Circadian rhythms and clocks in adipose tissues: current insights

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Abstract: Endogenous circadian timekeepers are found in most cells and organs of the body, including the different types of adipose tissues. This clock network orchestrates 24-hour rhythms of physiology and behavior to adapt the organism to daily recurring changes in the environment. Energy intake and expenditure as well as adipose physiology are under circadian control and, therefore, energy homeostasis and circadian clock function are closely linked. In this review, we summarize the current knowledge about the regulation and targets of adipocyte circadian clocks and how circadian rhythm disruption affects energy homeostasis and adipose tissue function. We provide a more detailed overview of metabolic phenotypes of different mouse models of circadian clock dysfunction and discuss the implications of (adipose) clock disruption on adipocyte–brain cross talk and metabolic homeostasis.

Keywords: food intake, metaflammation, clock genes, adipocyte–brain cross talk, adipokines

Introduction
Due to the earth’s rotation around its axis, life is embossed by two opposing daily recurring conditions, day and night. To deal with the resulting predictable changes, most species have developed circadian clocks (from Latin circa – about, dies – day) allowing an anticipation of daily recurring events. While a master pacemaker is located in the suprachiasmatic nucleus (SCN) of the hypothalamus, almost all peripheral tissues – including adipose tissues – and many brain nuclei harbor their own functional clocks.1,2 The SCN orchestrates these tissue clocks via the endocrine and nervous system to induce rhythmic behavior and physiology (Figure 1).

At the molecular level, circadian clocks are based on interlocked transcriptional–translational feedback loops of clock genes/proteins such as the transcription factors Clock (circadian locomotor output cycles kaput) and Bmal1 (brain and muscle ARNT-like protein 1, also known as Arntl) and the transcriptional modulators Period (Per1-3) and Cryptochrome (Cry1/2).3 The oscillation of the circadian clock machinery leads to rhythmic expression of tissue-specific programs of clock-controlled genes (CCGs) through activation of circadian promoter elements (E-boxes, D-boxes, and retinoid orphan response elements, ROREs). In mice, it was estimated that over 40% of all protein coding genes show circadian oscillations at least in one tissue.4 Many of them are involved in metabolic pathways, for example, glucose homeostasis and cholesterol and fatty acid (FA) metabolisms.5 Also, several adipokines, which will be discussed in the following text, have been shown to be directly regulated by the circadian clock, for example, leptin and adiponectin.6,7
To generate exact 24-hour rhythms, the circadian system has to be synchronized with the external light–dark cycle. The main synchronizer (or zeitgeber) of mammalian clocks is light. Irradiation information is integrated by intrinsically photosensitive retinal ganglion cells (ipRGCs) and transmitted to the SCN via the retinohypothalamic tract (Figure 1).8–10 For peripheral clocks, the timing of food intake is a zeitgeber to align peripheral tissues such as the liver or adipose tissue with energy availability.

Circadian rhythm disruption or genetic alterations in the clock gene machinery lead to pathophysiological effects ranging from sleep disorders to cardiovascular, mental, and metabolic impairments.11–13 Since energy homeostasis is centrally regulated, circadian misalignment of the brain–adipose axis might play an important role in the development of metabolic disorders.14,15 In this review, we will summarize the current knowledge about adipose clock function with a focus on energy homeostasis.

Adipose tissue circadian clocks
Like in most cells, circadian clocks are present in adipocytes regulating many essential adipose tissue processes. Lipolysis, adipogenesis, inflammation, brown adipose tissue (BAT) thermogenesis, as well as expression and secretion of several adipose hormones are under circadian control (Figure 2). Circadian rhythm disruptions alter adipose tissue physiology and may affect whole body homeostasis. Hence, adipose clocks are interesting targets for preventing and treating metabolic impairments in circadian rhythm disorders, for example, in shift workers.

In vitro and in vivo experiments reveal rhythmic expression of clock genes in different white adipose depots in rodents16–18 and humans.19–24 In line with this, several hundreds of genes display a diurnal expression rhythm in the adipose tissue of rodents16,17,25,26 and human,21 some of which are involved in core adipose functions such as lipolysis, adipogenesis, and metabolic inflammation (detailed genes are specified in Figure 2).18,21,25

The role of the circadian clock machinery in adipocyte physiology has been described in both in vitro and in animal studies. Knockdown of the clock genes, either Bmal1 or Rev-Erbα, in cells inhibits adipocyte differentiation while mutations of two other clock components, Per2 or retinoid orphan receptor α (RORα), increase adipogenesis.27–32 Both effects are mediated by peroxisome proliferator-activated receptor (PPARγ), a transcription factor crucial for terminal adipocyte differentiation, which is a direct adipose CCG.33,34 Interestingly, Bmal1 seems to have a bimodal impact on adipocyte differentiation. Whereas its knockdown leads to an upregulation of adipogenic genes during early differentiation (by suppression of the canonical Wnt pathway), fewer mature adipocytes survive at later stages.35

Interestingly, adipose PPARγ (and clock gene) expression rhythms are dampened under high-fat diet (HFD) conditions in male mice.36 This effect was not observed in female animals,37 in line with a persistent normal diurnal food intake...
Adipose clocks

**Adipose function in clock gene-mutant mice**

Many clock gene-mutant mouse strains show changes in adipose physiology (Table 1) often associated with altered body weight. Clock-Δ19 mutants show increased body weight and fat content with adipocyte hypertrophy.\(^{18,38,54,55}\) Furthermore, these mice are hyperphagic and display a dampened feeding rhythm.\(^{18,38}\) In Clock-Δ19, WAT expression of Atgl and Hsl is arrhythmic and overall low, and mice show lower lipolytic responses during fasting. Because of a decreased lipid utilization, blood glycerol and FFA concentrations are reduced.\(^{42}\) On the other hand, Clock-Δ19 mice show elevated circulating cholesterol and triglyceride levels, likely due to hepatic overproduction and elevated intestinal absorption.\(^{38,57}\) Of note, the metabolic consequences of this Clock mutation depend on the genetic background. While most experiments were performed in the original C57BL/6J background, Clock-Δ19 mice on a (melatonin-proficient) CBA background show normal body weight and fat content.\(^{38,40}\) Clock mutants on an ICR background also show increased food intake, but body weight is even lower than in wild-type littermates—a consequence of impaired lipid absorption.\(^{58,61}\)

Mice with mutations in the gene encoding the CLOCK partner protein BMAL1 show less ambiguous phenotypes with elevated adiposity, despite unaffected food intake.\(^{35,62-67}\) This could be explained by the WNT-mediated suppressive effect of Bmal1 on adipogenesis.\(^{35}\) However, since Bmal1 knockout (KO) mice show an early aging phenotype, the adipose phenotype can only be observed in young animals.\(^{66}\)

Genetic deletions in the negative limb of the circadian feedback loop provide varying results with regard to appetite and body weight control. While Cry double-mutant mice show dampened feeding rhythms on normal chow diet (NCD) and HFD, they are generally lighter and leaner than wild-type controls.\(^{7,40,68}\) Under HFD, however, they rapidly gain weight despite a lower food intake compared to wild types.\(^{40}\) This effect is explained by an enhanced potential of Cry-mutant adipocytes for lipid uptake and insulin-stimulated lipogenesis, making them highly susceptible to HFD-induced obesity.

**Per1- and Per2-mutant mice on the same genetic background** show an opposite body weight phenotype. Whereas Per1\(^{+/+}\) mice show reduced body weight, a mutation of Per2\(^{+/+}\) (Per2\(^{−/−}\) mice) results in elevated body weight.\(^{69,70}\) Despite different body weight effects, food intake is increased in both strains suggesting a higher metabolic rate in Per1 mutants.\(^{69}\) Per2\(^{+/−}\) mice on NCD show increased body weight.\(^{71}\) On HFD, all Per mutants display elevated body weight and fat mass without an increase in absolute food intake suggesting
alterations in energy efficiency. Food intake and body weight of Per1/Per2 of clock-deficient double mutants are elevated, indicating a dominant effect of mutation on energy efficiency. Body weight, fat mass, and adipose tissue phenotypes of clock gene-mutant mice are summarized in Table 1.

| Clock genes | Body weight | Adipose tissue | Food intake | Blood lipids | Adipokines | References |
|-------------|-------------|----------------|-------------|--------------|------------|------------|
| Bmal1       | ↓↑ on NC (age effect) | ↑ adiposity on NC and HFD | ↔ on NC and HFD | ↑ TGs (n.r.) | ↑ leptin, adiponectin, PAI-1 | 7,35,40,62,63,65,109 |
| Clock (C57BL/6) | ↑ on NC & HFD | ↑ adiposity on NC and HFD | ↑ on NC and HFD dampened rhythm | ↑ TGs and cholesterol on HFD | ↓ FFAs and glycerol (n.r.) | 38,42 |
| Clock (CBA) | ↔ on NC | ↔ adiposity on NC | ↓ on HFD | ↑ TGs and cholesterol on NC | ↓ leptin on NC | 59,60 |
| Clock (ICR) | ↔ on NC | ↔ adiposity on NC | ↓ on HFD | ↔ TGs and FFAs on NC | ↔ lepitin on NC | 61 |
| Cry1/2      | ↓ on NC and HFD | ↓ adiposity on NC and HFD | ↔ on NC | ↔ TGs, cholesterol and FFAs on NC | ↓ leptin on NC | 40,68,69 |
| Per1        | ↓ on NC | ↑ adiposity on HFD | ↑ on NC | ↑ adiponectin on NC | ↔ leptin on NC | 70 |
| Per2        | ↑ on NC | ↑ adiposity on HFD | ↔ on NC | ↔ adiponectin on HFD | ↓ leptin on NC | 70,72 |
| Per3        | ↔ on NC | ↑ adiposity on HFD | ↓ on HFD dampened rhythm | ↓ leptin (n.r.) | ↓ leptin on NC | 72 |
| Per1–3      | ↑ on HFD | ↑ adiposity on HFD | ↑ on chow (rel. to BW) and HFD | ↓ leptin & adiponectin on NC & HFD | ↓ leptin mRNa in WAT | 32,76 |
| RevErbα     | ↑ on NC and HFD | ↑ adiposity on NC and HFD | ↔ on chow |↓ TGs and glycerol on HFD | ↑ leptin on NC | 72,73 |
| RORα        | ↓ on NC and HFD | ↓ adiposity on NC and HFD | ↑ on HFD | ↑ LDL, cholesterol and NEFAs on NC and HFD | ↓ leptin mRNA in WAT | 72,73 |

Note: Arrows indicate increased (↑), unaltered (↔), and decreased (↓) levels compared to controls.

Abbreviations: n.r, not rhythmic; NC, normal chow; HFD, high-fat diet; FFA, free fatty acid; NEFA, non-esterified fatty acid; TG, triglyceride; BW, body weight; L/HDL, low/high-density lipoprotein.

Mice carrying mutations in genes involved in accessory loops of the clock machinery also show metabolic alterations. Mice with genetic deletion of the nuclear orphan receptor Rev-Erbα (Nr1d1) show normal to slightly increased body weight on NCD and an elevated body weight on HFD. Body fat percentage is increased on both diets. Consistently, treatment of obese wild-type mice with a synthetic REV-ERBα agonist reduces the obese phenotype due to increased energy expenditure and decreased expression of lipogenic genes. Opposite effects are seen if an activator of Bmal1 expression, Rorα, is mutated. Body weight, fat mass, and adipocytes of staggerer mice (carrying a Rorα loss-of-function mutation) are reduced independent of diet conditions. Although food intake of staggerer mice is elevated on HFD compared to controls, they do not show typical HFD-induced
obesity. This is associated with decreased expression of lipogenic genes (e.g., sterol regulatory element-binding protein [Srebp-1c] and FA synthase [Fas]) and increased expression of genes regulating oxidative metabolism (e.g., PPARγ coactivator 1 [Pgc-1α/β] and lipin1).

**Adipocyte-specific clock disruption**

A frequent problem in interpreting the phenotypes of clock gene-mutant mice is the difficulty to pinpoint which tissue clock determines the effect. This is particularly true for WAT, which is heavily influenced by systemic factors such as hormones and metabolic state. Therefore, although most clock gene mutants show alterations in adipose function, it cannot be excluded that these originate from outside the WAT. To study the specific role of the adipocyte clock itself, mouse models with CRE/loxP-driven deletion of the essential clock component Bmal1 have been developed. Comparable to conventional Bmal1 KO mice, animals carrying an aP2-Cre-driven deletion of Bmal1 primarily targeting WAT show hyperphagy and increased obesity independent of the type of diet. In line with this, they display increased serum leptin levels with dampened diurnal rhythmicity. In contrast, mice in which Bmal1 deletion is mediated by another adipose-targeting CRE driver, Adipoq (adiponectin), show a much milder phenotype with normal body weight on NCD, but an increased vulnerability to the obesogenic effects of HFD. In aP2-Cre × Bmal1-flox mice, the mechanisms of this change in metabolic state have been further characterized. Mutant mice show a lower concentration of polyunsaturated fatty acids (PUFAs) in WAT and plasma due to lower expression of the lipolytic gene Ces1d (carboxylesterase 1d) at CT4 and of Elovl6 (elongation of long-chain fatty acids) and Scd1 (stearoyl-coenzyme A desaturase 1), which catalyze the biosynthesis of long-chain PUFAs. All these genes contain circadian E-box regulatory elements in their promoter regions. PUFAs can cross the blood–brain barrier and inhibit appetite. The lower PUFA levels in aP2-Cre × Bmal1-flox mice could, therefore, explain the observed hyperphagy and elevated body weight. Indeed, aP2-Cre × Bmal1-flox mice fed with a PUFA-rich diet show wild-type-like food intake and body weight gain, energy expenditure, and normalized circadian expression of orexigenic and anorexigenic neuropeptides in the hypothalamus. Of note, ectopic activity of the aP2 driver has been reported in several tissues including the brain. Therefore, it will be important to investigate if these physiological changes are also observed in other adipose-specific clock mutants such as Adipoq-Cre × Bmal1-flox mice.

**The role of SCN pacemaker in adipose rhythms**

As mentioned earlier, signals derived from other tissues — and, thus, clocks in other tissues — may impinge on adipose rhythms. Of particular interest in this context is the SCN pacemaker itself as coordinator of all endogenous clocks and rhythms. Lesions of the SCN lead to arrhythmic locomotor activity, drinking behavior, and serum leptin levels. This dampened rhythm in oscillating leptin seems to be a direct effect of the SCN lesion and not of altered food intake since regularly scheduled meals have no effect on the rhythm of leptin. However, lesions of the SCN do not just affect the clock in the SCN but also destroy the SCN neuronal structure that serves as a relay of external light information to peripheral clocks. To bypass this issue, mouse models with (more or less) tissue-specific genetic deletion of SCN clock function have been developed. While under light-dark (LD) conditions peripheral clock gene and behavioral rhythms are preserved in SCN Bmal1-KO mice, they become in constant darkness behaviorally arrhythmic while peripheral clocks and hormone rhythms only gradually desynchronize and, thus, continue to cycle for some days. Microarray studies in WAT of these mice reveal that many adipose transcripts associated with lipid and carbohydrate metabolism lose their rhythm suggesting a dependence on SCN clock-driven rhythmic behavior independent of adipocyte clock function. Interestingly, expression of some immune genes gains rhythmicity in the absence of a functional SCN clock. This may indicate a counterregulatory action of cellular immunity by SCN and adipocyte clocks.

**Modulation of adipose rhythms by food**

The daily rhythm of food intake has a strong influence on metabolic homeostasis, which may in part be mediated by its synchronizing effects on peripheral clock function (Figure 1). Most studies investigating peripheral clock gene entrainment so far have focused on the liver, and the data for food resetting of adipose clocks are limited.

Nocturnal rodents, like mice and rats, with restricted access to food during the light phase — their normal rest phase — gain more weight than littermates with dark phase-restricted food intake. This effect does not concur with increased total food intake and it seems that the mistiming of food intake is the key to this maladaptation. Rest-phase feeding leads to higher lipogenic gene expression, abolishes the rhythm in lipolytic gene expression, and alters rhythms of circulating leptin and insulin, energy metabolism, and body
temperature. On the other hand, nighttime-restricted feeding leads to lower serum leptin, higher BAT thermogenesis, and lower VAT expression of pro-inflammatory cytokines. To which extent these adipose effects are direct consequences of alterations in energy supply or follow food-mediated resetting of adipocyte clocks remains to be explored.

**Adipokine rhythms**

WAT is a very active endocrine tissue. Depending on energy state, adipocytes secrete a large array of different peptides, the so-called adipokines, several of which show diurnal expression patterns. Besides peripheral targets, many adipokines enter the brain where they modulate central regulatory circuits of appetite control and energy expenditure, thus providing bottom-up feedback about the peripheral energy state of the body to ensure homeostasis (Figure 3). Disruption of this adipocyte–brain cross talk is associated with a broad range of mental and metabolic impairments. Disruption of adipocyte clocks remains to be explored.

The mRNA of leptin, the most prominent adipokine, is rhythmically expressed in adipocytes, and its circulating concentration rises during the night in humans and nocturnal rodents. In obesity, leptin serum concentrations are increased in mice and humans, while oscillations are dampened, probably due to the concomitant dampened feeding rhythm. Kettner et al showed rhythmic binding of BMAL1 to E-boxes in the Lep promoter that modulate C/EBPα-controlled Lep transcription in a daytime-dependent manner. The rhythmicity of leptin seems to be critical for energy homeostasis. Leptin-deficient (ob/ob) mice treated with leptin in anti-phase to their food intake rhythm gain more weight than mice treated in line with the feeding rhythm.

Other adipokines such as adiponectin also show diurnal oscillations. Adipoq mRNA expression in WAT and adiponectin blood concentrations peak during the active phase of mice and humans. Like leptin, adiponectin serum concentrations display dampened circadian and ultradian oscillations in obese subjects. The Adipoq promoter contains several E-box-like sequences and can be activated by CLOCK:BMAL. Interestingly, overexpression of human adiponectin in the liver of KK/Ta mice, which have low endogenous adiponectin levels, partly restores the circadian rhythm and free-running period of locomotor activity, indicating a role of adiponectin in central behavior regulation. Gene expression of this insulin-sensitizing adipokine is modulated by food intake; thus, its circadian action could be important for the circadian regulation of insulin sensitivity.

Some of the other adipokines that show diurnal expression rhythms are resistin, visfatin (nicotinamide phosphoribosyltransferase, Nampt), plasminogen activator inhibitor-1 (Serpine1, PAI-1), TNFα (tumor necrosis factor alpha), and IL-6 (interleukin-6). Expression of PAI-1 and visfatin is known to be directly controlled by the circadian clock. Additionally, visfatin can feed back on the circadian clock via the NAD+ salvage pathway. In murine WAT, mRNA expression of resistin and visfatin peaks during the dark phase, while mRNA expression of PAI-1 is highest during the light phase. Activity of plasma PAI-1, however, is highest at the beginning of the active phase. TNFα peaks in serum of lean rats during the inactive phase. Interestingly, if mice are fed a HFD, the peak of serum TNFα shifts to the active phase. IL-6 shows rhythmic gene expression in human subcutaneous WAT. Furthermore, diurnal oscillations of serum IL-6 are well documented in humans, but the situation is less clear in rodents.

To which extent these adipose-derived cytokine oscillations contribute to metabolic inflammatory processes (see the following text) remains to be explored.

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**Figure 3** Adipose–brain cross talk in the regulation of energy homeostasis. Rhythmically regulated adipokines cross the blood–brain barrier to affect appetite and energy expenditure regulating circuits in the mediobasal hypothalamus (MBH). The circadian disruption leads to lower serum leptin, higher BAT thermogenesis, and lower VAT expression of pro-inflammatory cytokines.
It should be noted that even in the absence of rhythmic hormone concentrations time-of-day-dependent effects can be achieved in target tissues. For example, leptin transport into the brain and the mRNA expression of its receptor, LepR (or ObR), show diurnal rhythmicity in the hypothalamus.129,130 This could indicate circadian rhythms in the central sensitivity to leptin and interpretation of the leptin signal (circadian gating). Indeed, destruction of LepR-expressing neurons in the arcuate nucleus leads to dampened feeding rhythms and to arrhythmicity of locomotor activity in DD.131 Interestingly, LepR is also expressed in the SCN of humans and rodents indicating a possible direct effect of leptin on the central pacemaker132–134 (note the reported absence of LepR in the SCN reported by Caron et al135).

Circadian rhythms and metaflammation

Metaflammation (short for metabolic inflammation) depicts a low-grade chronic inflammatory state resulting from over-nutrition and playing a crucial role in the development of obesity-associated insulin and leptin resistance and cardiovascular complications.136 Metaflammation originates from WAT, but later spreads to other peripheral tissues such as liver, skeletal muscle, pancreatic islets, and the hypothalamus.136,137

Under obese conditions, the number of WAT infiltrating macrophages releasing pro-inflammatory cytokines (M1 macrophages) increases.138 Furthermore, pro-inflammatory cytokine production is enhanced by elevated activation of pro-inflammatory kinases like c-Jun N-terminal kinase, inhibitor of xB kinase, and protein kinase R and their signaling cascades.139,140 Additionally, the inflammasome and toll-like receptors, components of the innate immune system, are activated during metaflammation.141–143 The triggers and mechanisms of metaflammation are still poorly understood, but it has been noted that in obesity, feeding rhythms are dampened leading to an uninterrupted inflammatory stimulation.144,145 Time-restricted HFD access, on the other hand, has been shown to not just improve diurnal oscillations of clock gene expression but also to prevent increased macrophage infiltration and increased cytokine production in WAT.146

Circadian clock KO mice seem to be more susceptible for pro-inflammatory stimulus. LPS treatment of bone marrow-derived macrophages leads to a higher induction of TNFα and IL-1β expression in Per1/2 mutant mice.147 Furthermore, Per1/2-mutant mice show a higher percentage of M1 macrophages in WAT. HFD-fed mice with myeloid-specific disruption of Per1/2 display elevated gene expression of TNFα, IL-6, and IL-1β and increased macrophage infiltration in WAT. This seems to be mediated by PPARγ, which is downregulated in Per1/2-mutant mice. Myeloid-specific deletion of Bmal1 results in elevated numbers of inflammatory monocytes in WAT and BAT, increased body weight gain, and impaired glucose tolerance.148 It will be interesting to follow up this clock-inflammation lead as a potential target for the treatment of metaflammation.

Conclusion

Many important adipose functions including adipose differentiation, lipid metabolism, and adipokine expression are controlled by the adipocyte circadian clock or systemic factors such as food intake and SCN-dependent hormones. Vice versa adipose-derived factors, the adipokines, can modulate circadian appetite and energy metabolism rhythms in the brain. Circadian rhythm disruption, thus, has double effects on energy homeostasis; it affects WAT function via resetting of adipocyte clocks and alters central metabolic regulation directly and through modulation of adipokine signaling. This circadian aspect of the adipocyte–brain cross talk may have an important role in the regulation of obesity-associated metaflammation.

Considering the rising numbers of patients with metabolic disorders, targeting the circadian clock system may be a promising preventive and therapeutic approach.31,149,150 In particular, those adipokines that do not lose their central anorexigenic properties during obesity, like nesfatin or adiponectin, may have chronotherapeutic potential.151 Other approaches may affect clock proteins directly such as the nuclear orphan receptor REV-ERBα for which small molecule modulators have already been developed.79

Author contributions

All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

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