# Metabolism and Circadian Rhythms - Implications for Body Weight

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**Abstract:** Mammals have developed an endogenous circadian clock located in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus that responds to the environmental light-dark cycle. Similar clocks are found in peripheral tissues, such as the liver and adipose tissue, regulating cellular and physiological functions. The circadian clock has been reported to regulate metabolism and energy homeostasis, including lipogenic and adipogenic pathways. This is achieved by mediating the expression and/or activity of certain metabolic enzymes and transport systems. In return, enzymes and transcription activators interact with and affect the core clock mechanism. Animals with clock gene mutations that disrupt cellular rhythmicity have provided evidence to the relationship between the circadian clock and metabolic homeostasis. In addition, clinical studies in obese patients accentuate the link between the circadian clock and metabolism. This review will focus on the inter-connection between the circadian clock and metabolism with implications for body weight and how the circadian clock is influenced by hormones that regulate metabolism.

**Keywords:** Clock, body weight, obesity, nutrition, metabolism, circadian rhythms.

## INTRODUCTION

The biological clock regulates the expression and/or activity of enzymes and hormones involved in metabolism. In turn, metabolic factors and some nutrients feed back to entrain circadian clocks. Moreover, disruption of circadian rhythms leads to metabolic disorders. This review will summarize recent findings concerning the relationship between metabolism and circadian rhythms in mammals with implications for body weight. Regulation of circadian rhythms may help combat obesity which has become a serious public health problem.

## CIRCADIAN RHYTHMS

Rotation of earth around its axis imparts light and dark cycles. Organisms on earth evolved to predict these cycles and restrict their activity to either the night or day, being nocturnal or diurnal, respectively. By developing an endogenous circadian (*circa* - about and *dies* - day) clock, which is entrained to external stimuli, organisms on earth ensure that physiological processes are performed at the optimal time during the circadian cycle [1]. In mammals, most of the physiological and behavioral systems, such as sleep-wake cycle, cardiovascular activity, endocrine system, blood pressure, body temperature, renal activity, gastrointestinal tract activity, hepatic metabolism, are regulated by the circadian clock [1, 2].

## THE CENTRAL BIOLOGICAL CLOCK

In mammals, the central circadian clock is located in the hypothalamic suprachiasmatic nuclei (SCN). The SCN clock is composed of single-cell circadian oscillators, which are synchronized and generate coordinated circadian outputs that regulate rhythms in the periphery [3-6]. Similar clock oscillators have been found in peripheral tissues, such as the liver, intestine, retina, adipose tissue, etc. [2, 7-9] **(**Fig. **1)**. Complete destruction of SCN neurons abolishes circadian rhythmicity in the periphery, as it leads to loss of synchrony among individual cells and damping of the rhythm at the population level [10, 11].

As the endogenous rhythms in the SCN are approximately 24 h, it is necessary to entrain the circadian pacemaker each day to the external light-dark cycle to prevent drifting out of phase. Light, a potent synchronizer for the SCN [12], is perceived by the retina and the signal is transmitted *via* the retinohypothalamic tract (RHT) to the SCN [2, 13, 14]. In response, the SCN sends signals to peripheral oscillators to maintain rhythmicity in these tissues. The SCN accomplishes this task *via* neuronal connections or circulating humoral factors [15] **(**Fig. **1)**. Although the mechanisms are not fully understood, several humoral factors expressed cyclically by the SCN, such as transforming growth factor α (TGFα) [16], prokineticin 2 (PK2) [17], and cardiotrophin-like cytokine (CLC) [18], have been shown to inhibit nocturnal locomotor activity when injected intracerebroventricularly. Indeed, all these factors peak during the daily period of locomotor quiescence. In turn, SCN rhythms can be altered by neuronal and endocrine inputs [19] (see below).

In each peripheral tissue, the fraction of cyclically expressed transcripts ranges between 5-20% of the total population and the vast majority of these genes are tissue-specific [1, 9, 20-26]. These findings emphasize the circadian control over a large portion of the transcriptomes in peripheral tissues. For a peripheral tissue, such as the liver, signals from the central SCN clock or the local endogenous clock may control rhythmic gene expression [27, 28].

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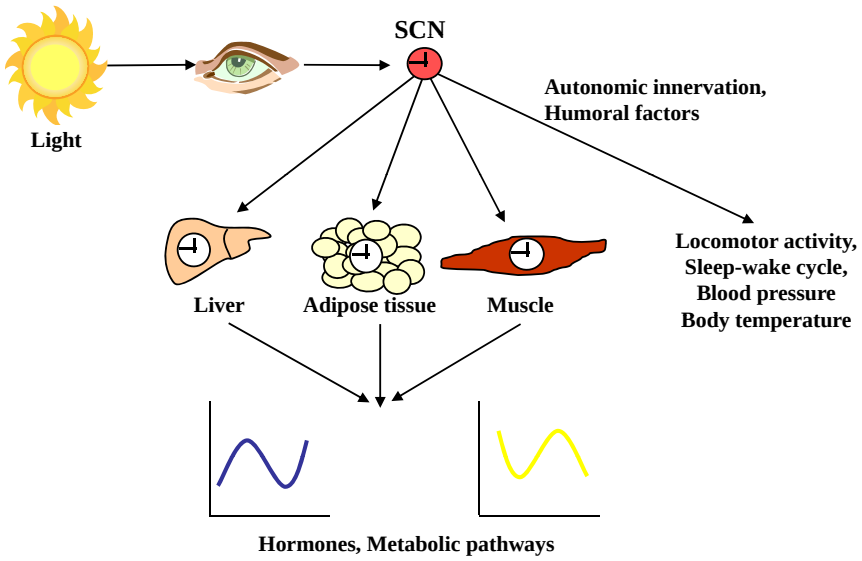


**Fig. (1).** Control of the circadian clock over peripheral tissues. Light resets the suprachiasmatic nuclei (SCN) *via* the retinohypothalamic tract (RHT). The SCN then dictates entrainment of peripheral tissues and physiological system *via* humoral factors or autonomic innervation. As a result, tissue specific hormone expression and secretion and metabolic pathways exhibit circadian oscillation.

## SCN EFFERENTS

SCN fibers have been shown to terminate in and around the arcuate nucleus (ARC), in the ventromedial hypothalamus (VMH), and in the ventral part of the lateral hypothalamus (LH), suggesting an interaction with areas involved in food intake and organization of activity [29] (Fig. **2**). However, the SCN provides its most intense output to the subparaventricular zone (SPZ) and dorsomedial hypothalamus (DMH) [30, 31]. The SPZ and DMH project to other regions in the brain, including the paraventricular nucleus (PVN), the LH, ventrolateral preoptic nucleus (VLPO), and medial preoptic area (MPOA) that regulate corticosteroid release, wakefulness/feeding, sleep, and thermoregulation, respectively (Fig. **2**). Destruction of the ventral SPZ (vSPZ) reduces circadian rhythms of sleep-wakefulness and locomotor activity, but has little effect on circadian regulation of body temperature [32]. Conversely, degeneration of the dorsal SPZ (dSPZ) disrupts circadian regulation of body temperature with minimal effect on sleep-wakefulness and locomotor activity [32]. Ablation of DMH cell bodies, which are innervated by SCN and SPZ neurons, results in severe impairment of circadian-regulated sleep-wakefulness, locomotor activity, corticosteroid secretion, and feeding [33]. Thus, DMH and VMH constitute a gateway between the SCN master pacemaker and brain centers involved in feeding regulation and organization of activity [34] (Fig. **2**).

The SCN can control energy homeostasis by providing its output to pre-autonomic neurons located in the ventral and dorsal borders of the PVN which are connected to the parasympathetic and sympathetic systems [35, 36]. Studies have shown that many inter-neurons, which project from the SCN to the PVN, contain γ-aminobutyric acid (GABA) as neurotransmitter and inhibit the PVN [37, 38]. The SCN uses outputs via the PVN to control glucose metabolism in the liver and via the MPOA to control lipid metabolism in adipose tissue [39-41]. Thus, the SCN is capable of controlling peripheral tissues not only by the secretion of humoral signals but also by affecting the two branches of the autonomic nervous system, i.e., the sympathetic and parasympathetic systems.

## SCN AFFERENTS

From the sites where visceral sympathetic (the dorsal horn) and parasympathetic information enters the brain (the nucleus tractus solitarius (NTS)), no direct projections are known to reach the SCN. Autonomic information is transmitted first to the PVN and then to the SCN. Gastrin-releasing peptide, a mediator of both feeding and locomotor activity, mediates light-like resetting of the SCN [42]. Peptide tyrosine-tyrosine ($PYY_{3-36}$) has also been shown to correlate with alterations to wakefulness and sleep architecture [43]. The effect of these gut-derived polypeptides on the SCN is presumably mediated via vagal afferents that travel through the autonomic nervous system to the SCN.

This seems to be different for areas free of the blood–brain barrier (circumventricular organs) that can directly sense metabolites and hormones in the blood stream. The ventromedial ARC (vmARC) is considered the site where information from the circulation can reach the hypothalamus, either via its connection with the circumventricular median eminence (ME) or through hormones that cross the blood brain barrier and bind to its membrane-bound receptors. The dense reciprocal interaction between the vmARC and the SCN provides the anatomical basis for the link between circulating metabolic information and the SCN [29]. This anatomical connection between vmARC and SCN may form the basis upon which the SCN is informed about circulating hormones and the vmARC about the time of the day (Fig. **2**).

Leptin can be the bridge between energy homeostasis and circadian control, due to its circadian oscillation and expression of its receptor in several hypothalamic regions. Receptors for leptin and ghrelin are present on SCN cells [29, 44, 45], so it is possible that these hormones bind

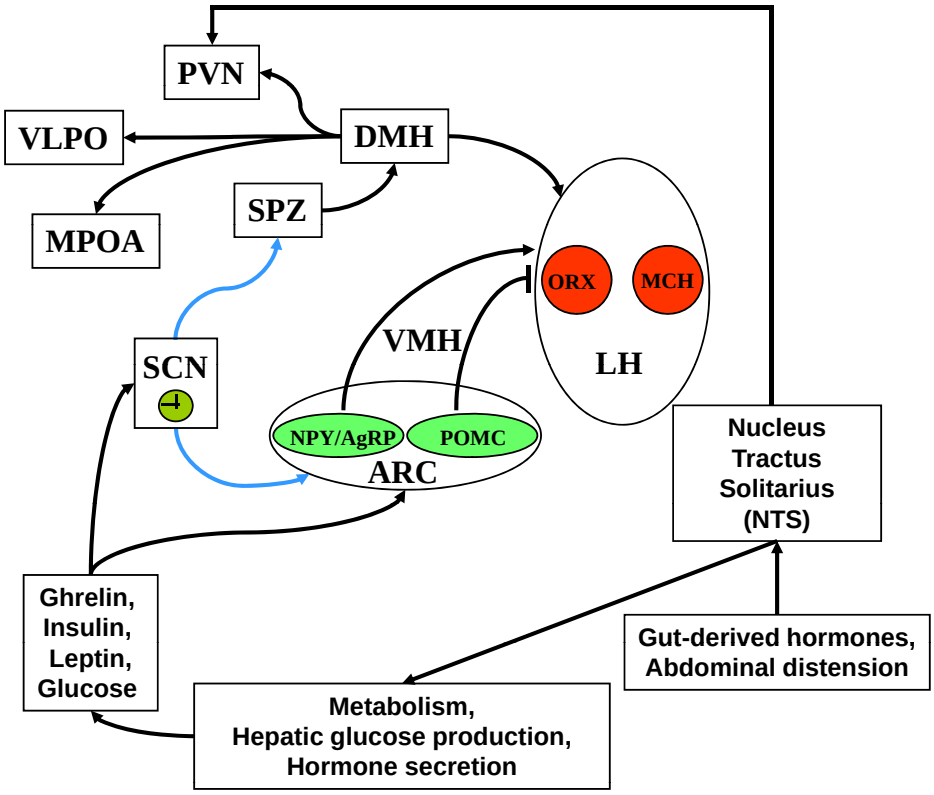


**Fig. (2).** SCN afferents and efferents. The SCN sends neuronal connections mainly to the ARC and SPZ (blue arrows). Hormones and nutrients may affect the ARC directly. The ARC controls expression of orexins and MCH in LH. The SPZ innervates the DMH, which, in turn, innervates PVN, MPOA, VLPO, and LH. AgRP, agouti-related protein; ARC, Arcuate nucleus; DMH, dorsomedial hypothalamus; LH, lateral hypothalamus; MCH, melanin concentrating hormone; MPOA, medial preoptic area; NPY, neuropeptide Y; ORX, orexins; POMC, proopiomelanocortin; PVN, paraventricular nucleus; SCN, suprachiasmatic nuclei; SPZ, subparaventricular zone; VLPO, ventrolateralpreoptic area; VMH, ventromedial hypothalamus.

directly to SCN neurons, similarly to their effect on NPY/AgRP-neurons in the ARC. Activation of ventromedial ARC (vmARC) neurons by systemic administration of the ghrelin mimetic GH-releasing peptide-6 combined with SCN tracing showed that vmARC neurons transmit feeding-related signals to the SCN [29]. Administration of ghrelin to SCN slices or SCN explants *in vitro* caused phase shifts in gene expression. However, administration of ghrelin to wild-type mice only caused phase shifts after 30 h of food deprivation, whereas intraperitoneal injection of ghrelin did not cause phase shifts in wild-type mice fed *ad libitum* [46]. Thus, it is still not clear whether ghrelin and leptin affect the SCN directly or through their effect on the ARC, which is then relayed to the SCN (Fig. **2**).

Thus, there are three possible pathways by which metabolic information may reach the SCN: 1) the sympathetic and parasympathetic branches of the autonomic nervous system; 2) hormones that cross the blood brain barrier; 3) neuronal connection with other nuclei that receive information through connections with circumventricular organs.

## THE MOLECULAR CIRCADIAN CLOCK

The circadian clock in mammals is an intracellular mechanism sharing the same molecular components in SCN neurons and peripheral cells [47]. Generation of circadian rhythms is dependent on the concerted co-expression of specific clock genes, which exhibit a 24-h oscillation in cells. Many clock gene products function as transcription factors, which possess PAS (PER, ARNT, SIM) and basic helix-loop-helix (bHLH) domains involved in protein-protein and protein-DNA interactions, respectively. These factors ultimately activate or repress their own expression and, thus, constitute a self-sustained transcriptional feedback loop. Changes in subcellular localization, concentration, posttranslational modifications (phosphorylation, acetylation, deacetylation, SUMOylation), and delays between transcription and translation lead to the approximate 24-h cycle [1, 2, 48, 49].

In mammals, the first clock gene identified, encodes the transcription factor circadian locomotor output cycles kaput (CLOCK) [50], which dimerizes with brain and muscle-Arnt-like 1 (BMAL1) to activate transcription (Fig. **3**). CLOCK and BMAL1, two PAS-bHLH transcription factors, can activate transcription upon binding to E-box (5'-CACGTG -3') and E-box-like promoter sequences [2]. BMAL1 can also dimerize with other CLOCK homologs, such as neuronal PAS domain protein 2 (NPAS2), to activate transcription and sustain rhythmicity [51, 52]. PERIOD (PER1, PER2, and PER3) and two CRYPTOCHROME (CRY1 and CRY2) proteins operate as negative regulators and inhibit CLOCK:BMAL1-mediated transcription [6, 53, 54] (Fig. **3**). Recent studies have demonstrated that CLOCK has histone acetyltransferase activity [55, 56]. Indeed, cyclic

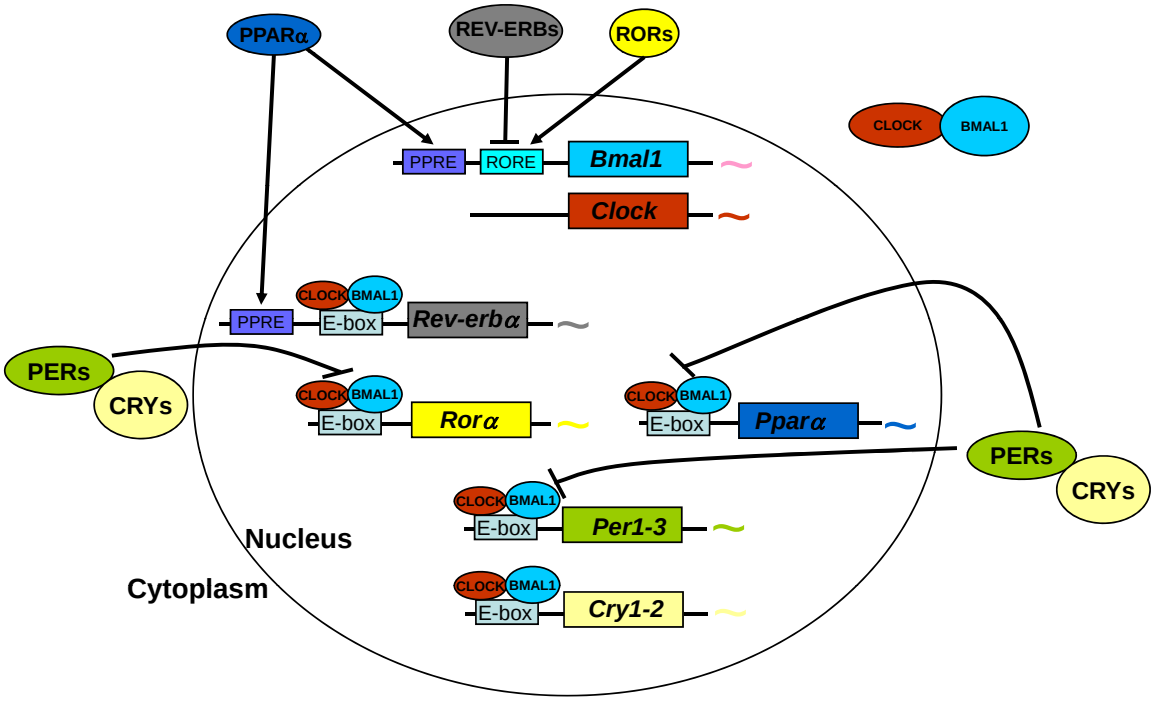


**Fig. (3).** The core mechanism of the mammalian circadian clock and its link to lipid metabolism. The CLOCK:BMAL1 heterodimer binds to enhancer E-box sequences and activates transcription. PERs and CRYs inhibit CLOCK:BMAL1, resulting in decreased transcription. CLOCK:BMAL1 heterodimer induces the transcription of *Rev-erbα*, *Rorα*, and *Pparα*. RORα stimulates and REV-ERBα inhibits *Bmal1* transcription, acting through ROR elements (RORE). PPARα activates transcription of *Rev-erbα* and *Bmal1* by binding to peroxisome proliferator-response elements (PPRE).

histone acetylation and methylation have been observed on the promoters of several clock genes [56-60]. In addition to the aforementioned factors, several other proteins are crucial in order to sustain clock function. Casein kinase I epsilon (CKIε) is thought to phosphorylate the PER proteins and, thereby, enhance their instability and degradation [48, 61-63]. CKIε also phosphorylates and partially activates BMAL1 [64].

## EFFECT OF THE BIOLOGICAL CLOCK ON METABOLISM

Hypothalamic orexigenic neuropeptides, such as neuropeptide Y (NPY) and Agouti-related protein (AgRP), and anorexigenic neuropeptides, such as proopiomelanocortin (POMC), are expressed according to a pronounced diurnal rhythm [65]. Many hormones involved in metabolism, such as insulin, glucagon, adiponectin, corticosterone, leptin, and ghrelin, have been shown to exhibit circadian oscillation [66]. Leptin, an adipocyte-derived circulating hormone that acts at specific receptors in the hypothalamus to suppress appetite and increase metabolism, is extremely important in obesity. Leptin exhibits striking circadian patterns in both gene expression and protein secretion, with peaks during the sleep phase in humans [67]. Neither feeding time nor adrenalectomy affects the rhythmicity of leptin release. However, ablation of the SCN has been shown to eliminate leptin circadian rhythmicity in rodents, suggesting that the central circadian clock regulates leptin expression [68]. In addition, SCN-lesioned rats, as opposed to intact animals, showed no elevation in plasma free fatty acids after intraperitoneal administration of leptin, suggesting a role for SCN in leptin function [69].

In addition to the endocrine control, the circadian clock has been reported to regulate metabolism and energy homeostasis in peripheral tissues [66, 70]. This is achieved by mediating the expression and/or activity of certain enzymes and transport systems [71, 72] involved in the different metabolic pathways, such as cholesterol metabolism, drug and toxin metabolism, the citric acid cycle, and glucose metabolism [34, 73-76]. Recently, a comprehensive survey of nuclear receptor mRNA profiles in white and brown adipose tissue, liver, and skeletal muscle in mice revealed that ~50% of the known nuclear receptors exhibit rhythmic expression [77]. As these receptors sense various lipids, vitamins, and fat-soluble hormones, they serve as direct link between nutrient-sensing pathways and the circadian control of gene expression. Similarly, glucose uptake and the concentration of adenosine triphosphate (ATP) in the brain and peripheral tissues have been found to fluctuate around the circadian cycle [41, 74, 78]. Lesion of rat SCN abolishes diurnal variations in whole body glucose homeostasis [79], altering rhythms in glucose utilization rates and endogenous hepatic glucose production.

## EFFECT OF METABOLISM ON CIRCADIAN RHYTHMS

Adenosine monophosphate (AMP)-activated protein kinase (AMPK), an important low energy sensor, has been found to phosphorylate Ser-389 of CKIε, resulting in increased CKIε activity and degradation of mPER2. mPER2

degradation leads to a phase advance in the circadian expression pattern of clock genes in mice [80]. In addition, AMPK$\gamma_3$ subunit knockout mice exhibits impaired expression profile of clock-related genes, such as *Per1* and *Cry2*, in skeletal muscle in response to 5-amino-4-imidazole-carboxamide riboside (AICAR), an AMPK activator, as well as a diurnal shift in energy utilization [81]. As AMPK has been implicated in feeding regulation [82] and it serves as an energy sensor, it could be one of the links that integrates the circadian clock with metabolism.

Another protein, recently found to link metabolism with the circadian clock, is SIRT1. SIRT1 is an $NAD^+$-dependent histone deacetylase involved in transcriptional silencing, genome stability, and a key factor in the longevity response to caloric restriction [83, 84]. It seems that after binding to E-box, CLOCK and CBP/p300 acetylate histones H3 and H4 [55] and BMAL1 leading to binding of PER/CRY complex [85] and PER2 acetylation [86]. SIRT1 interacts directly with CLOCK and deacetylates BMAL1 and PER2 [86-88] leading to PER2 phosphorylation and degradation and a new cycle can begin. In addition, CLOCK:BMAL1 heterodimer regulates the circadian expression of NAMPT (nicotinamide phosphoribosyltransferase), a rate-limiting enzyme in the $NAD^+$ salvage pathway. SIRT1 is recruited to the *Nampt* promoter and contributes to the circadian synthesis of its own coenzyme [89]. Most recently, it has been shown that AMPK enhances SIRT1 activity by increasing cellular $NAD^+$ levels, resulting in the deacetylation and modulation of the activity of downstream SIRT1 targets [90].

Thus, it turns out that the levels of $NAD^+$ regulate circadian rhythms [91]. Indeed, CLOCK and its homolog NPAS2 can bind efficiently to BMAL1 and consequently to E-box sequences in the presence of reduced nicotinamide adenine dinucleotides (NADH and NADPH). On the other hand, the oxidized forms of the nicotinamide adenine dinucleotides ($NAD^+$ and $NADP^+$) inhibit DNA binding of CLOCK:BMAL1 or NPAS2:BMAL1 [91, 92]. The ratio of $NAD(P)^+$/NAD(P)H, which dictates the binding of CLOCK/NPAS2:BMAL1 to E-boxes, could cause phase-shifts in gene expression [71, 91, 92].

## CIRCADIAN RHYTHMS AND LIPID METABOLISM

Circadian clocks have been shown to regulate the physiology of inguinal white adipose tissue, epididymal white adipose tissue, and brown adipose tissue [9, 93, 94]. Clock and adipokine genes, such as resistin, adiponectin, leptin, and visfatin, exhibit circadian expression in visceral fat tissue [95]. In addition, *Fatty acid transport protein 1* (*Fatp1*), *fatty acyl-CoA synthetase 1* (*Acs1*), and *adipocyte differentiation-related protein* (*Adrp*) all exhibit diurnal variations in expression [96].

BMAL1 activity has been shown to be involved in the control of adipogenesis and lipid metabolism in mature adipocytes. Embryonic fibroblasts from *Bmal1*$^{-/-}$ knockout mice failed to differentiate into adipocytes. In addition, loss of BMAL1 expression led to a significant decrease in the expression of several key adipogenic/lipogenic factors, such as peroxisome proliferator-activated receptor γ2 (PPARγ2), adipocyte fatty acid-binding protein 2 (aP2), CCAAT enhancer binding protein α (C/EBPα), C/EBPδ, sterol regulatory element-binding protein 1a (SREBP-1a), phosphoenolpyruvate carboxykinase (PEPCK), and fatty acid synthase (FAS). Furthermore, over-expression of BMAL1 in adipocytes increased lipid synthesis activity. These results indicate that BMAL1, a key protein in the core clock mechanism, plays important roles in the regulation of adipose differentiation and lipogenesis in mature adipocytes [97]. The role of BMAL1 in adipogenesis is further emphasized, as its expression is negatively regulated by the transcription factor reverse erythroblastosis virus α (REV-ERBα) [98], and positively regulated by retinoic acid receptor-related orphan receptor α (RORα) and RORγ [99] *via* the ROR response element (RORE) [100]. Both REV-ERBs and RORs have been implicated in adipogenesis and lipid metabolism [101]. Interestingly, CLOCK:BMAL1 heterodimer regulates the expression of both *Rev-erbα* and *Rorα* [98, 99, 102] (Fig. **3**). Mice deficient in RORα or REV-ERBα have impaired circadian rhythms of locomotor activity and clock gene expression [98, 99]. In addition to REV-ERBs and RORs, peroxisome proliferator-activated receptor α (PPARα), provides another example of a reciprocal link between circadian and lipid metabolic processes. PPARα regulates the transcription of genes involved in lipid and glucose metabolism upon binding of endogenous free fatty acids. The CLOCK:BMAL heterodimer mediates transcription of PPARα, which subsequently binds to the peroxisome-proliferator response element (PPRE) and activates transcription of *Bmal1* [103-105] (Fig. **3**).

The PPARγ coactivator, PGC-1α, a transcriptional coactivator that regulates energy metabolism, is rhythmically expressed in the liver and skeletal muscle of mice. PGC-1α stimulates the expression of *Bmal1* and *Rev-erbα*, through coactivation of the ROR family of orphan nuclear receptors [106, 107]. Mice lacking PGC-1α show abnormal diurnal rhythms of activity, body temperature, and metabolic rate due to aberrant expression of clock genes and those involved in energy metabolism. Analyses of PGC-1α-deficient fibroblasts and mice with liver-specific knockdown of PGC-1α indicate that it is required for cell-autonomous clock function [106]. Acetylated PGC-1α is also a substrate for SIRT1 [90]. Thus, PPARα, PPARγ, REV-ERBα, RORα, and PGC-1α are key components of the circadian oscillator that integrate the mammalian clock and lipid metabolism. The inter-connection between the clock core mechanism and lipogenic and adipogenic pathways emphasizes why clock disruption leads to metabolic disorders.

## CIRCADIAN RHYTHMS AND METABOLIC DISORDERS

Disruption of circadian rhythms in the SCN and peripheral tissues leads to metabolic disorders [108-110]. Evidence suggests that loss of circadian rhythmicity of glucose metabolism may contribute to the development of metabolic disorders, such as type 2 diabetes, in rodents [111-113] and humans [114, 115]. For example, daily cycles of insulin secretion and glucose tolerance are lost in patients with type 2 diabetes [115, 116], as are daily variations in plasma corticosterone levels and locomotor activity in streptozotocin-induced diabetic rats [111, 112]. The oscillations of clock (*Bmal1*, *Per1*, *Per2*, *Cry1*, *Cry2* and *Dbp*) and adipokine genes were mildly suppressed in the adipose tissue of obese KK mice and greatly suppressed in

the adipose tissue of obese, diabetic (KK-$A^y$) mice [95]. Similarly, obese diabetic mice exhibit circadian oscillation of most genes in the liver, but some genes had attenuated, but still rhythmic, expression [117]. In addition, in type 1 diabetes patients, lipolysis is increased earlier in the evening than in healthy controls and remains elevated throughout the night, indicating that lipolysis shows a distinct circadian rhythm that is altered in type 1 diabetes patients [118]. These findings point to the tight relationship between disruption of circadian rhythms and metabolic disorders.

The most compelling linkage between metabolic disorders and the circadian clock is demonstrated in clock gene-mutant and knockout animals. Several strains with varying effects on metabolism have thus far been examined. Homozygous C57BL/6J *Clock*$^{\Delta 19}$ mice, with a truncated exon 18 and deleted exon 19 of the *Clock* gene, have a greatly attenuated diurnal feeding rhythm, are hyperphagic and obese, and develop a metabolic syndrome of hyperleptinemia, hyperlipidemia, hepatic steatosis, and hyperglycemia [119]. Loss of circadian rhythms in *Clock*$^{\Delta 19}$ mutant mice was accompanied by attenuated expression of hypothalamic peptides associated with energy balance, such as ghrelin and orexin [119]. In addition, *Clock*$^{\Delta 19}$ mice had altered gluconeogenesis and increased insulin sensitivity [120, 121]. In *Clock*$^{\Delta 19}$ on an Jcl:ICR background, serum levels of triglyceride and free fatty acids were significantly lower than in wild-type control mice, whereas total cholesterol and glucose, insulin, and leptin levels did not differ [122]. Unlike C57BL/6J *Clock*$^{\Delta 19}$ mutant mice [119], neither male nor female Jcl:ICR *Clock*$^{\Delta 19}$ mutant mice were obese, and they mostly had low or normal fasting plasma glucose rather than hyperglycemia, low plasma free fatty acids rather than hyperlipidemia, and normal plasma leptin rather than hyperleptinemia. Combination of the *Clock*$^{\Delta 19}$ mutation (Jcl:ICR) with the leptin knockout (*ob/ob*) resulted in significantly heavier mice than the *ob/ob* phenotype [123]. However, in Jcl:ICR *Clock*$^{\Delta 19}$ mutant mice, high fat diet amplified the diurnal variation in glucose tolerance and insulin sensitivity and obesity was attenuated through impaired dietary fat absorption [122]. Triglyceride content in the liver was significantly less increased in Jcl:ICR *Clock*$^{\Delta 19}$ mutant mice fed a high-fat diet compared with wild-type mice. Jcl:ICR *Clock*$^{\Delta 19}$ mutant mice had attenuated daily rhythms of *Acsl4* (acyl-CoA synthetase long-chain 4) and *Fabp1* (fatty acid binding protein 1) gene expression in the liver under both normal and high-fat diet conditions compared to wild-type mice, which could have led to the attenuated accumulation of triglycerides in the liver under a high-fat diet [124]. In *Clock*$^{\Delta 19}$ mutant, melatonin producing mice of the BALB/c/CBA background, relative weight of epigonadal fat compared with body weight was not significantly different between male wild-type and mutant mice fed a high-fat diet [121]. Although the effects on metabolism were variable, due to strain differences, the over all picture is that disruption of the *Clock* gene leads to disruption of metabolic pathways.

*Bmal1*$^{-/-}$ knockout mice are lean. However, similarly to C57BL/6J *Clock*$^{\Delta 19}$mutant mice, *Bmal1*$^{-/-}$ knockout mice exhibit suppressed diurnal variations in glucose and triglycerides as well as abolished gluconeogenesis. Although recovery from insulin-induced hypoglycemia was impaired in C57BL/6J *Clock*$^{\Delta 19}$ mutant and *Bmal1*$^{-/-}$ knockout mice, the counter-regulatory response of corticosterone and glucagon was retained [120]. Thus, CLOCK and BMAL1 regulate the recovery from insulin-induced hypoglycemia, glucose tolerance, insulin sensitivity, and fat absorption.

Mutation in another central clock gene *Per2* (*mPer2*$^{-/-}$ mice), exhibits no glucocorticoid rhythm even though the corticosterone response to hypoglycemia is intact. Although food consumption is similar during the light and dark periods on high fat diet, *mPer2*$^{-/-}$ mice develop significant obesity [125]. *mPer2*$^{-/-}$ mice also exhibit increased bone density in mice [126]. As bone and adipose tissue share a common ontogeny, it is possible that these findings may also have implications for adipogenesis [127].

## EFFECT OF HIGH-FAT DIET ON CIRCADIAN RHYTHMS

Few studies show that a high-fat diet leads to minimal effects on circadian gene expression in visceral adipose tissue and liver [128, 129]. However, recent studies show that high-fat diet leads to rapid changes in both the period of locomotor activity in constant darkness and to increased food intake during the normal rest period under light-dark conditions [130]. These changes in behavioral rhythmicity correlate with disrupted clock gene expression within hypothalamus, liver, and adipose tissue, leading to altered cycling of hormones and nuclear hormone receptors involved in fuel utilization, in mice, rats, and humans [130-135]. Furthermore, a high-fat diet modulates carbohydrate metabolism by amplifying circadian variation in glucose tolerance and insulin sensitivity [120].

In addition to the disruption of clock gene expression, high-fat diet induced a phase delay in clock and clock-controlled genes [134]. AMPK has been found to phosphorylate Ser-389 of CKIε, resulting in mPER2 degradation and a phase advance in the circadian expression pattern of clock genes [80]. As the levels of AMPK decline under HF diet, it is plausible that the changes seen in the expression phase of genes under HF diet are mediated by changes in AMPK levels [134]. In addition to its effect on gene expression, high-fat feeding led to impaired adjustment to local time by photic resetting. These results correlated with reduction in c-FOS and pERK expression in the SCN in response to light-induced phase shifts [136].

## CIRCADIAN RHYTHMS AND OBESITY

Fluctuations in body weight have been associated with changes in day length in various species, suggesting a central role for the circadian clock in regulating body weight. For example, in Siberian hamsters, modulation of body weight depends on photoperiod acting *via* the temporal pattern of melatonin secretion from the pineal gland [137, 138]. In studies performed on sheep, adipose tissue leptin levels were modulated by day length independently of food intake, body fatness, and gonadal activity. In addition, increasing the length of the photoperiod resulted in increased activity of the lipogenesis-promoting proteins lipoprotein lipase and malic enzyme, independent of the nutritional status [139, 140]. In humans, studies have demonstrated an increased incidence of obesity among shift workers [141-143].

Clock and adipokine genes, such as resistin, adiponectin, leptin, and visfatin, exhibit circadian expression in visceral fat tissue [95]. The expression of these mediators is blunted in obese patients [68, 144, 145]. In obese subjects, leptin retains diurnal variation in release, but with lower amplitude [146]. Leptin 24-h levels were lower in obese compared with non-obese adolescent girls, suggesting that blunted circadian variation may play a role in leptin resistance and obesity [147]. Circadian patterns of leptin concentration were distinctly different between adult women with upper-body or lower-body obesity, with a delay in peak values of leptin of approximately 3 h in women with upper-body obesity [148]. Indeed, leptin and the leptin receptor knockouts in animals or mutations in humans have been demonstrated to produce morbid, early onset obesity, hypoleptinemia, hyperphagia, hyperinsulinemia, and hyperglycemia [149-152]. Similarly to leptin, the rhythmic expression of resistin and adiponectin was greatly blunted in obese (KK) and obese, diabetic (KK-$A^y$) mice [95]. 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 [145]. In obese subjects, adiponectin levels were significantly lower than lean controls, although the obese group had significantly higher average pulse height and valley concentrations [153]. In rats, melatonin, a synchronizer of the SCN clock, decreased weight gain in response to high-fat diet and decreased plasma leptin levels within 3 weeks. These effects were independent of total food consumption [154]. Thus, it seems that the circadian clock plays a major role in determining body weight probably by influencing the expression and secretion of hormones.

A potential role of the *Clock* polymorphisms in obesity and other metabolic disorders has been suggested. A significant association was found between the different *Clock* haplotypes and obesity, nonalcoholic fatty liver disease, the metabolic syndrome, type 2 diabetes, and cardiovascular disease [155-157]. In addition, the *Clock* gene CGC haplotype may be protective for the development of obesity [156]. However, the 3111T/C single nucleotide polymorphism (SNP) of the *Clock* gene is not associated to human obesity and/or binge eating disorder, but it seems to predispose obese individuals to a higher BMI [158]. Thus, polymorphism in the *Clock* gene is associated with obesity and metabolic disorders.

## SUMMARY AND CONCLUSIONS

Western lifestyle leads to high food consumption, inactivity during the active period, enhanced activity in the rest period, and shortened sleep period. This lifestyle may cause disrupted circadian rhythms leading to obesity and metabolic disorders. Disruptions of rhythms together with genetic background increase the risk to develop health complications. Unfortunately, circadian rhythms in metabolism are often overlooked in both treatments and design of clinical and animal studies. Resetting the biological clock by food or feeding time may lead to better functionality of physiological systems preventing metabolic disorders and promoting well-being.

## ACKNOWLEDGEMENTS

This study was supported by grants from Nutricia Research Foundation (grant no. 2008-16, 2009-E3) and the Binational USA – Israel Science Foundation (BSF) (grant no. 2007101).

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Received: July 13, 2009 Revised: July 23, 2009 Accepted: July 24, 2009