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 behavioral 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. (Circ Res. 2010;106:447-462.)

Key Words: clock ■ circadian ■ metabolic syndrome

The metabolic syndrome (MS) is comprised of several metabolic abnormalities, including central (intra-abdominal) obesity, dyslipidemia, hyperglycemia, and hypertension. This syndrome has become a major public health challenge worldwide; an estimated 25% to 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 high-density lipoprotein cholesterol, high blood pressure, and impaired fasting glycemia. Elevated circulating inflammatory and/or thrombotic markers (C-reactive protein, tumor necrosis factor-α, interleukin-6, and plasminogen activator inhibitor type 1) or reduced levels of antiinflammatory molecules such as adiponectin are further markers of MS.2,4

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 behavior and sleep are associated with increased hunger, decreased glucose and lipid metabolism, and broad
changes in the hormonal signals involved in satiety. Recently, Sheer et al demonstrated adverse cardiometabolic end points in human subjects who underwent forced misalignment of behavioral and circadian cycles, simulating the conditions of jet lag and shift work within a controlled clinical setting. Against this backdrop of human studies, advances in the field of experimental genetics have uncovered the fundamental molecular mechanism governing these 24-hour circadian rhythms of physiology, revealing that all mammalian processes that persist under constant conditions with a period of 24 hours are controlled by a network of autoregulatory transcription–translation feedback loops.

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.

The following are terms discussed in this review:

- **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 rhythms**: 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 (ie, 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.

### Adverse Effects of Alterations in Circadian Rhythms: Clinical Evidence

The decrease in sleep duration in the United States has occurred over the same time period as the increase in the prevalence of metabolic disease (reviewed previously). 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 and various other confounding variables are taken into account. For instance, reduced sleep duration in children is associated with increased risk of being overweight. 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 (reviewed previously). For example, healthy subjects who underwent six consecutive nights of sleep restricted to 4 hour exhibited impaired insulin sensitivity following a glucose challenge. 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. Both hormones may also impact energy expenditure (reviewed previously). Curiously, individuals diagnosed with night eating syndrome appear to have greater propensity toward obesity. 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, was proposed to cause clock gene dysfunction, and effective treatments of sleep apnoea have been found to improve glucose metabolism and energy balance. In addition, the circadian oscillation of leptin was found to be disrupted in narcoleptic patients, which may predispose them to weight gain. 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. 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 to 55 years old men and women, respectively. 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 hour 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 proinflammatory cytokines, including interleukin-6 and tumor necrosis factor-α.30,31 This point is of interest because obesity is recognized to involve a low-grade inflammatory state (reviewed previously32). 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 behavioral and circadian cycles. Their behavioral cycle was extended to a 28-hour day, under dim light, with 14-hour rest, and fasting alternated with 14 hours of wakefulness, interspersed with 4 evenly spaced and isocaloric meals. When subjects ate and slept ∼12 hours 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 was elevated, and sleep efficiency was 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 behavioral 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 (eg, 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 whereas many depressed patients, particularly bipolar patients, show delayed sleep phase,38 and depression is also a comorbidity 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 2 Bmal1 haplotypes are associated with type 2 diabetes and hypertension.42 Polymorphisms within other clock core genes (ie, 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/Pbe1 [pre–B-cell colony enhancing factor 1]), 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.

Molecular and Hierarchical Organization of the Clock

The Core Molecular Clock Network

Forward genetics and positional cloning enabled identification of the first mammalian circadian clock gene and provided an entry point into a molecular understanding of the clock mechanism.51,52 The core molecular clock is composed of a transcription–translation feedback loop that oscillates with 24-hour rhythmicity (Figure 1). The driving force is the positive limb of the clock comprised of the bHLH-PAS (basic helix-loop-helix–Period-Arnt-Single-minded) transcription factors CLOCK (circadian locomotor output cycles kaput), and its paralog NPAS2 (neuronal PAS domain protein 2), and BMAL1/ARNTL (brain and muscle aryl-hydrocarbon receptor nuclear translocator-like 1/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-erba and Rora.62–65 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.62–64,66 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
mice display a much more pronounced loss of circadian rhythmicity compared to their single mutant counterparts. Although 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.

Central Clock Organization
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, see87,88). The SCN controls physiological and behavioral 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 behavioral activity, namely with the supraventricular area, the arcuate nucleus and the lateral hypothalamic area.91

The SCN clock is entrained by light through the retinohypothalamic tract (Figure 2). Photic input provides a dominant time-keeping signal (zeitgeber), orienting the animal each day to geophysical time. Endogenous period length is not precisely 24 hour (humans are longer, whereas mice are shorter), thus the daily entrainment to light is a critical mechanism to maintain organismal synchrony with the external environment (Figure 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 (Figure 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 contro-
versy concerning the involvement of circadian oscillators in food anticipatory activity (FAA) because the behavior persists in Bmal1 nullizygous mice.\(^9\) Interestingly, FAA appears to involve the melanocortin signaling pathway, because restriction failed to increase wakefulness before food presentation in melanocortin-3 receptor–null mice.\(^1\) Rather than localization to a single nucleus of hypothalamus, the food entrainable oscillator may in fact involve a more dispersed network of cell groups.\(^1\) Furthermore, the FAA may constitute a metabolic oscillator responsive to peripheral neural or circulating signals elicited by food ingestion.\(^1\) An interesting question remains concerning whether macronutrient flux in the postprandial state may participate in establishing FAA (reviewed in\(^\) and\(^\)). A related question is whether nutrient signaling per se may affect core properties of the SCN pacemaker.

**Peripheral Clocks**

Molecular analyses have revealed that the clock network is also widely expressed throughout nearly every tissue/cell type in vertebrates (Figure 3).\(^1\) Original studies by Schibler and colleagues demonstrated cell autonomous clock gene oscillation within fibroblasts ex vivo.\(^1\) 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 24-hour rhythmic expression, and a large proportion of these genes have a role in metabolic processes (reviewed previously\(^\)). 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.\(^1\) 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.\(^1\) Many nuclear receptors expressed in liver and white and brown adipose tissues also display rhythmic patterns of expression.\(^1\) 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 gastroin-
testinal tract enzymes may ensure that factors involved in nutrient absorption are expressed in anticipation of daily episodes of food ingestion. In addition, adipose enzymes involved in fatty acid storage peak coincident with feeding (reviewed previously). Moreover, components of gluconeogenesis, glycolysis, and fatty acid metabolism cycle with a peak during the subjective night in mouse liver. 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. 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-erba overexpression within liver. 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 nonautonomous signals.

Multiple signals related to feeding, and even fasting, may entrain peripheral clocks. Indeed, 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 mitogen-activated protein kinase (phorbol esters, fibroblast growth factor, endothelin) and glucocorticoid receptors (dexamethasone) and even glucose; dissecting how these signaling pathways converge on the clock remains an area of intensive investigation (reviewed previously).

**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. The reduced forms of these cofactors increase DNA binding, whereas 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.

The gene encoding the rate-limiting enzyme in NAD biosynthesis, nicotinamide phosphoribosyltransferase (NAMPT), displays circadian rhythmicity in peripheral tissues, including liver and white adipose tissue, 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 ClockΔ10/Δ19 and Bmal1−/− mice, whereas they are 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 sirtuin (SIRT1), an NAD-dependent and nutrient responsive deacetylase, which has also recently been described as a novel regulator of circadian clock function. 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 pharmacological 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 regulates both NAD production and SIRT1 activity, whereas, 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 AMP-activated protein kinase–dependent manner, and SIRT1 has been demonstrated in numerous tissues to be increased during fasting or caloric restriction. 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. 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, though it is not yet known whether extracellular NAMPT is regulated in a circadian manner, thereby influencing downstream processes on a systemic level. Recent evidence has also implicated the other NAD-dependent sirtuin family members (SIRT2–7) in a variety of metabolic processes; 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 (PPARγ) and the coactivator 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. It is interesting to note that SIRT1 promotes fat mobilization during fasting by binding to and repressing PPARγ. PGClα also displays circadian oscillations in liver and skeletal muscle and upregulates the transcription of Bmal1 and Rev-erba. Mice lacking PGClα have abnormal diurnal locomotor activity rhythms, body temperature, and metabolic rate, along with altered expression of clock and metabolic genes. PGClα 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 PGClα, 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 to develop therapies targeting disease states involving disruption of both rhythms and metabolism, such as type 2 diabetes.

From Circadian Disruption to Metabolic Disease

What Have We Learned From the Experimental Models?

How might circadian misalignment impact the metabolic comorbidities 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 activity. 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 previously). 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 mutant mice, which express a loss of function mutation in Clock, have yielded new insight in this field. In addition to disruptions in sleep and circadian behavior, 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. 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 mutant mice, and dual-energy X-ray absorptiometry scanning and fat pad weight both demonstrated significant increases in fat and lean mass relative to controls following high fat feeding. It is likely that the obese phenotype results, at least in part, from altered rhythms of neuropeptides in the hypothalamus, because ghrelin, CART (cocaine- and amphetamine-regulated transcript), and orexin are all expressed at constitutively low levels in the Clock mutant mice. In addition, the anorectic neuropeptide POMC was decreased throughout the entire light/dark cycle in hypothalami of young Clock mutant before the onset of weight gain and overt diabetes and is consistent with a deficit in the central homeostatic regulation of weight constancy. Because the original Clock 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 mutant mouse with the melatonin-producing CBA strain to produce the “ClockMEL” mouse. Interestingly, in this model, the restoration of melatonin did not rescue gene expression rhythms in liver or muscle. Such studies underscore the importance of strain background in the evaluation of metabolic phenotype. For example, when introduced onto the ICR strain, the Clock 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. Disruption of other circadian clock genes also leads to metabolic alterations. For example, gene disruption in Bmal1 induces an abnormal metabolic phenotype characterized by impaired gluconeogenesis, hyperleptinemia, glucose intolerance, and dyslipidemia. In addition, Per2 knockout mice develop increased weight gain on high-fat diet (HFD). Conversely, mice deficient in the circadian deadenylase nocturnin 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.

Although clock genes impact metabolic homeostasis, a reciprocal effect of metabolic disruption on circadian rhythms also exists, because diet-induced obesity per se alters circadian behavioral and molecular rhythms in C57BL/6J mice. 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. Interestingly, genetically obese animals are resistant to weight gain when feeding is restricted to the active (dark) phase. In agreement with these observations, recent evidence demonstrated that circadian timing of food intake contributes to weight gain. Indeed, mice fed a HFD only during the 12-hour light phase gain significantly more weight compared to isocalorically fed mice provided food only during the 12-hour dark phase. Further studies are necessary 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 behavioral alterations, including both a decrease in inactive phase activity, core body temperature, feeding and adiposity. Taken together, these observations largely based on 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.

Clock Disruption in Adipose Tissue

Excess adipose tissue and altered body fat distribution, rather than adiposity per se, is an important risk factor for obesity-related disorders. Excess intra-abdominal fat rather than
subcutaneous fat (central versus peripheral obesity) is associated with MS and cardiovascular disease. 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.

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, whereas BMAL1 deletion in adipose cell lines resulted in impaired adipogenesis. Furthermore, heme, the REV-ERBα/β natural ligand, has long been known to enhance adipocyte differentiation in vitro. Activation of SIRT1, which regulates the clock network, may increase insulin sensitivity and reduce the inflammatory response in adipocytes, 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. In addition, HFD also alters the cyclic expression and function of core clock genes and clock-controlled genes in adipose tissue, resulting in disrupted fuel use. Of further interest, clock gene disruption targeted to adipocytes, however it is unclear whether the effect is direct or not.

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 body mass index. However, biopsies from human fat likely represent a heterogeneous 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, including the rhythmic oscillation of genes involved in glucocorticoid turnover. In agreement, human adipose biopsies removed at different zeitgeber times reflect different levels of expression consistent with the observed circadian rhythmicity found in cell-culture studies.

Further studies are necessary 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 adipose tissue and/or misalignment with meal times may lead to inappropriate expression patterns of enzymes involved in lipid metabolism such as lipoprotein lipase. 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 versus muscle) but also within distinct adipose depots (visceral versus subcutaneous). It is further possible that differences of circadian gene expression patterns within visceral adipose tissue and subcutaneous adipose tissue depots may contribute to cell-autonomous differences in inflammatory, lipogenic, and/or lipolytic pathways within these locales. 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 (reviewed previously). Interestingly, both have been proposed to be involved in the etiology of the MS.

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 (reviewed previously). Because 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 on the feeding rhythm. Interestingly, in obese humans, disruption of the 24 hour profiles of leptin and adiponectin was observed compared to healthy lean subjects. These adipokines play major roles in fuel partitioning and insulin sensitivity but also regulate immunity. Indeed, leptin was the first adipokine found to control energy balance. The metabolic effects of leptin are thought to primarily involve its actions within brain whereas adiponectin functions primarily within peripheral target tissues. Many of the metabolic effects of leptin and adiponectin involve activation of AMP-activated protein kinase signaling in muscle/liver. Following the discovery of leptin, a growing list of adipokines has been identified, some of which also exert proinflammatory roles. Because many adipokines are expressed in a circadian fashion in humans, 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. Interleukin-6 and tumor necrosis factor-α plasma levels, which are increased in obesity, may also impair circadian clock gene oscillations and promote sleep.

Circadian regulation may extend to effects within adipose tissue on endoplasmic reticulum (ER) stress, an important component of the inflammatory response in this tissue. Obesity results in conditions that increase demand on the ER in metabolic tissues including liver, adipocytes and pancreas, resulting in a persistent inflammatory state. 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. In adipose tissue, ER stress is involved in adipo-
Clock Disruption and Impaired Glucose Tolerance

Disruption in the normal cyclic pattern of glucose tolerance is a hallmark of type 2 diabetes, 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. For example, oral glucose tolerance is impaired in the evening compared to the morning, an effect which is believed to be attributable to a combination of both decreased insulin secretion and altered insulin sensitivity in the evening. The ‘dawn phenomenon’ is also a well-described phenomenon where glucose levels are known to peak before the onset of the active period. Furthermore, studies in rats have revealed that the SCN is critical for the maintenance of diurnal variations in glucose metabolism.

Although 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 have now demonstrated that genetic variants of the melatonin receptor may be involved in abnormal glucose homeostasis and that NAD metabolism may influence the production of reactive oxygen species. Interestingly, the endoplasmic reticulum chaperone protein BiP, a key protein involved in the ER stress response, is expressed in a circadian manner in flies. It has also been reported that clock genes may influence the production of reactive oxygen species. Thus, disrupted synchrony of stress response gene expression may alter adipose tissue function and thereby contribute to insulin resistance. ER stress may also be induced in brain following high fat feeding, thereby contributing to leptin resistance and perhaps circadian and sleep disturbances (reviewed previously).

In addition to the impact of white adipose tissue 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. As numerous genes including nuclear receptors exert circadian expression profiles in brown adipose tissue, alterations of circadian oscillator genes in fat tissue, and glucose-stimulated insulin secretion in islets. Indeed, NAMPT-deficient (Namp−/−) mice showed impaired glucose tolerance attributable to a defect in glucose-stimulated insulin secretion, which was corrected by intraperitoneal administration of NMN, and mice overexpressing SIRT1 specifically in their β-cells displayed improved glucose tolerance and increased glucose-stimulated insulin secretion. 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.

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. Conversely, pressure overload–induced hypertrophy and diabetes mellitus result in alterations in the circadian clock within the heart. It has also been reported that clock genes, a phenomenon that may be partially explained by the altered diurnal variation in epinephrine and norepinephrine in these mice. Interestingly, the mutated mice also showed a reduced response to immobilization stress compared to wild-type mice. Thus, expression of the core clock within vasculature has been shown to impact blood pressure and thromboocclusive response. Clock genes may influence the temporal incidence of clinical cardiovascular events by regulating the magnitude of the early morning rise in blood pressure. In addition, 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. 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. Effects of the clock system on blood pressure may also involve the modulation of aldosterone biosynthesis by Per1. Furthermore, Anea et al demonstrated that Bmal1-knockout and Clock mutant mice present a loss of vascular adaptation and predisposition to thrombosis, both hallmarks of endothelial dysfunction. The endothelial dysfunction in Bmal1-knockout mice has been related to defects in Akt and nitric oxide signaling. 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 expression.
mutation or Bmal1 deficiency but rather the result of behavioral disruption in these animals.222

As noted above, NAD+ regulation has recently emerged as a major factor coupling circadian rhythms and metabolic signaling pathways. Because 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.225

Cardiomyocytes must adapt rapidly to changes in circulating fatty acid, the primary fuel source for contraction. In this regard, PPAR signaling is important in the control of cardiac energy metabolism.226 Of note, it was also demonstrated that the circadian clock within the cardiomyocyte is essential for responsiveness of the heart to fatty acids.227-229 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.230 These mice developed abnormalities in blood pressure and heart rate in parallel with a reduction of diurnal variation in the sympathetic nerve activity.230 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.230 Thus, the temporal environment may be integrated within the heart by PPARγ.230 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.231

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 antihypertensive effects at night.232 In contrast, only modest decrease antihypertensive effects were detected in studies involving a single lowering heart rate throughout the 24-hour period.232 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.230 For instance, RARα and RXRα interact with CLOCK and MOP4 resulting in repression of CLOCK/MOP4: BMAL1 activity in vascular cells.218 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.218 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, antithrombin, factor VII, protein S, and fibrinogen, have been demonstrated to fluctuate in a circadian manner in humans. Among these factors, plasminogen activator inhibitor type 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 prothrombotic state.235 Circadian control of plasminogen activator inhibitor type 1 gene expression by the REV-ERBα in liver may contribute to the circadian variation in fibrinolysis,236 an effect that may also involve interactions between the cyclolike factor (CLIF) and CLOCK.237 Thus, further studies on the circadian gene control of fibrinolysis may shed new light on factors contributing to the prothrombotic state.

Circadian Rhythms and Hepatic Function
Lever also plays a key role in the development of metabolic syndrome (reviewed previously238). BMAL1 and CLOCK control gene expression of enzymes critical in liver and influence both glucose and lipid homeostasis (reviewed previously239). 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.146 Some of the effects of BMAL1 and CLOCK in liver may involve direct regulation of phosphoenolpyruvate carboxykinase (Pepck).145 In addition, circadian gene expression in hepatocytes is altered in mouse models of type 2 diabetes240 and by high fat feeding.9,241 Moreover, HFD induced a phase delay of components of the adiponectin signaling pathway.241 Alterations in circadian control of adiponectin signaling may reduce its protective effects241 and thereby increase susceptibility to steatosis, a major risk factor in cardiovascular disease.242

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.243 For example, several proteins involved in lipid metabolism (such as hepatic cytochrome P450 cholesterol 7α-hydroxylase, 3-hydroxy-3-methylglutaryl coenzyme A [HMG-CoA] reductase, or apolipoprotein AIV) show diurnal variation in both humans and rodents.105 Interestingly, Rev-erba 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.244 In mouse liver, Rev-erba 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.244 In addition, Rev-erba also controls the daily expression of genes involved in cholesterol and lipid homeostasis through circadian modulation of SREBP (sterol regulatory element-binding protein) signaling.245

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-erba, which is expressed in cells from the immune system such as macrophages and other cell types, may impact the inflammatory response140 (also see elsewhere246). Intriguingly, REV-ERBα increases the tumor necrosis factor-α-induced nuclear factor-κB response,
whereas RORα impedes it. As rhythmic mRNA expression of the clock genes is dampened in peripheral leukocytes of patients with type 2 diabetes, this impairment might be involved in its pathogenesis.

Conclusion

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. In this context, strategies to improve alignment between the cycles of sleep/wakefulness and feeding/fasting may ameliorate physiological processes including appetite 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.

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