with loss of function of the Ncor1 – Hdac3 complex are leaner than normal, showing increased sensitivity to the hormone insulin as a result of increased energy expenditure. So, contrary to the common perception that disruption of normal daily rhythms (for example, in shift workers) is metabolically deleterious, these results indicate that alterations of normal circadian physiology could lead to favourable metabolic changes - changes that could combat diseases of nutritional excess, including cardiometabolic disorders (95).

Circadian clocks synchronize diverse biological processes in organism to 24-hour light-dark cycles. The rhythmic activation of selective pathways enables the organisms to optimize their ability to store and generate chemical energy, to minimize environmental stresses, and to reproduce through cell growth and division cycles. In mammalian tissues, major metabolic pathways exhibit robust diurnal rhythms, including glucose and lipid metabolism as well as mitochondrial fuel oxidation. This temporal organization of energy metabolism in relation to circadian timing adds a new dimension to the mechanisms that maintain energy homeostasis. Our recent study demonstrated that the transcriptional coactivator PGC-1α is a key factor that integrates clock and metabolic pathways (96). PGC-1α stimulates the expression of clock genes, notably Bmal1 (Arntl) and Rev-erbα (Nr1d1), through coactivation of the ROR family of orphan nuclear receptors. Mice lacking PGC-1α show abnormal diurnal rhythms of activity, body temperature and metabolic rate (97).

## Nuclear Receptors, Circadian Clock and Metabolism

Mammalian genomes contain 48–49 nuclear receptor (NR) genes encoding a large family of proteins that function as ligand-inducible transcription factors (98). This family includes receptors for steroid and thyroid hormones, retinoic acid, vitamin D, oxysterols, and fatty acid derivatives as well as a number of orphan receptors for which no physiological ligands have been identified. Nuclear receptors (NRs) form a large family of transcription factors that include both ligand-inducible and orphan receptors (99).

Whole-body energy homeostasis depends on a tight and coordinated control of key metabolic processes. Nuclear receptors are transcription factors which, in response to activation by endogeneous (from intermediate metabolism) or exogenous (food-derived) small fat-soluble molecules, such as hormones or fatty acids, control clusters of genes involved in major regulatory pathways. Most of them (Rev-erbs and RORs, PPARs, FXRs (NR1H), ERRs (NR3B), for instance) display cyclical expression in liver, adipose tissue and/or skeletal muscle, indicating that they may entrain and synchronise transcriptional metabolic networks in a circadian manner. In this context, the two core clock components Rev-erbα and RORα may play a central role in orchestrating the temporal coordination of metabolism (100).

They are also important regulators of lipid and lipoprotein metabolism, adipogenesis and vascular inflammation. Moreover, they cross-talk with several other nuclear receptors controlling energy homeostasis. Therefore, Rev-erbα and RORα may play a central role in the coordination of metabolic processes and circadian outputs.

Rev-erbα expression is induced during the adipogenic process (101,102) and ectopic Rev-erbα expression in 3T3L1 preadipocytes promotes their differentiation into mature adipocytes and enhances lipid storage (101). This action of Reverbα is further enhanced by treatment with the PPARγ ligand rosiglitazone. PPARγ ligands induce Rev-erbα expression and Rev-erbα over-expression increases the expression of the PPARγ target genes aP2 and CCAAT/enhancer-binding protein (c/EBP)α. Together, these data indicate a role for Rev-erbα in adipocyte differentiation and physiology and suggest that Rev-erbα can directly link the adipogenic process and the master clock system, and could play a role in the well-documented link between sleep disorders and weight gain.

A major characteristic of Rev-erbα expression is its robust circadian regulation *in vitro* in serum-synchronised fibroblasts (103), *ex vivo* in primary cultures of rat hepatocytes (104), as well as *in vivo* in brain, liver, muscle, pancreas and white and brown adipose tissue (75,104-107). RORα also displays a strong diurnal expression pattern in adipose tissue, whereas RORγ (NR1F3) oscillates in the liver (107). RORβ (NR1F2) is also expressed in the SCN and the retina. It is interesting to note that not all ROR isotypes peak at the same time (107). Since the three ROR isoforms do not all cycle in the same tissues, we hypothesise that the ratio between each isoform of ROR and Rev-erb might affect the regulation of the positive limb in a tissue-specific manner and may offer the opportunity to finetune the circadian network to nutrient and energy metabolism.

Several other nuclear receptors (such as PPARs) and their coregulators (i.e. PGC1) show strong circadian patterns in numerous tissues and, although not belonging to the core clock machinery, they might transduce circadian signals into metabolic adjustments. Feeding

time is one of the major cues in the synchronisation of the peripheral clock (108), and food access restriction to the light phase inverses the phase of circadian expression in mouse liver without affecting SCN circadian gene expression. Remarkably, glucocorticoids interfere in that process since mice harbouring a hepatospecific deletion of the glucocorticoid receptor [GR] (NR3C1) invert their circadian liver gene expression more rapidly after the day-time shift of food availability. Interestingly, Rev-erbα expression is down-regulated by glucocorticoids (104), and Rev-erbα may therefore also play a role in phase adjustment to food availability (109).

The fasting/feeding transition is one of the most predictable daily changes. It implies coordinated metabolic adjustments in several metabolic tissues in order to maintain fuel supply and energy homeostasis at all times. PPARγ and SREBP1c play a crucial role in adipose tissue by regulating the balance between lipogenesis and lipolysis. Similarly, PPARα, SREBP1c, PGC1 and HNF4α modulate expression of genes involved in fatty acid (FA) oxidation, glycolysis/gluconeogenesis and play crucial roles in the glucose vs. FA oxidation switch. Interestingly, most of them (except for HNF4α) oscillate diurnally in the liver, skeletal muscle and/or adipose tissue. PPARα has been shown to oscillate diurnally (110) and is able to bind to the Bmal1 promoter and regulate its expression (111), while the clock/Bmal1 heterodimer reciprocally regulates PPARα (112). Both PPARα and PPARγ increase Rev-erbα expression. In addition, a recent report demonstrated that PGC1α enhances Rev-erbα transcription through RORα transcriptional activity potentiation (97).

The expanding functional links between NRs and circadian clocks open novel perspectives for understanding the hormonal regulation of the mammalian circadian system as well as for exploring the role of circadian clocks in the pathogenesis of NR-related diseases such as cancer and metabolic syndrome (99).

## Circadian Rhythms in Adipose Tissue

Obesity is one of the most profound public health problems today, and although much has been learned regarding the regulation of body weight and adiposity, the prevalence of obesity continues to rise. Simplistic explanations based on nutritional overconsumption, poor diet and/or lack of physical activity are inadequate to explain this dramatic rise in obesity prevalence. Current treatments for obesity have been largely unsuccessful in maintaining long-term weight loss, demonstrating the urgent need for new insight into mechanisms that may lead to obesity and altered metabolism. Recently, a number of studies have provided support for a link between the altered sleep/wake patterns associated with our '24-h' lifestyle and obesity. At the heart of the association between sleep and obesity may be a molecular mechanism intrinsic to all eukaryotic cells and organisms, namely the circadian clock (113,114).

Over the course of a 24-h period, the adipocyte must reciprocally adjust rates of triglyceride synthesis (lipogenesis) and storage with rates of triglyceride breakdown (lipolysis). Although diurnal variations in adipose metabolism are undoubtedly influenced by neurohumoral factors, the circadian clock within the adipocyte likely plays a significant role by altering sensitivity of the adipocyte to specific stimuli (e.g. insulin, adrenaline) throughout the day, or by altering the capacity of the adipocyte for triglyceride storage (e.g. influencing expression of lipid-stabilizing proteins, such as perilipin) (115).

Numerous environmental influences promote adipocyte proliferation and differentiation, and the early stages of these processes are governed by a complex interaction of adipocyte-enriched transcription factors. An early signal of adipocyte differentiation is the expression of the C/EBP family of transcription factors (116), which are induced by pro-adipogenic signals, such as insulin, glucocorticoids, IGF-1 and other growth factors, as well as fatty acids.

C/EBP-β has also been shown previously to undergo light entrainable circadian rhythms in the eye (117), suggestive of regulation by the circadian clock. Consistent with these observations, we find that *c/ebpβ* exhibits dramatic diurnal variations in expression in murine epididymal adipose. Taken together, these observations suggest that C/EBP-β, a critical factor in adipocyte differentiation and a putative clock-controlled gene, may be a novel link between the circadian clock, adipocyte biology and global adiposity.

Rev-erbα is an orphan nuclear receptor that has also been implicated in adipocyte differentiation. It is highly expressed in adipose tissue, and its gene expression is specifically induced in differentiating adipocytes. During adipogenesis, Rev-erbα gene expression initially declines and subsequently increases. Remarkably, Rev-erbα protein levels are nearly the opposite, increasing early in adipogenesis and then markedly decreasing in adipocytes. The Rev-erbα protein is necessary for the

early mitotic events that are required for adipogenesis. The subsequent reduction in Rev-erbα protein, due to increased degradation via the 26S proteasome, is also required for adipocyte differentiation because Rev-erbα represses the expression of PPARγ2, the master transcriptional regulator of adipogenesis. Thus, opposite to what might be predicted from Rev-erbα. Gene expression, Rev-erbα protein levels must rise and then fall for adipocyte differentiation to occur. Thus, the dynamic expression of Rev-erbα is an important determinant of adipocyte differentiation (118).

There are multiple lines of evidence that suggest a close relationship between circadian rhythms and adipose biology. Indeed, multiple aspects of adipose-related physiology display daily variation. For instance, in humans 24-h rhythms have been reported in the circulating blood-borne concentration of leptin and adiponectin (119), which are members of the fat-derived hormone family, the adipokines. Leptin exhibits striking circadian patterns in both gene expression and protein secretion, with peaks in leptin expression occurring during the sleep phase of the sleep–wake cycle in humans (120).

Leptin retains diurnal variation in release even in altered metabolic states such as obesity, although the amplitude of peak release is lower in obese subjects (121). Along with leptin, a number of additional adipocyte-specific factors have been demonstrated to exhibit rhythmic expression, including acylation stimulating protein (*asp*), adipsin (*d.f*), resistin (*rstn*), adiponectin (*apm1*) and visfatin (*pbef1*).

In humans, circulating adiponectin levels exhibit both ultradian pulsatility and a diurnal variation. In the latter case, the pattern of adiponectin release is out of phase with leptin with a significant decline at night, reaching a nadir in the early morning (119).

It is now clearly understood that different adipose depots have markedly different influences over body metabolism; for example, visceral fat is a much better predictor of metabolic syndrome than subcutaneous fat (122). This differential function of adipose depots also appears to extend to circadian rhythmicity, as the phase of clock gene rhythms in adipose tissue is dependent upon anatomical location (115,123). These findings suggest that the intracellular clock gene system acts in visceral adipose tissues as well as liver and is influenced by the conditions of obesity/type 2 diabetes and pioglitazone treatment (124).

The role of the peripheral circadian clock mechanism within the adipocyte represents an exciting new field of study in pursuit of the causes of increasing obesity prevalence. Elucidation of the link between the adipocyte-specific circadian clock and obesity may have profound implications on the timing of obesity therapies.

## Circadian Rhythms in Cardiovascular Function

Several aspects of cardiovascular physiology and the incidence of cardiovascular events, such as sudden cardiac death, myocardial infarction, unstable angina, ventricular tachycardia, and ischemic and hemorrhagic stroke, are subject to diurnal variation, peaking in the early morning hours. The early morning surge in blood pressure, accompanied by a decline in endothelial function, coincides with the peak incidence in clinical cardiovascular events. The corresponding oscillations in gene and protein expression of known regulators of vascular physiology highlight the potential importance of the vascular clock in the described diurnal variation of the incidence of cardiovascular events (125).

The existence of a circadian rhythm in the function of human blood vessels has long been recognized. Two aspects of vascular function, vascular tone and thrombus formation, have been studied in respect to the daily cycle. Studies have previously described a circadian variability in both sympathetic tone and vascular reactivity to adrenergic receptor agonists. Other studies have shown that vascular tone exhibits a circadian variation in humans (126).

Both the master clock in the SCN and the peripheral clocks, including those within the vasculature, impose a rhythm in several mediators of vascular function. The SCN can exert its effect both directly into the vasculature and indirectly by synchronizing peripheral clocks. Glucocorticoids, catecholamines, angiotensin II, and eNOS activity vary with time within the day. This temporal variation is responsible for a diurnal variation in vasoacting responses, resulting in the diurnal rhythm of blood pressure. Moreover, circadian clock function is necessary for physiological angiogenesis and thrombogenesis. Circadian clocks inhibit Akt signaling and resulting vascular senescence and promote endothelial progenitor cell mobilization to maintain angiogenesis. Vascular luminal remodeling and composition requires functional circadian clocks for physiological thrombogenesis (125).

Circadian misalignment has been implicated in the development of obesity, diabetes mellitus, and cardiovascular disease. Time-of-day–dependent synchronization of organisms with their environment is mediated by circadian clocks. This cell autonomous mechanism has been identified within all cardiovascular-relevant cell types, including cardiomyocytes. Recent molecular- and genetic-based studies suggest that the

cardiomyocyte circadian clock influences multiple myocardial processes, including transcription, signaling, growth, metabolism, and contractile function. Following an appreciation of its physiological roles, the cardiomyocyte circadian clock has recently been linked to the pathogenesis of heart disease in response to adverse stresses, such as ischemia/reperfusion, in animal models (127).

Accumulating evidence suggests that intrinsic circadian clocks exert some level of control over heart rate. Through maintenance of a controlled environment for a prolonged period of time, Hu et al have reported that circadian rhythms in heart rate variability are driven by an intrinsic mechanism in humans (128).

Myocardial metabolism and contractile function are interlinked (129). Imbalances/impairment of energy metabolism adversely affects cardiac function (130). Conversely, periods of increased cardiac output are balanced by increased metabolic fluxes, thereby meeting energetic demands (131). What is becoming increasingly clear is that metabolism is inseparably interlinked with the circadian clock.

The possibility that a molecular mechanism intrinsic to the cardiomyocyte, such as the circadian clock, may contribute to cardiovascular disease is the subject of contemporary investigation. A principal role of cell autonomous circadian clocks is to allow anticipation of changes in extracellular/environmental stimuli. The presence of such a mechanism enables the cell/organ/organism to react to a stimulus with appropriate timing and response. Therefore, impairment of the clock mechanism may lead to responses outside of the normal physiological range. Interestingly, although essentially all mammalian cell types possess functional circadian clocks, this mechanism appears to be regulated in a cell-type specific manner (132). This raises the possibility that dyssynchronization between distinct cell types (eg, cardiomyocytes, vascular smooth muscle cells, endothelial cells) could occur within a given system/organ (eg, heart) during specific physiological/pathological situations. Whether prolonged circadian dyssynchronization contributes toward the pathogenesis of cardiovascular disease is an interesting hypothesis, Sole and Martino have recently hypothesized that inappropriate elevations in pressure during the sleep/inactive phase may significantly contribute toward the development of pathological hypertrophy, and ultimately contractile dysfunction (ie, cardiomyopathy) (133).

The early stage of fibrosis development involves degradation of the existing extracellular matrix by extracellular matrix metalloproteinases (MMPs). Numerous pathological conditions associated with fibrosis, such as heart failure, myocardial infarction, and hypertension, have also been reported to be associated with increased levels and activities of MMPs (134-136). The activity of MMPs is normally counterbalanced by the presence of tissue inhibitors of matrix metalloproteinases (TIMPs). During times of extracellular matrix breakdown MMP levels have been shown to be elevated and TIMP levels repressed. This is followed by a decrease in MMP activity (by TIMP inhibition) and collagen deposition (137).

Two MMPs (*mmp14 and mmp24*) and 2 TIMPs (*timp1 and timp3*) were identified as being circadian clock regulated (138). Previous research has demonstrated that activation of the SMAD complex (SMAD2/SMAD3/SMAD4) induces the transcription of several types of collagen, including type I, type III, and type VI (139). Interestingly, the expression levels of both *smad3* and *smad4* appear to be influenced by the cardiomyocyte circadian clock (138). Consistent with alterations in SMAD complex components, a large number of collagen genes were identified as clock regulated (*col3a1, col4a1, col4a2, col5a1*, and *col6a3*). Collectively, these data suggest a possible role for the cardiomyocyte circadian clock in modulating cardiac fibrosis, which may in turn influence the pathogenesis of cardiovascular disease (138).

The cardiomyocyte circadian clock has emerged as a molecular mechanism influencing multiple critical myocardial processes. This mechanism profoundly influences myocardial gene expression, signaling, metabolism, and contractile function in a time-of-day–dependent manner. More specifically, the cardiomyocyte circadian clock appears to regulate heart rate, growth, triglyceride and glycogen metabolism, and contractility, as well as modulate the responsiveness of the myocardium to extracellular factors/stimuli, such as fatty acids and β-adrenergic signaling. This mechanism is altered in multiple animal models of cardiovascular disease and modulates the severity of myocardial damage in response to adverse/pathological stresses (eg, ischemic episodes). It is therefore tempting to speculate that dyssynchrony of the cardiomyocyte circadian clock during shift work, diabetes mellitus, and/or obesity contributes to the pathogenesis of cardiovascular disease (127).

In addition to representing a risk factor for cardiovascular disease, core circadian components might be important for the repair of cardiovascular tissue. The process of regeneration of blood vessels depends on proliferation of endothelial and smooth muscle cells, both of which harbor functional clock (140). Accordingly, the circadian proteins might be involved in regulation of the cell cycle checkpoints in a way similar to other tissues (ie, liver) and thus contribute to tissue repair. Recent report demonstrating the presence of functional circadian clock

in cultured bone marrow–derived mouse and human mesenchymal stem cell provides the rationale for potential exploiting of the circadian machinery to facilitate heart repair (141,142).

## Circadian Clock and Metabolic Syndrome

The incidence of the metabolic syndrome (MetS) 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 (73).

In fact, the general disregard for timing, in cycles of feeding, sleep, and activity, is equally pervasive in our modern patterns of reduced sleep, shift work, and 24/7 activity. Thus, it should no longer escape us to recognize that we have taken timing for granted, despite its centrality to maintaining metabolic health (143).

Dysregulation of these pathways owing to, for instance, a sedentary lifestyle and high caloric intake, results in the development of cholesterol abnormalities (dyslipidemia), insulin resistance, obesity and hypertension. These disorders often occur simultaneously and have therefore been grouped under the term 'metabolic syndrome' (Fig. 3). Recent studies show that deletion of the *Clock* and *Bmal1* genes results not only in circadian disturbances, but also in metabolic abnormalities of lipid and glucose homeostasis—a phenotype resembling the metabolic syndrome (144).

Circadian clocks and energy metabolism are linked because the disruptions of the clockwork lead to alterations in metabolism and vice versa (145). Mutation in the *Clock* gene leads to MetS in mice (146), and in humans Clock polymorphisms have been associated with obesity and MetS (147,148). Cellular metabolic states can serve as a link between stimuli from the habitat and drive for the clockwork, because the reduced forms of nicotinamide adenine dinucleotide cofactors stimulate DNA binding of the NPAS2-ARNTL (149) and CLOCK-ARNTL (150) heterodimers, whereas the oxidized forms inhibit the binding (151). *Npas2*-deficient mice have reduced ability to adapt to restricted feeding (152), whereas *Clock*-deficient

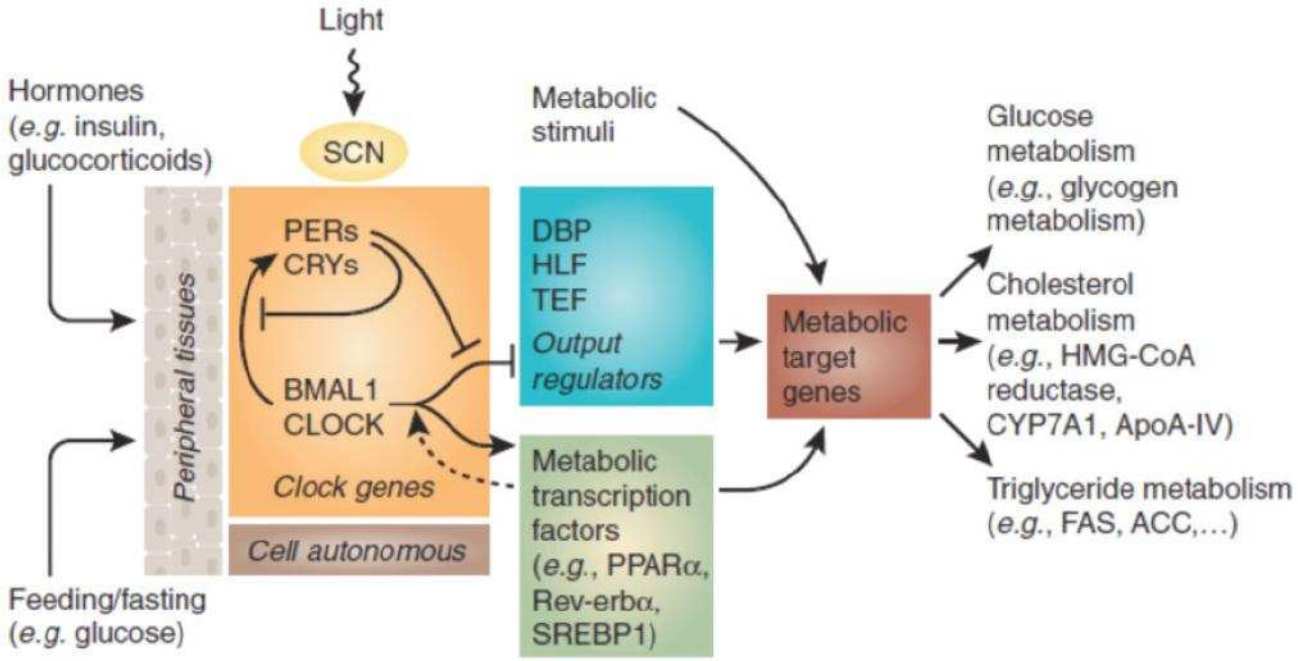


**Figure 3**. Transcriptional control of metabolic pathways by circadian oscillators (Adapted with permission from Nature Publishing Groups) (144).

mice adapt to it even better than do wild-type mice (153), suggesting a key role of NPAS2.

Some examples of adipose-specific molecules implicated in MetS are leptin, adipsin, resistin, adiponectin and visfatin, all of them exhibiting circadian rhythmicity. Glucocorticoids are also key factors in the pathogenesis of MetS that show circadian rythmicity in human adipose tissue (154). Adiponectin, defined as the 'guardian angel' against MetS disturbances (155), exhibits both ultradian pulsatility and a diurnal variation. The daily pattern of this adipocytokine is out of phase with leptin, showing a significant decline at night and reaching a nadir in the early morning (156).

Circadian rhythms in the ability of cardiomyocyte to use fatty acids have also been considered essential in the development of MetS (157). Thus, the inability of cardiomyocyte to cope with the periodical increases in fatty acid availability and results in accumulation of intracellular long-chain fatty acid derivatives, causing contractile dysfunction of the heart. Of particular relevance to MetS is the effect that circadian system has on blood pressure (BP), endothelial and hemostatic function. BP varies diurnally, rising during the day and dipping at night. The loss of this pattern is correlated with insulin resistance and is associated with increased end-organ damage (158).

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 Mets involves the introduction of artificial light and work into the night-time, in addition to the pervasive rise in voluntary sleep curtailment. Indeed, these common disorders of circadian behavior and sleep are associated with increased hunger, decreased glucose and lipid metabolism, and broad changes in the hormonal signals involved in satiety. 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. 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.

## Bench to Bedside Application

It is becoming more and more evident that circadian proteins play important roles in the processes of cell proliferation and control of response to genotoxic stress both at the cellular and organismal levels. The molecular systems and pathways affected by the circadian clock include extracellular signals, checkpoint control, cell proliferation, and DNA damage response, highlighting their importance and potential translational applications (159).

The recent discovery of actual molecular clock-work mechanisms inside virtually all of our cells has added time as a critical fourth dimension of cellular physiology. These principles highlight several important bench-to-bedside applications. Diurnal molecular variation holds considerable promise for novel discovery of physiologically important biomarkers for aiding in understanding, diagnosing, and/or treating human disease. However, in translating research from bench to bedside, the differences between nocturnal animals and diurnal humans must be considered. The risk/benefit ratio of some therapeutic strategies is not the same across the 24-hour diurnal cycle.

Disregard for diurnal rhythms may contribute to differences in therapeutic efficacy, which may be observed, between nocturnal animal models and human patients. Clinical trials should routinely take into account the differing safety and efficacy profiles over 24-hour daily cycles. Chronotherapeutics offers an approach, which may enhance drug efficacy, reduce side effects, attain better patient compliance and perhaps reduce costs even for long established drugs.

These studies also show that sleep disruption or an inappropriately synchronized wake/sleep schedule may be an important environmental determinant affecting the expression of a disease phenotype (160).

## Conclusion

Synchrony between external and internal diurnal rhythms and harmony among the molecular rhythms within the cell is essential for normal organ biology. The substrate and enzymatic components of a given metabolic pathway must be present not only in the right compartmental space within the cell but also at the right time. Cell physiology is 4 dimensional. Harmony between our biology and our environment is a key to good health.

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