REVIEW

Circadian mechanisms in adipose tissue bioenergetics and plasticity

Chelsea Hepler and Joseph Bass

Department of Medicine, Division of Endocrinology, Metabolism, and Molecular Medicine, Feinberg School of Medicine, Northwestern University, Chicago, Illinois 60611, USA

The circadian clock plays an essential role in coordinating feeding and metabolic rhythms with the light/dark cycle. Disruption of clocks is associated with increased adiposity and metabolic disorders, whereas aligning feeding time with cell-autonomous rhythms in metabolism improves health. Here, we provide a comprehensive overview of recent literature in adipose tissue biology as well as our understanding of molecular mechanisms underlying the circadian regulation of transcription, metabolism, and inflammation in adipose tissue. We highlight recent efforts to uncover the mechanistic links between clocks and adipocyte metabolism, as well as its application to dietary and behavioral interventions to improve health and mitigate obesity.

Circadian clocks are endogenous timing systems that allow organisms to anticipate and adapt to environmental changes that occur over the 24-h day. Circadian disruption is strongly linked to the development of metabolic disease (Maury et al. 2014). Individuals who experience shift work, jet lag, and sleep disorders develop glucose intolerance, insulin resistance, and weight gain (Wang et al. 2014). Obesity is associated with late night eating and an extended feeding window beyond 12 h in humans and feeding during the light period in rodents (Kohsaka et al. 2007; Vujović et al. 2022). Conversely, aligning mealtime to the daylight in humans and active/dark period in rodents leads to improved glucose homeostasis and reduced adiposity (Arble et al. 2009; Sutton et al. 2018; Cienfuegos et al. 2020). A molecular understanding of how circadian rhythms regulate energy homeostasis in relation to the light/dark cycle is of critical importance in obesity and metabolic disorders.

The circadian clock is encoded by a conserved transcription–translation feedback oscillator that regulates thousands of clock-controlled genes (Takahashi 2017). At the molecular level, the circadian clock activator complex CLOCK/BMAL1 regulates expression of the repressors PER/CRY, which feed back to inhibit their own transcription (Kornmann et al. 2007). An additional feedback loop consisting of REV-ERB/ROR regulates transcription of *Bmal1*. In mammals, the central pacemaker located in the suprachiasmatic nucleus (SCN) of the hypothalamus is the primary regulator of circadian rhythms in the body (Herzog et al. 1998). Peripheral tissues also possess their own clocks that interact with tissue-specific transcription factors to control physiology in distinct metabolic organs (Bass and Lazar 2016). Unlike the central SCN clock, which is exclusively entrained by light, peripheral clocks exhibit unique plasticity in that they can be entrained by shifts in mealtime and the macronutrient content of diet (Kohsaka et al. 2007). RNA splicing as well as post-transcriptional and post-translational levels of regulation contribute to circadian oscillations in physiology (Cox and Takahashi 2019). Clocks work in different tissues often in opposing ways. For instance, within the liver, clocks induce pathways important in gluconeogenesis and lipid oxidation during fasting, whereas in the pancreas, the clock regulates postprandial secretion of insulin essential in nutrient uptake and storage (Lamia et al. 2008; Marcheva et al. 2010). Temporal separation imposed by circadian clocks leads to distinct phases of reductive and oxidative metabolic processes over the 24-h day.

The adipose tissue clock regulates rhythmic activation and repression of metabolic pathways in a reciprocal manner and responds to various environmental cues to meet metabolic demand (Fig. 1).The original discoveries that clock disruption is strongly linked to diet-induced obesity and that high-fat feeding at the wrong time of day accelerates obesity reveal a central role of circadian pathways in adipose tissue homeostasis (Turek et al. 2005; Kohsaka et al. 2007). Emerging studies focusing on new insights into adipocyte cellular plasticity as well as circadian control of adipose tissue metabolism, architecture, and remodeling form the focus of this review.

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Corresponding author: j-bass@northwestern.edu
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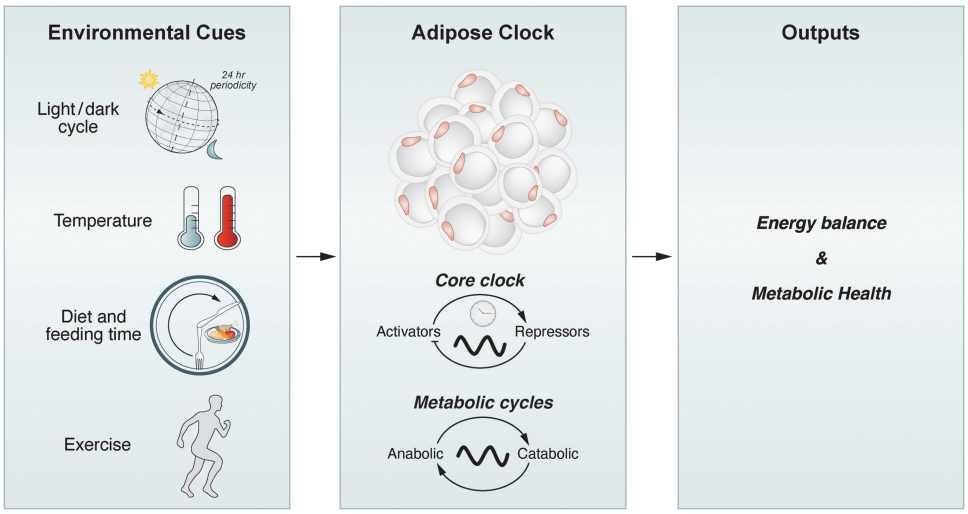

Figure 1. Environmental inputs and physiological outputs of the adipose tissue clock. The adipose tissue circadian clock coordinates the daily timing of metabolic reactions in anticipation of the 24-h light/dark cycle. The clock integrates various environmental cues to meet metabolic demand.

Function and plasticity of distinct adipose tissue depots: separate regions of circadian control

Adipose tissue is a dynamic organ that stores energy in the form of triglycerides and releases fatty acids in response to energy demands. In addition, adipose tissue secretes various hormones, cytokines, and metabolites that contribute to systemic nutrient balance and energy homeostasis. The physiological significance of adipose tissue is highlighted in individuals with excess adipose tissue (obesity) or an abnormal deficiency of fat tissue (lipodystrophy). In either of these conditions, the dysfunctional storage of lipid in adipose tissue is accompanied by insulin resistance, dyslipidemia, inflammation, hepatic steatosis, and increased risk of cardiovascular disease (Hepler and Gupta 2017).

Body fat distribution is one of the best predictors of metabolic health in obesity (Karpe and Pinnick 2015). White adipose tissue (WAT) is anatomically organized into subcutaneous or visceral compartments. Obese individuals who store excess adiposity in the visceral compartment are at a greater risk of developing metabolic syndrome than those who accumulate adiposity in the subcutaneous regions. This association is likely due to functional differences between depots, such as adipokine secretion, lipolytic capacity, inflammation, and adipogenesis (Lee et al. 2013). Females tend to preferentially accumulate fat in the subcutaneous region as compared with visceral; however, despite having greater adiposity than males, females have a decreased risk for metabolic disease compared with males (Palmer and Clegg 2015). Menopause is associated with a redistribution of fat mass from subcutaneous to visceral depots, implicating a role for sex hormones in determining adipose tissue distribution (Toth et al. 2000). In support of this, removal of the testes during sex reassignment therapy is more metabolically protective as compared with cross-sex hormone therapy alone in transwomen (Nelson et al. 2016).

The way adipose tissue expands during caloric excess is a critical determinant of health. Pathologic expansion through enlargement of existing adipocytes (hypertrophy) is associated with inflammation, hypoxia, and metabolic dysfunction (Sun et al. 2011). As adipocytes reach their maximal storage capacity, lipids "spill over" into nonadipose organs, such as the liver, where they accumulate and contribute to insulin resistance. Conversely, expansion of adipose tissue through increased adipogenesis from preadipocytes (hyperplasia) is associated with less inflammation and improved metabolic health. During weight gain, females expand subcutaneous depots through adipocyte hyperplasia, whereas males predominately accumulate fat mass through hypertrophy (Tchoukalova et al. 2010). A subset of obese individuals who do not have cardiometabolic disease, termed "metabolically healthy obese," have reduced visceral WAT accumulation, smaller adipocyte size, and less inflammation (Klöting et al. 2010). As discussed further below, healthy adipose tissue is associated with high amplitude of circadian clock genes, preserved metabolic rhythms, and diurnal proliferation of progenitors, whereas obesity and metabolic disease have flattening of these oscillations (Fig. 2).

Mammals also have brown and beige adipocytes, which are specialized to dissipate energy in the form of heat. Brown adipose tissue (BAT) exists in distinct depots, whereas beige adipocytes are recruited in existing subcutaneous depots upon activation of β-adrenergic receptors (Cannon and Nedergaard 2004). Thermogenic adipocytes appear during physiologic stimuli such as cold exposure, exercise, and cachexia. Both the master clock in the SCN and the cell-autonomous adipocyte clock are involved in thermogenic activation. Brown and beige adipocytes are characterized by their multilocular appearance and high mitochondrial content, as well as the presence of uncoupling protein 1 (UCP1), which facilitates proton leak across the inner mitochondrial membrane for heat

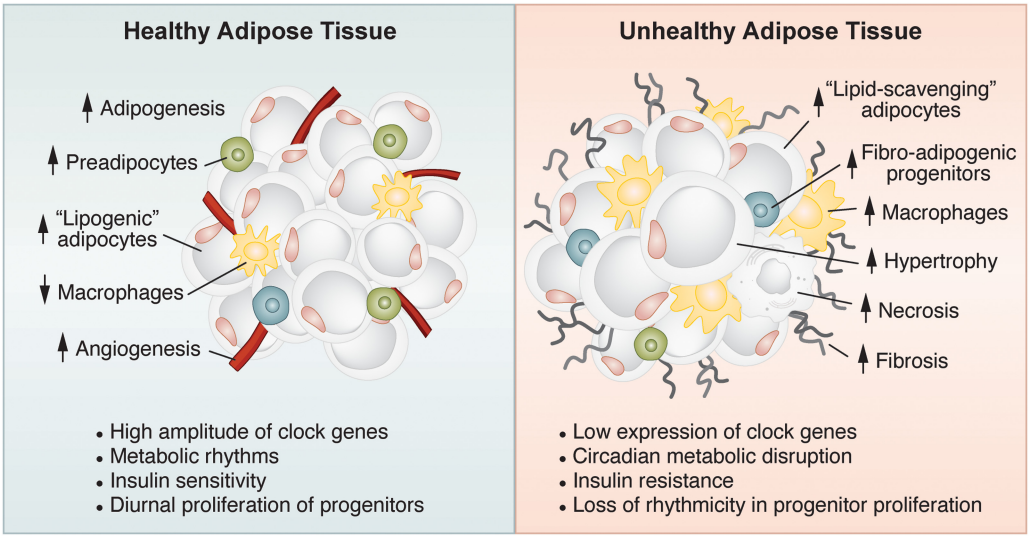

Figure 2. Characteristics of metabolically healthy and unhealthy adipose tissue. "Healthy" adipose tissue is characterized by smaller adipocytes that are enriched for lipogenic gene expression with increased adipogenesis, angiogenesis, and insulin sensitivity. Preadipocytes are abundant in this tissue, and macrophage levels are low and predominantly anti-inflammatory. In contrast, "unhealthy" adipose tissue is marked by hypertrophic adipocytes that are enriched for lipid-scavenging genes with decreased adipogenesis. This tissue is associated with increased presence of fibro-adipogenic progenitors and proinflammatory macrophages and displays increased inflammation, fibrosis, and insulin resistance. In lean animals, adipose tissue health is positively linked with high amplitudes of circadian clock genes, metabolic rhythms, and diurnal proliferation of progenitors, whereas these properties are lacking in adipose tissue of obese animals.

generation. However, several UCP1-independent mechanisms of thermogenesis have been identified, including futile creatine cycling, accumulation of thermogenic reactive oxygen species (ROS), proton leak through the ATP/ADP carrier (AAC), calcium-dependent ATP hydrolysis, and futile lipid cycling (Chouchani et al. 2019). Promising studies in mice have revealed that brown and beige adipocytes are significant regulators of weight, glucose homeostasis, and hepatic health (Seale et al. 2011; Shao et al. 2016). Several studies have confirmed the presence of thermogenic adipose tissue in humans that can be activated and contributes to nutrient homeostasis (Cypess et al. 2009; Virtanen et al. 2009). Consequently, there is a significant focus on investigating how thermogenic mechanisms in adipocytes are regulated and can be activated to improve human health.

The diverse spectrum of adipocyte subpopulations: unique plasticity in response to environmental changes

WAT is composed of diverse cell types including adipocytes, adipocyte progenitors, immune cells, and endothelial cells. Adipocytes themselves are considerably heterogeneous, as is readily apparent during histological analysis of adipocyte size and number of lipid droplets per cell. In addition, the type and appearance of fat cells differ based on anatomical location within each depot. For example, in the inguinal depot, there is a greater frequency of beige adipocytes and smaller white adipocytes near the lymph node (Barreau et al. 2016). The existence of cellular heterogeneity within adipocytes and stromal cells has vast implications for interpreting experimental data and understanding the complex metabolic functions and circadian regulation of adipose tissue.

The advancement of single-nucleus RNA sequencing has allowed for the unbiased discovery of transcriptionally distinct adipocyte subtypes in adipose tissues. In epididymal WAT, three major adipocyte populations have been identified: lipogenic adipocytes (LGAs), characterized by high levels of genes involved in lipid biosynthesis and insulin response; lipid-scavenging adipocytes (LSAs), which express high levels of genes involved in lipid uptake and transport; and stressed lipid-scavenging adipocytes (SLSAs), which have high expression of genes involved in stress response, hypoxia, and autophagy (Sárvári et al. 2021; Emont et al. 2022). The antithermogenic transcription factor *Zfp423* was enriched in the SLSAs, which may have a functional role in regulating the cellular identity and function of these cells. In lean mice, LGAs are the smallest and constitute the majority of all adipocytes, whereas SLSAs exist in low abundance and are hypertrophic. During high-fat diet (HFD) feeding, LSAs and SLSAs increase in frequency and gain expression of genes involved in inflammation and fibrosis. It is unclear whether the clock has a role in regulating the frequencies of subpopulations or their unique functions. The core clock gene *Nr1d2* (REV-ERBβ) is one of the top enriched genes in SLSAs as compared with other adipocyte populations, and its expression is significantly lower in LGAs. This could indicate a functional role for REV-ERBβ SLSAs or potentially constitutively elevated REV-ERBβ with clock disruption in this subpopulation.

Thermogenic adipocytes also are heterogeneous, as initially observed through differences in UCP1 expression among brown fat cells within the interscapular depot

(Cinti et al. 2002). Chen et al. (2019) discovered an uncharacterized subpopulation of beige adipocytes that is regulated independently of β-adrenergic receptor signaling. Glycolytic beige fat (g-beige fat) in the inguinal depot expresses high levels of genes involved in glycolysis and has elevated glycolytic metabolism. This indicates that thermogenic adipocytes exist as a heterogeneous population, though it is unclear whether these diverse adipocytes represent different stages of differentiation/activation or distinct subpopulations with stable gene expression and function. In addition, it remains to be determined whether other subpopulations within beige adipocytes might be uncovered under distinct stimuli. In a recent study, two UCP1$^+$ beige adipocyte populations in inguinal WAT were identified that can be separated by high or low expression of de novo lipogenesis (DNL) genes (*Acly* and *Fasn*) (Holman et al. 2023). The DNL-high beige adipocytes only emerged after long-term cold exposure (2 wk), and the expression of these genes in beige adipocytes was attenuated in aged mice.

In the interscapular BAT, Song et al. (2020) identified two major populations of brown adipocytes: *Adipoq*-high brown adipocytes (BA-H), which have elevated expression of *Ucp1*, *Adrb3*, and genes involved in succinate metabolism, high oxidative metabolism, and smaller lipid droplets, and *Adipoq*-low brown adipocytes (BA-L), which are characterized by decreased *Ucp1*, increased genes involved in futile creatine cycling and fatty acid uptake, and decreased mitochondrial respiration. Cold exposure and β3-adrenergic receptor agonism promoted the conversion of BA-L to BA-H cells, whereas thermoneutrality converted BA-H to BA-L adipocytes. This indicates that distinct UCP1-dependent and creatine-dependent thermogenic mechanisms may be programmed in individual brown adipocyte subpopulations, as opposed to cells having the ability to generate heat through both pathways at the same time. This also provides evidence that adipocyte subpopulations with distinct gene signatures in thermogenesis can interconvert under specific stimuli. *Nfil3* (E4BP4) is a negative component of the clock that is enriched in the BA-H cells compared with the BA-L cells. E4BP4 could have a functional role in BA-H cells, or its enrichment could be a result of the sequencing performed at only one time point. This could reveal either enrichment of clock genes in one population or differences in timing of the clock between subpopulations.

Distinct functions of adipocyte progenitors in remodeling

Prior to the emergence of commercially available single-cell RNA sequencing, several studies investigated the existence of cellular heterogeneity within the WAT progenitor pool. Some of the earliest observations concluded that adipocytes originate from perivascular mesenchymal cells that express mural cell markers (e.g., smooth muscle actin [SMA] and platelet-derived growth factor receptor β [PDGFRβ]) (Napolitano 1963; Cinti et al. 1984; Tang et al. 2008; Gupta et al. 2012; Vishvanath et al. 2016). Analysis of adipose tissue from mice that express a reporter under the control of the adipocyte lineage-determining transcription factor *Pparg* revealed that only a subset of PDGFRβ$^+$ cells expresses *Pparg* (Tang et al. 2008). This is consistent with the notion that adipocyte mesenchymal stem cells have a hierarchical organization consisting of stem cells and more committed preadipocytes but also suggests the possibility that distinct perivascular cells with nonadipogenic functions may exist. PDGFRβ$^+$ cells separated on the basis of the preadipocyte commitment factor *Zfp423* reveal that a subset of perivascular cells that are *Zfp423*$^-$ has enrichment of inflammatory genes and low adipogenic capacity (Vishvanath et al. 2016). Another study found that a subset of PDGFRα$^+$ cells that expressed high levels of CD9 has a profibrotic and proinflammatory gene signature with low adipogenic potential (Marcelin et al. 2017). These observations demonstrate that distinct perivascular cell populations in adipose tissue contribute to adaptation and remodeling of adipose tissue through unique adipogenic and nonadipogenic functions.

Single-cell RNA sequencing has led to the identification and characterization of subsets of adipocyte precursors that contribute to adipose tissue physiology. A unified nomenclature of the subpopulations has not been established; however, it has been widely observed that two major populations of adipocyte progenitors (DPP4$^+$ fibro-adipogenic progenitors and DPP4$^-$ preadipocytes) exist in WAT depots (for a detailed review, see Maniyadath et al. 2023). In the visceral depot, the fibro-adipogenic progenitors secrete antiadipogenic, profibrotic, and proinflammatory signals, whereas the preadipocyte population undergoes spontaneous adipogenesis in culture and upon transplantation (Hepler et al. 2018). Circadian genes *Per1* and *Nfil3* were both enriched in the DPP4$^+$ fibro-inflammatory progenitors. In addition, visceral fat harbors a mesothelial cell population (PDGFRα$^+$;CD9$^+$;LY6C$^-$), though it appears these cells may not contribute to visceral adipogenesis (Burl et al. 2018; Westcott et al. 2021). Further stratification of precursor populations reveals additional subsets within these groups; however, it is unclear whether this deeper stratification corresponds with functional diversity (Ferrero et al. 2020). Computational analyses that infer differentiation trajectories predict that the fibro-adipogenic progenitor population creates preadipocytes before becoming fully committed into mature adipocytes (Merrick et al. 2019). However, these fibro-adipogenic progenitors isolated from the visceral depot of adult mice have low adipogenic capacity in vitro and in vivo when transplanted into lipodystrophic mice (Hepler et al. 2018; Zhang et al. 2022a). Lineage tracing studies using a *DPP4*^{CreER} mouse showed that these progenitors give rise to preadipocytes and mature adipocytes in visceral WAT during HFD feeding (Stefkovich et al. 2021). However, the frequency of labeling mature adipocytes from DPP4$^+$ cells following 18 wk of HFD feeding is low in visceral depots when compared with the expected turnover rate of ~10%–30% of visceral adipocytes arising from de novo adipogenesis (Wang et al. 2013; Vishvanath et al. 2016). This indicates that the DPP4$^+$ fibro-adipogenic progenitor population in visceral WAT is capable of

undergoing adipogenesis, albeit at a low rate. More precise lineage tracing strategies will be required to further understand the contribution of fibro-adipogenic progenitors to adipose tissue physiology.

In the inguinal WAT depot, both the DPP4$^+$ and DPP4$^-$ populations are adipogenic in vitro and upon transplantation into mice (Merrick et al. 2019; Shao et al. 2021). The DPP4$^+$ fibro-adipogenic population is highly proliferative and gives rise to DPP4$^-$ cells upon transplantation (Merrick et al. 2019). Two additional smaller subpopulations were identified and functionally characterized as antiadipogenic: adipose-regulatory cells (Aregs; CD142$^+$ and SCA1$^+$) and aging-dependent regulatory cells (ARCs; CD36$^+$ and Lgals3$^+$) (Schwalie et al. 2018; Nguyen et al. 2021). During HFD feeding, subcutaneous WAT is largely refractory to undergoing adipogenesis; however, cold exposure stimulates beige adipocyte formation in the inguinal depot predominately from de novo beige adipogenesis (Shao et al. 2019). Kajimura and colleagues (Oguri et al. 2020) identified a subset of DPP4$^-$ progenitors in iWAT marked by PDGFRα, SCA1, and CD81 that gives rise to beige adipocytes upon exposure to cold.

Lineage tracing studies uncovered that classical brown adipocytes are derived from *Myf5*$^+$ progenitors shared with skeletal muscle but not white or beige adipocytes (Seale et al. 2008). Single-cell analysis of interscapular BAT identified *Trpv1*-expressing vascular smooth muscle cells (VSMCs; αSMA$^+$, SCA1$^-$, and PDGFRα$^-$) as progenitors that differentiate into brown adipocytes in response to cold (Shamsi et al. 2021). In contrast, Burl et al. (2022) identified a *Pdgfra*-expressing population, termed ASC1, that does not express *Trpv1* but appears poised to differentiate in response to cold exposure. This population expresses *Col5a3*, *Cxcl14*, *Apod*, and *Bmper* and is localized in the tissue parenchyma and associated with capillaries. The investigators also identified a *Dpp4*-expressing population of stromal cells, termed ASC2, that is enriched for *Pi16*, *Cd55*, and *Fn1* and bears a resemblance to DPP4$^+$ cells in WAT. Single-cell analysis of aortic perivascular adipose tissue, which resembles interscapular BAT, identified a fibro-adipogenic progenitor population (*Pdgfra*$^+$;*Ly6a*$^+$;*Dpp4*$^+$;*Pi16*$^+$;*Pparg*$^-$), a preadipocyte population (*Pdgfra*$^+$;*Ly6a*$^+$;*Pparg*$^+$;*Acta2*$^+$), and a VSMC progenitor population (*Myh11*$^+$;*Pdgfra*$^-$;*Pparg*$^+$) (Angueira et al. 2021).

Collectively, these studies have expanded our knowledge of the existence of distinct adipocyte progenitor populations that have functionally diverse roles. Moving forward, careful consideration should be made when analyzing adipocyte progenitor cells during cell culture assays, flow cytometry analysis, or histology to capture individual subpopulations. The frequencies and identities of populations may change during environmental or genetic perturbation. New populations may emerge under certain stimuli, such as in response to cold exposure, caloric restriction (CR), obesity, and aging. In addition, efforts should be made to investigate individual contributions of subpopulations when using genetic lines of mice that label all populations (i.e., *Pdgfr*α-*Cre*, *Pdgfr*β-*rtTA*, and *SMA-CreERT2*). Finally, single-cell RNA sequencing analyses with follow-up investigation of cellular function are often only performed at a single time point, often from samples harvested in animals during the rest (light) period. It is worth noting that most genes are rhythmic, and downstream processes (discussed further below) such as post-transcriptional modifications, metabolic pathways, proliferation, and signaling responses are regulated by time of day. Going forward, it will be important to gain insight from sequencing and downstream analyses at multiple time points within subpopulations to elucidate how time of day elicits adipose depot-specific responses within cells of different origins and functions.

Adipose tissue-resident immune cell populations and their heterogeneity

Immune cells appear in adipose tissue early during development before adipocytes are formed, when adipose tissue resembles a dense mass of blood vessels (Zhang et al. 2022a). In lean individuals, adipose tissue-resident immune cells contribute to metabolic homeostasis and tissue remodeling (Ferrante 2013). During changes in nutritional states or aging, the immune cell profile in adipose tissue shifts and can contribute to fibrosis, inflammation, and insulin resistance (Wu et al. 2007a; Zamboni et al. 2014). This occurs in part through secretion of cytokines by adipocytes and adipocyte progenitors that recruit and activate immune cells, which alters the composition of resident immune cell populations.

Adipose tissue macrophages comprise the largest and best-studied population of immune cells in adipose tissue. Macrophage polarization was traditionally defined to M1 or M2 states according to functional demand in response to environmental changes (Benoit et al. 2008). During infection, macrophage polarization to M1 state is proinflammatory and defends against host pathogens. In contrast, M2 macrophages are anti-inflammatory and participate in repairing damaged tissue. M1 macrophages accumulate during diet-induced obesity (DIO) in adipose tissue and are marked by high expression of CD11c (Lumeng et al. 2007). However, recent advances in single-cell RNA sequencing have challenged the notion that macrophages exist in bimodal M1 or M2 identities and instead show a continuum of transcriptional states with unique properties (Xu et al. 2013; Burl et al. 2018; Hill et al. 2018; Jaitin et al. 2019; Weinstock et al. 2019; Sárvári et al. 2021; Cottam et al. 2022). In visceral WAT, macrophages can be clustered into several distinct categories based on the expression of genes involved in perivascular or nonperivascular localization, phagocytosis, lipid handling, extracellular matrix remodeling, and cell cycle. Lipid-associated macrophages (LAMs) are enriched for genes involved in lipid metabolism and phagocytosis with localization to crown-like structures (CLSs). In response to HFD feeding, LAMs proliferate, and both the perivascular and nonperivascular macrophages adopt a LAM-like signature (Sárvári et al. 2021). A similar diverse spectrum of macrophages was observed in BAT (Gallerand et al. 2021). Heterogeneity in other immune

cell subpopulations in adipose tissue, including monocytes, T cells, B cells, innate lymphoid cells, and dendritic cells, has been investigated (for a complete review, see Duerre and Galmozzi 2022).

Role of clocks in adipogenesis

The degree of insulin resistance is positively correlated with adipocyte size (Salans et al. 1968). Adipocyte progenitors isolated from metabolically healthy obese individuals have a higher propensity to undergo adipogenesis as compared with unhealthy obese individuals with hypertrophic adipocytes (Gustafson and Smith 2012). These observations suggest that hypertrophic obesity is due at least in part to impairment of adipocyte progenitors to undergo adipogenesis. Consistent with this notion, enhancing adipogenesis during development or in adulthood protects against metabolic disease during caloric excess (Shao et al. 2018; Zhang et al. 2022a). Thus, understanding the molecular regulation of adipogenesis may lead to new therapeutic targets that improve health.

Adipogenesis is the process whereby adipocyte precursors restrict their fate to the adipocyte lineage and undergo differentiation into mature lipid-laden adipocytes. PPARγ is the master regulator of adipocyte differentiation (Chawla and Lazar 1994; Tontonoz et al. 1994). Several other transcription factors, including ZFP423, C/EBPα, and C/EBPβ, are also involved in adipocyte differentiation (Ghaben and Scherer 2019). There is robust circadian clock activity in adipocyte progenitors (Wu et al. 2007b). The expression of several clock genes is further induced early in the process of adipogenesis (Chawla and Lazar 1993; Austin et al. 1998; Fontaine et al. 2003). Early studies identified *Nr1d1* as being essential during 3T3L1 adipogenesis (Chawla and Lazar 1993; Wang and Lazar 2008; Kumar et al. 2010). Loss of the circadian clock activators CLOCK or BMAL1 leads to a decrease in the expression of the clock repressors and *Nr1d1*. However, ablation of BMAL1 has been reported to exhibit mixed results on adipogenesis. One study found that ablation of *Bmal1* in embryonic fibroblasts leads to impaired adipocyte differentiation (Shimba et al. 2005). Other reports have revealed that deletion of BMAL1 in several cell types, including 3T3L1 preadipocytes, embryonic fibroblasts, and C3H10T1/2 mesenchymal stem cells, leads to increased adipogenesis through suppression of canonical Wnt signaling (Guo et al. 2012; Nam et al. 2015). In addition, visceral preadipocytes isolated from ClockΔ19 mice have an increased propensity to undergo adipocyte differentiation through transcriptional regulation of glucocorticoid-induced leucine zipper (GILZ) by CLOCK (Zhu et al. 2018). Deletion of the clock repressor *Per2* in MEFs or 3T3L1 adipocytes leads to an increase in adipogenesis through direct derepression of PPARγ (Grimaldi et al. 2010). Similarly, genetic deletion of the paralog *Per3* in inguinal adipocyte progenitors increases adipogenesis through derepression of *Klf15* (Aggarwal et al. 2017). Conversely, deletion of *Cry1* in 3T3-L1 cells and C3H10T1/2 or of *Rora* in MEFs reduces adipogenesis (Duez et al. 2009; Sun et al. 2018).

These differing findings on the role of the preadipocyte circadian clock in adipogenesis may have arisen for several reasons. The circadian clock is encoded by an autoregulatory transcriptional feedback circuit whereby genetic ablation of one transcription factor perturbs the expression of other circadian clock proteins and in turn produces opposing effects on downstream rhythmic processes. Therefore, an observed effect on adipocyte differentiation after deletion of one clock factor may result in indirect and compensatory regulation of adipogenesis by another component of the clock. Individual clock transcription factors may also regulate adipogenesis through interaction with other nonclock proteins. Furthermore, in vitro adipogenesis assays are not completely representative of in vivo adipogenesis. Mice with global mutations in *Clock* (Turek et al. 2005), *Bmal1* (Shimba et al. 2011), *Per2* (Yang et al. 2009), *Per3* (Dallmann and Weaver 2010), *Nr1d1* (Delezie et al. 2012), and *Cry1/2* (Barclay et al. 2013) exhibit features of metabolic syndrome, dyslipidemia, adipocyte hypertrophy, and ectopic lipid accumulation in response to HFD. These models of genetic clock mutations do not exhibit a complete absence of adipocytes, which indicates that adipogenesis at least during development is not inhibited by manipulation of core clock genes. A more comprehensive investigation into the role of clocks in adipogenesis in vivo using genetic tracing and manipulation of circadian clock components specifically in preadipocytes is warranted.

A functioning rhythmic circadian clock is an essential regulator of the multiday process of adipocyte differentiation. Individual preadipocytes undergo adipogenic commitment—as defined by an increase in PPARγ abundance above a critical threshold—in daily bursts as opposed to all at once (Zhang et al. 2022b). The precise timing of irreversible commitment to differentiate in individual cells is during the rising phase of REV-ERBα, which corresponds to the inactive/sleep period in mice. Cells that do not reach the threshold for adipogenic commitment on the first rising phase of REV-ERBα may try again the next day. Knockdown of *Bmal1*, which reduces *Nr1d1*, leads to commitment to differentiate in one peak, as opposed to multiple daily bursts when the clock is rhythmic. This may in part explain why ablation of Bmal1 appears to increase the rate of adipocyte differentiation in vitro (Guo et al. 2012). Clock-mediated restriction of differentiation commitment to the rest phase is gated by C/EBPα, which has two BMAL1/CLOCK-regulated E-boxes upstream of its promoter (Ahrends et al. 2014; Zhang et al. 2022b). This suggests that dysregulation of circadian clocks, such as during shift work and obesity, may impair adipogenesis through disruption of rhythmic differentiation commitment.

In healthy animals, adipocyte differentiation-inducing hormones, such as glucocorticoids, oscillate in a circadian manner. Loss of daily rhythms in glucocorticoids correlates with increased obesity and metabolic disease. Glucocorticoid receptor agonism is a standard component of the adipogenic cocktail for the first 48 h to induce differentiation in vitro (Pantoja et al. 2008). Interestingly, adipocyte commitment does not occur under normal 12-h on/12-h

off circadian glucocorticoid cycles in vitro (Bahrami-Nejad et al. 2018). In mice, flattening of glucocorticoid oscillations results in increased visceral fat mass and visceral adipocyte number, suggesting increased adipogenesis. At the molecular level, glucocorticoids may control the rhythmic expression of adipogenic targets through direct interaction with the core clock repressor CRY1 (Lamia et al. 2011). Alternatively, glucocorticoid signaling may modulate adipogenesis through inhibition of COP1, a ubiquitin ligase that functions as a hub to modulate nutrient-responsive transcription factors (Rizzini et al. 2019). The persistent elevation in glucocorticoids during Cushing's disease and chronic stress may explain how these disorders result in increased adiposity (Bahrami-Nejad et al. 2018).

The circadian clock temporally regulates metabolic pathways and cellular processes in part through control of metabolite levels that may modulate adipogenesis at the post-transcriptional level. For example, the clock regulates mitochondrial oxidative function through controlling nicotinamide adenine dinucleotide (NAD^+) biosynthesis through control of the rate-limiting enzyme in NAD^+ biosynthesis, nicotinamide phosphoribosyl transferase (NAMPT) (Ramsey et al. 2009; Peek et al. 2013). During adipogenesis, nuclear NAD^+ levels decline along with an induction of cytoplasmic NAD^+ synthesis (Ryu et al. 2018). NAD^+ also functions as an obligate cofactor of the poly (ADP-ribose) polymerase 1 (PARP-1), a regulator of C/EBP activity (Luo et al. 2017). The decline in nuclear NAD^+ during the early stage of differentiation results in reduced PARP-1 activity, increased C/EBPβ binding to chromatin, and enhanced adipogenesis. Consistent with these findings, depletion of NAD^+ in preadipocytes leads to an increase in adipogenesis (Sánchez-Ramírez et al. 2022). However, terminal differentiation involves activation of SIRT1 by NAD^+ (Sánchez-Ramírez et al. 2022). This indicates an additional potential link between rhythmic metabolite oscillation and adipogenesis. Therefore, temporal and spatial control of NAD^+ synthesis, which is regulated by the circadian clock, is critical in adipogenesis.

Diurnal proliferation of adipocyte progenitors

It has been suggested that adipocyte differentiation requires mitotic clonal expansion to maintain the precursor pool (Tang et al. 2003). Labeling with BrdU demonstrates that visceral adipocyte progenitors (Lin^-;$CD29^+$;$CD34^+$;$Sca\text{-}1^+$) proliferate in response to HFD feeding (Jeffery et al. 2015). However, only a small fraction of newly generated adipocytes is labeled with BrdU after HFD feeding (Jeffery et al. 2016). This indicates that the majority of visceral adipogenesis during HFD feeding may not require precursor proliferation. In support of this finding, the visceral fibro-adipogenic subpopulation proliferates in response to HFD, whereas the preadipocyte cells have a low rate of proliferation (Marcelin et al. 2017; Hepler et al. 2018).

Adipocyte progenitors (Lin^-;$CD34^+$) undergo diurnal proliferation, with elevated EdU incorporation during the onset of the light period (ZT0) as compared with the onset of the dark period (ZT12) (Ribas-Latre et al. 2021). HFD feeding or deficiency of *Clock* results in abolished diurnal regulation of progenitor proliferation and instead results in constitutively elevated proliferation. Therefore, there may be a metabolically healthy benefit to suppressing progenitor proliferation during the dark period (ZT12). The visceral preadipocyte subpopulation with high adipogenic capacity has low expression of CD34 (Hepler et al. 2018; Buffolo et al. 2019). Consistent with this, the visceral progenitor population with high proliferative capacity during HFD feeding appears to be the fibro-adipogenic cells rather than the preadipocytes (Hepler et al. 2018). A more refined analysis of the rhythmic proliferative capacity of subpopulations (adipocyte progenitors and preadipocytes) in adipose in response to HFD feeding is warranted.

Rhythmic transcription and metabolism in adipocytes

Adipocytes have intrinsic circadian clocks that oscillate even in the absence of the central pacemaker (Fig. 3; Kolbe et al. 2016; Friedrichs et al. 2018). Several thousands of genes display diurnal rhythmic expression in adipose tissue, including metabolic genes, adipokines, and adipocytokines (Zvonic et al. 2006; Shostak et al. 2013; Zhang et al. 2014). BMAL1/CLOCK generates rhythms in C/EBPα-mediated transcription of *Leptin* and PPARγ/PCG1α-mediated expression of *Adiponectin* (Barnea et al. 2015; Kettner et al. 2015). Jonckheere-Terpstra-Kendall (JTK) cycle analysis of adipocyte-specific gene expression at a 4-h resolution using *Adiponectin-Cre;NuTRAP* mice housed at thermoneutrality revealed that 28% of genes in brown adipocytes and 17.3% of genes in inguinal adipocytes oscillate (Hepler et al. 2022). In brown adipocytes, most of the rhythmic transcripts reach maximal abundance during a single phase of the circadian cycle around ZT1, corresponding to the early rest period in mice. Oscillating genes in inguinal adipocytes display two phases of maximal amplitude around ZT7 and ZT22. Analysis of chromatin accessibility using assay for transposase-accessible chromatin by sequencing (ATAC-seq) identified a considerable number of ATAC-seq peaks as rhythmic (16% and 12.7% of identified peaks oscillate in brown adipocytes and inguinal adipocytes, respectively). Motif enrichment analyses revealed rhythmic chromatin opening in areas occupied by transcription factors including PPARγ, C/EBP, ESRRβ, and EBF that regulate energetic, adipogenic, and inflammatory gene networks. This indicates that temporal ordering of cellular processes in adipocytes are regulated at the genomic and transcriptomic levels, which may be coordinated by the cell-autonomous adipocyte circadian machinery.

The rhythmic expression of transcription factors and metabolic enzymes in adipose tissue leads to functional changes in metabolism throughout the day (Fig. 2). Synchronized adipocytes display cell-autonomous rhythms in lipolysis and response to isoproterenol (Pendergrast et al. 2023). Mice that undergo maximal exercise during the early

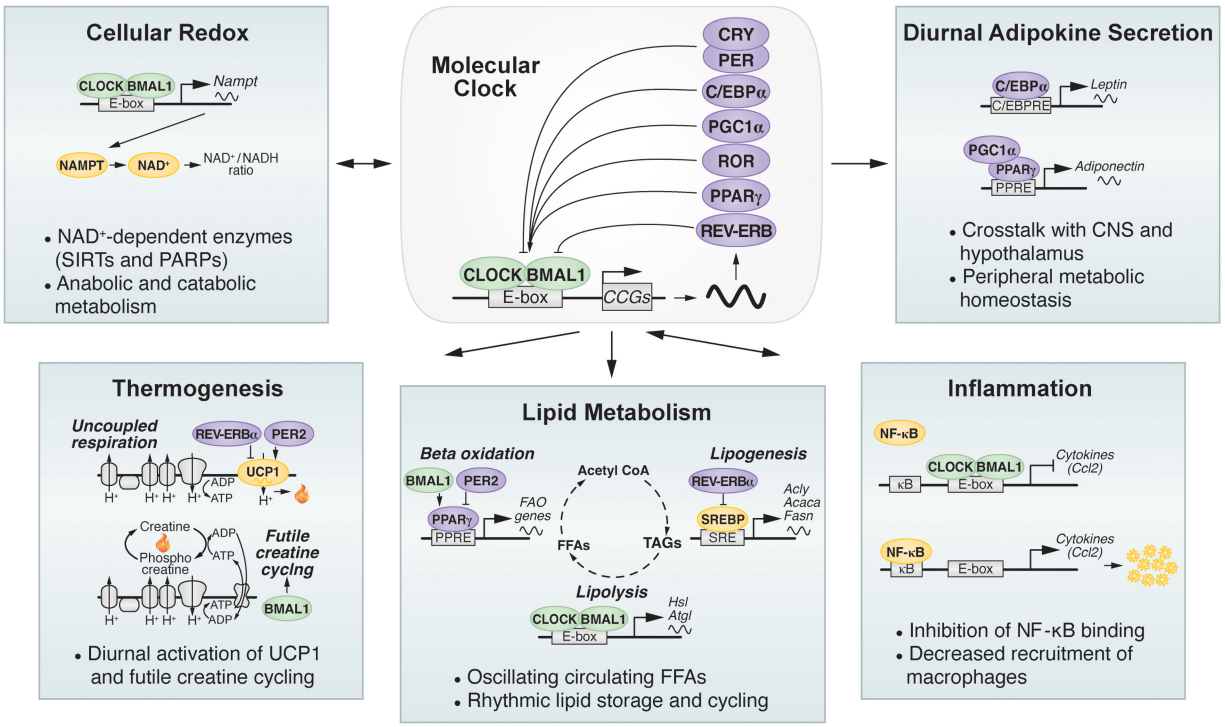

Figure 3. Circadian regulation of physiology in adipocytes. The core circadian clock in adipocytes comprises multiple feedback loops of transcription and translation that coordinate various metabolic activities via direct and indirect outcomes. Highlighted here are direct outputs of the clock on adipokine secretion, cellular redox, metabolism, and inflammation. The clock is also influenced by bidirectional signals from nutrient signaling and inflammatory pathways, which serve as regulators to link circadian rhythms with metabolic changes.

active phase have increased serum NEFAs and expression of thermogenic genes in inguinal WAT, as opposed to those that undergo exercise during the early rest phase (Pendergrast et al. 2023). This highlights a transcriptomic and metabolic response in WAT in response to timing of exercise, which may be coordinated by the adipocyte clock control of mitochondrial lipid oxidation (Peek et al. 2013). Metabolomics profiling at a 4-h resolution over the 24-h day revealed that several metabolites oscillate in adipose tissues (Dyar et al. 2018). The most highly abundant metabolites in adipose tissues relative to other tissues were lipids. In BAT, enriched rhythmic metabolite pathways included pyrimidine, dipeptide, and lysolipid metabolism, whereas in WAT, guanidino/acetamido and lysine pathways were rhythmic. HFD feeding for 10 wk led to a change in the abundance and rhythmicity of metabolites across tissues. Several metabolites gained rhythmicity in WAT, including many fatty acids. In BAT, HFD feeding led mainly to loss of metabolite rhythmicity and gain in cycling of some metabolites involved in lysine-, purine-, and guanine-containing pathways. Analyses of adipocyte-specific rhythmic gene expression and metabolite abundance in mice with a genetically disrupted adipocyte clock under distinct environmental conditions (e.g., lean/obese, fasted/fed, or thermoneutral/cold) would reveal the contribution of the adipocyte clock to rhythmic adipose tissue metabolism.

The redox coenzyme NAD^+ functions as a hub, linking core clock oscillations with pathways involved in metabolism, chromatin remodeling, RNA processing, and inflammation in adipocytes (Yamaguchi and Yoshino 2017). NAD^+ levels are regulated in part through clock control of the NAD^+ salvage biosynthesis enzyme *Nampt*. NAMPT has also been reported to circulate in the blood as extracellular NAMPT (eNAMPT), with reduced circulating levels following ablation of *Nampt* in adipocytes (Yoshida et al. 2019). Adipocyte-derived eNAMPT is elevated during fasting, whereas circulating eNAMPT oscillates and peaks during the dark period when mice feed (Park et al. 2023). Intraperitoneal administration of NAMPT suppresses fasting-induced hyperphagia (Park et al. 2023). Therefore, adipocyte-derived eNAMPT may have a role in appetite during fasting.

The recent identification of SLC25A51 as the mitochondrial NAD^+ transporter suggests that deficiency of NAD^+ biosynthesis in cytosol might in turn contribute to impaired mitochondrial respiration in *Bmal1*-deficient animals (Peek et al. 2013; Kory et al. 2020; Luongo et al. 2020). Loss of *Nampt* in adipocytes using *Adiponectin-Cre* leads to a marked decrease in levels of NAD^+ and salvage pathway intermediates (Yamaguchi et al. 2019). Ablation of *Nampt* in adipocytes also results in a decrease in the amplitude of clock gene expression and loss of rhythmicity of TCA cycle intermediates, particularly in BAT (Basse et al. 2023). These mice have impaired adrenergic-mediated lipolysis, severe cold intolerance during cold exposure when fasting, and increased adipose tissue inflammation and mitochondrial dysfunction (Yamaguchi et al. 2019; Franczyk et al. 2021).

Mice with deletion of *Nampt* only in UCP1$^+$ cells have normal cold tolerance, despite these mice having impaired mitochondrial function in BAT. This suggests that the impairment in WAT lipolysis during fasting is the primary driver of cold intolerance. However, it is unclear whether compensatory UCP1-independent thermogenesis rescues the body temperature phenotype in mice lacking *Nampt* in brown adipocytes. In support of this, mice lacking *Nampt* in all adipocytes have an increase in the expression of genes involved in futile creatine cycling in BAT and have improved cold tolerance under fed conditions (Basse et al. 2023). NAD^+ levels also feed back to the clock to influence circadian physiology through deacetylation of PER2 by the NAD^+-dependent class III histone deacetylase SIRT1 (Nakahata et al. 2009; Ramsey et al. 2009; Levine et al. 2020). This raises the possibility that some of the actions of NAD^+ within thermogenic adipocytes may involve changes in the activity of SIRT1, PER2, and CLOCK/BMAL1. Together, these findings reveal that rhythmic NAMPT-mediated NAD^+ biosynthesis is critical in adipocyte metabolic flexibility and whole-body energy homeostasis.

Adipocyte clocks and obesity

Genetically obese (ob/ob or KK-Ay) mice have altered or dampened oscillations of clock genes in adipose tissues (Ando et al. 2005, 2011). HFD feeding dampens rhythmicity of several components of the core clock in a tissue-specific manner (Kohsaka et al. 2007). In particular, the expression of the clock repressors is suppressed in WAT during DIO (Hong et al. 2018). Obesity is characterized by chronic low-grade inflammation with increased secretion of cytokines and infiltration of immune cells (Klöting and Blüher 2014). This chronic inflammatory state in WAT is a major contributor to insulin resistance and metabolic dysfunction (Hardy et al. 2011). Activation of NF-κB results in marked inhibition of the clock repressors and relocalization of CLOCK/BMAL1 genome-wide (Hong et al. 2018). Conversely, inhibition of NF-κB signaling through genetic deletion of IKKβ leads to up-regulation of the repressors of the circadian core genes in visceral WAT (Hong et al. 2018). In addition, BMAL1 recruitment to target genes is restored by inhibition of NF-κB in adipocyte progenitors (Maury et al. 2021). This suggests that the mechanism for reduced expression of the clock repressors in WAT results at least in part from sustained activation of NF-κB during obesity. Impaired PPARγ activity also disrupts *Bmal1* expression via reduced methionine and glutamine uptake, which results in decreased H3K27ac and H3K4me3 at the *Bmal1* promoter (Wang et al. 2022).

The original discovery that *Clock* mutant mice become obese and develop metabolic disease revealed a strong link between circadian disruption and obesity (Turek et al. 2005). Genetic deletion of the essential clock activator *Bmal1* also leads to increased weight gain early in life (Lamia et al. 2008). This increase in weight gain in both models is attributable predominately to expansion of visceral adiposity with hypertrophic adipocytes and decreased lipolysis (Shostak et al. 2013). Whole-body ablation of several other components of the clock affects susceptibility to DIO (Tsang et al. 2017). However, global manipulation of circadian clock components often affects daily rhythms in feeding and activity, which confound metabolic analyses specific to peripheral organs.

Adipocyte-specific deletion of the clock activator *Bmal1* using *Adiponectin-Cre* results in increased weight gain during HFD feeding (Paschos et al. 2012). Mice lacking an adipocyte clock display increased food intake during the light period and decreased energy expenditure throughout the day prior to divergence in body weight. The investigators found that the increased food intake was associated with temporal changes in circulating polyunsaturated fatty acids (PUFAs), which are sensed by the hypothalamic feeding centers. In support of this, PUFA supplementation during HFD feeding corrects the greater food intake during light period and normalizes weight gain in adipocyte *Bmal1* knockout mice.

The defect in energy expenditure in mice lacking an adipocyte clock appears to be independent of *Ucp1*. In fact, *Ucp1* expression is slightly elevated in mice lacking an adipocyte clock (Paschos et al. 2012; Hasan et al. 2021; Xiong et al. 2023). In addition, mice lacking *Bmal1* in brown adipocytes using *Ucp1-Cre* have mildly reduced surface temperature of the BAT region (Hasan et al. 2021). Analysis of BAT from these mice revealed slightly dampened oscillations in genes involved in lipid utilization and a decrease in acetyl CoA, creatine, and metabolites required for creatine synthesis (glycine, arginine, and S-adenosylmethionine [SAM]). This indicates that the defect in energy expenditure may involve clock-mediated control of thermogenic pathways, such as creatine metabolism. Tissue creatine abundance and expression of several enzymes involved in creatine synthesis and futile cycling are rhythmic in adipose tissue and are also reduced in inguinal WAT (Hepler et al. 2022). Interestingly, mice lacking an adipocyte clock do not display a difference in food intake during HFD feeding when housed at thermoneutrality but still have elevated adiposity. Supplementing these mice with 2% creatine in the HFD restores the excess weight gain, loss of glucose homeostasis, and amounts of iWAT creatine and SAM in adipocyte *Bmal1* knockout mice. In addition, genetic overexpression of *Bmal1* in adipocytes (*Adiponectin-rtTA;TRE-Bmal11*) attenuates weight gain during HFD feeding and increases adipose tissue creatine levels (Hepler et al. 2022). These results reveal that futile creatine cycling is rhythmic and is regulated by the intrinsic adipocyte clock.

Global deletion of *Nr1d1* results in excess weight gain and adiposity during chow and HFD feeding (Delezie et al. 2012). Conversely, treatment with a synthetic REV-ERB agonist in wild-type mice reduces adiposity through increasing energy expenditure (Solt et al. 2012). However, whether such effects are selective for REV-ERB has been challenged (Dierickx et al. 2019). Mice with adipocyte-specific deletion of *Nr1d1* with *Adiponectin-Cre* display elevated weight gain and adiposity only during HFD feeding (Hunter et al. 2021). However,

adipocyte *Nr1d1* knockout mice do not develop metabolic disease during DIO. Instead, these mice have preserved glucose homeostasis accompanied by less WAT macrophage infiltration and fibrosis. Despite the increased white fat depot mass, these mice do not have a difference in adipocyte size compared with control mice. This suggests that deletion of adipocyte *Nr1d1* promotes healthy WAT remodeling and preserves adipocyte metabolic function during HFD feeding.

Circadian clocks in time-restricted feeding

A significant amount of the positive energy balance that occurs during DIO can be attributed to increased food intake at the incorrect circadian time; i.e., the light period in mice (Kohsaka et al. 2007). Individuals who experience circadian desynchrony through shift work, jet lag, nocturnal feeding, and sleep disorders are at an increased risk of developing obesity and diabetes (Wang et al. 2014). Conversely, restricting feeding to the active period in mice or daylight in humans improves metabolic health and reduces adiposity, highlighting an interconnection between meal timing and energy homeostasis (Arble et al. 2009; Hatori et al. 2012; Chaix et al. 2014; Sutton et al. 2018). The effects of time-restricted feeding (TRF) are independent of age but dependent on sex in mice (Chaix et al. 2021). Male mice tend to have a greater benefit in weight and glucose tolerance from TRF as compared with females, which respond primarily with improved metabolic health. Mice with global deletion of both *Cry1* and *Cry2* show improvements in body weight, adiposity, oxygen consumption (normalized to body weight), glucose tolerance, and circulating lipids in response to TRF (Chaix et al. 2019). However, the hepatic transcriptome and metabolome as well as hepatic steatosis were not altered by TRF in mice lacking *Cry1/2*. This suggests that the clock may play a role in at least some metabolic benefits driven by TRF. Analysis of liver-specific deletion of *Bmal1* or *Rev-erbα/β* revealed that TRF improved metabolic health and body weight in mice lacking a hepatic clock (Chaix et al. 2019). Collectively, these results indicate that the clock in nonhepatic tissues may promote metabolic benefits during TRF.

In mice, TRF consistently leads to decreased adiposity, increased adiponectin, decreased leptin, and an improved serum lipid profile (Hatori et al. 2012; Chaix et al. 2019). In addition, histological analysis after TRF shows less visceral WAT inflammation, decreased white adipocyte size, and less whitening of BAT (Chaix et al. 2014). RNA sequencing of several tissues in mice after 7 wk of TRF during HFD feeding revealed that BAT, epididymal WAT, and inguinal WAT had the largest transcriptional response to TRF as compared with other tissues (Deota et al. 2023). In epididymal WAT, TRF led to up-regulation in rhythmic gene pathways involved in glucose and fatty acid metabolism, TCA cycle, and oxidative phosphorylation and down-regulation in rhythmic pathways involved in inflammation, cell cycle, and immune activation. TRF in BAT led to increased rhythmicity in pathways involved in thermogenesis, adipogenesis, and autophagy. Therefore, adipose tissues have a robust and depot-specific response to TRF through modulation of metabolic pathways, tissue inflammation, and cellular processes.

The improved adipose tissue remodeling after TRF is consistent with a leaner phenotype; however, adipose tissue may also play a causative role in the metabolic benefits driven by TRF. While HFD feeding dampens rhythms in core clock genes, TRF in mice restores the expression of several clock genes in adipose tissues (Hepler et al. 2022; Deota et al. 2023). Analysis of transcriptional rhythms in subcutaneous adipose tissue in obese humans revealed that TRF resulted in increased expression of *Clock* and *Nr1d2* and reduced expression of *Per1* and *Nr1d1* (Zhao et al. 2023). This suggests that the adipocyte clock may mediate the healthy adipose tissue remodeling and metabolism in response to TRF. Genetically ablating *Bmal1* in adipocytes prevents the improved glucose tolerance and blunted weight gain responses to TRF (Hepler et al. 2022). Therefore, the adipocyte clock activator *Bmal1* is required for the metabolic response to TRF.

Mice display a diurnal rhythm of BAT thermogenesis, which is highest during the start of the active/dark period (van den Berg et al. 2018). Analysis of mice with adipocyte-specific deletion of the antithermogenic transcription factor zinc finger protein 423 (*Zfp423*) revealed that enhancing adipocyte thermogenesis is sufficient to improve metabolic benefits during HFD feeding restricted to the inactive/light period (Hepler et al. 2022). Therefore, a reduction in adipocyte thermogenesis during the light period is involved in metabolic decline when mice feed during the inactive/light period. A major mechanism through which *Zfp423* knockout mice have increased thermogenesis is through enhanced futile creatine cycling. Mice lacking the rate-limiting enzyme involved in creatine synthesis, glycine amidinotransferase (*Gatm*), in adipocytes have reduced adipose tissue creatine and futile creatine cycling (Kazak et al. 2017). These mice lacking *Gatm* in adipocytes do not display improved health in response to dark/active period TRF (Hepler et al. 2022). This reveals that rhythmic futile creatine cycling in adipocytes is an essential mechanism that drives metabolic benefits during TRF to the dark/active period. Mice that were fed during the light/inactive period did not have increased futile creatine cycling during feeding (Hepler et al. 2022). Therefore, adipose tissue thermogenesis through creatine metabolism aligns with the light/dark cycle rather than feeding time. A TRF regimen after HFD feeding also restores the expression of genes involved in futile cycling of lipogenesis and lipolysis in visceral WAT (Bushman et al. 2023).

Caloric restriction and aging: dependence on circadian phase

Aging leads to a decline in circadian function across tissues, particularly in adipose tissue. The age-related increase in inflammation is first detectable through increased immune cell activation in adipose tissue depots

during middle age (Schaum et al. 2020). In addition, diurnal uptake and abundance of lipids in adipose tissues are blunted in aged mice (Held et al. 2021; In Het Panhuis et al. 2022). CR is a dietary intervention that results in extension of life span and improved health across species (Weindruch et al. 1986). A hallmark of CR is a decrease in adiposity, which is associated with improvement in adipose tissue metabolism and secretory profiles and a delay in age-related conditions (Miller et al. 2017; Feng et al. 2023). When provided a hypocaloric diet, mice consolidate nearly all their food intake within a couple of hours of when food becomes available (Acosta-Rodríguez et al. 2017). Thus, CR experiments performed in mice often involve a combination of a self-imposed temporal restriction of their feeding rhythm in addition to calorie restriction. However, providing a CR diet either all at once at the onset of the dark/active period or evenly distributed throughout the active/dark period results in a greater degree of life span extension as compared with mice that receive CR during the light/inactive period or distributed throughout the 24-h day (Acosta-Rodríguez et al. 2022). Similarly, in *Drosophila*, alignment of calorie restriction with intrinsic circadian behavioral cycles produces greater effects on healthspan (Ulgherait et al. 2021). This demonstrates the importance of circadian alignment of feeding time during CR for extending life span across species.

CR provided under a TRF protocol increases the amplitude of core clock genes in the liver and leads to up-regulation of circadian clock genes in WAT (Patel et al. 2016b; Sato et al. 2017; Pak et al. 2021). Mice with global deletion of *Bmal1* do not exhibit increased life span in response to CR (Patel et al. 2016a). However, mice lacking *Cry1* and *Cry2* have a similar weight loss and glucose tolerance improvement during 30% CR given during the dark period (Mezhnina et al. 2022). Therefore, the effect of CR on health and life span involves interplay between circadian systems and metabolism across tissues. The precise role of the adipose clock in the metabolic response to CR requires further investigation.

Role of the central and adipocyte circadian clock in activation of thermogenesis

Activation of BAT occurs primarily via the release of norepinephrine from nerve endings that activate β3-adrenergic receptors in brown adipocytes. Mice and humans have a diurnal rhythm in fatty acid and glucose uptake by BAT, with the peak in uptake occurring at waking (van den Berg et al. 2018). The diurnal uptake by BAT is abolished by denervation but conserved at thermoneutrality in mice. The central clock is a major regulator of sympathetic activation of BAT through a process that is also sensitive to photoperiod (Bartness et al. 2001). Mice exposed to prolonged light have reduced sympathetic input into BAT and decreased glucose and lipid uptake by BAT (Kooijman et al. 2015). Conversely, mice lacking *Bmal1* in the ventromedial hypothalamus have enhanced BAT activity through increased β3-adrenergic receptor activation (Orozco-Solis et al. 2016). Interestingly, mice lacking β-adrenergic receptors maintain a diurnal rhythm in circadian clock genes and *Ucp1* despite having impaired body temperature regulation during cold exposure (Bachman et al. 2002; Razzoli et al. 2018). This indicates that β-adrenergic signaling is dispensable for maintaining circadian rhythmicity in BAT but is required for cold-induced thermogenesis.

The ability of mice to maintain body temperature when exposed to cold depends on time of day. Mice are better able to defend their body temperature in response to cold exposure during the dark/active period when *Nr1d1* is low (Gerhart-Hines et al. 2013). *Nr1d1* represses *Ucp1* in brown adipocytes, and global ablation of *Nr1d1* leads to loss of rhythms in body temperature and BAT activity. Conversely, mice with global ablation of *Per2* are less tolerant to cold temperatures and have reduced *Ucp1* in BAT (Chappuis et al. 2013). Mice with brown adipocyte-specific deletion of *Nr1d1* and *Nr1d2* are better able to maintain their body temperature during fasting after 1 wk of cold exposure (Adlanmerini et al. 2019). Ablation of REV-ERBs leads to derepression of REV-ERB target genes, including genes involved in DNL. Futile cycling of fatty acid oxidation and lipogenesis during β3-adrenergic stimulus is a UCP1-independent thermogenic mechanism (Mottillo et al. 2014). Mechanistically, circadian repression by REV-ERBs drives rhythmicity of *Srebp1*, a master regulator of DNL gene expression (Adlanmerini et al. 2019).

Mice lacking the core clock activator *Bmal1* in brown adipocytes using *Ucp1-Cre* display decreased body temperature at 22°C and 4°C (Chang et al. 2018; Hasan et al. 2021). The thermogenic impairment is independent of *Ucp1*, since mice lacking *Bmal1* in brown adipocytes have compensatory elevated UCP1 and increased levels of several electron transport chain subunits (Hasan et al. 2021). However, the BAT has a whitened appearance, and several enzymes involved in lipid utilization are decreased in brown adipocyte *Bmal1* knockout mice. Metabolomics analysis revealed that mice lacking *Bmal1* in BAT have decreased abundance of acetyl CoA, glucogenic amino acids, creatine, ATP, and ADP. Collectively, this suggests that the thermogenic defect in mice lacking a brown adipocyte clock involves impaired lipid metabolism and futile creatine cycling with an intact mitochondrial electron transport chain.

Clocks in adipose tissue inflammation and immune cells

Increased adipose tissue inflammation is a hallmark of circadian clock dysfunction during obesity, shift work, and chronic sleep disruption (Xiong et al. 2021). Circadian alignment of feeding time (Lee et al. 2021) or CR (Kosteli et al. 2010) after HFD feeding reduces accumulation macrophages in visceral WAT. In turn, inflammation affects the clock in a reciprocal manner through altered expression of clock genes and shifts in the phase of the clock (Hergenhan et al. 2020). The circadian clock regulates rhythms in immune function and controls several

inflammatory and metabolic pathways. The magnitude of inflammatory responses, including NF-κB, interferon, and infection, depends on time of day (Gibbs et al. 2012, 2014; Spengler et al. 2012; Greenberg et al. 2020). Generally, inflammatory stimuli tend to evoke a greater response in mice during the light period as opposed to the dark period, though the precise timing of these effects is likely specific to individual cell type and stimuli. There is evidence that the core clock in hepatocytes may control non-cell-autonomous interaction between hepatocytes and nonparenchymal cells within the liver, including macrophages and endothelial cells (Guan et al. 2020, 2023). It seems likely that similar intercellular communication will be seen between adipocytes and other cell types during genetic clock manipulation within adipose tissue.

Ly6C-high monocytes display a *Bmal1*-dependent diurnal oscillation in the circulation, with the peak occurring around ZT4–ZT8 (Nguyen et al. 2013). BMAL1 suppresses the expression of several cytokines, and mice lacking *Bmal1* in monocytes display higher serum levels of IL-1β, IL-6, IFN-γ, and CCL2 (Nguyen et al. 2013). Macrophages lacking *Bmal1* also lose rhythmicity in cytokine secretion and have constitutively higher levels of IL-6, CCL5, and IL-12 (Gibbs et al. 2012). BMAL1$^{-/-}$ mice have elevated expression of interferon-stimulated genes in response to a TLR7 agonist in the skin (Greenberg et al. 2020). Therefore, BMAL1 largely acts to limit the inflammatory activation of NF-κB and interferon. Mechanistically, BMAL1 recruits the polycomb repressor complex 2 (PRC2), which leads to trimethylation of histone H3 at lysine 27 (H3K27Me3) at the promoter region of several cytokines to suppress their expression (Nguyen et al. 2013). The BMAL1:CLOCK heterodimer also interacts with RelB, a subunit of NF-κB (Bellet et al. 2012). In contrast, CLOCK appears to be a positive regulator of the NF-κB response (Spengler et al. 2012; Bellet et al. 2013).

Work mostly in global circadian mutant mice and cell culture has indicated that PER1, CRY1/2, REV-ERBα, and RORα are predominately anti-inflammatory (Hergenhan et al. 2020). PER1 binds to PPARγ on the *Ccr2* promoter to inhibit its expression (Wang et al. 2016). CRY1 binds to adenylyl cyclase to limit cAMP production and downstream NF-κB activation (Narasimamurthy et al. 2012). REV-ERBs recruit receptor corepressor (NCoR)–HDAC3 complexes to suppress cytokine production (Lam et al. 2013). RORα promotes transcription of IκBα, a major inhibitor of NF-κB (Delerive et al. 2001). Conversely, the role of PER2 in inflammatory responses is less clear. Mice with global ablation of *Per1* and *Per2* have elevated macrophage inflammation (Xu et al. 2014). Mice lacking only *Per2* have decreased circulating IFN-γ and IL-1β in response to LPS (Liu et al. 2006). However, overexpression of *Per2* in OR6 cells counteracts viral replication of hepatitis C virus (Benegiamo et al. 2013).

In visceral WAT of chow-fed mice, hematopoietic cells (CD45$^+$) display a diurnal pattern of proliferation with elevated proliferation at ZT0 compared with ZT12 (Ribas-Latre et al. 2021). In contrast, CD45$^+$ cells in iWAT from chow-fed mice show a very low level of proliferation with no difference at ZT0 and ZT12. However, it is unclear which immune cell population in adipose tissue undergoes diurnal proliferation and whether the clock has a role in this process.

In mice lacking *Bmal1* in myeloid cells using *Lyz2-Cre*, HFD feeding for 1 wk leads to excess accumulation of Ly6C-high macrophages in visceral WAT and BAT (Nguyen et al. 2013). Ablation of *Bmal1* in macrophages with *LysM-Cre* has no effect on weight gain after 12 wk of HFD feeding (Paschos et al. 2012). However, long-term HFD feeding in these mice results in decreased energy expenditure, increased weight gain, and insulin resistance associated with increased macrophage infiltration into adipose tissue (Nguyen et al. 2013). The body weight phenotype appears only after 14 wk of HFD feeding. Therefore, the clock in myeloid cells, which includes monocytes and macrophages, plays a role in susceptibility to inflammation and metabolic dysfunction during long-term HFD feeding. Mice lacking *Per1/2* in myeloid cells after bone marrow transplant have elevated visceral WAT CD11c$^+$ macrophage infiltration and have exaggerated visceral adiposity and insulin resistance (Xu et al. 2014). Primary adipocytes cocultured with *Per1/2* knockout macrophages have increased expression of cytokines and decreased expression of *Adipoq*. Therefore, Period proteins in myeloid cells regulate adipose tissue inflammation and insulin sensitivity through cross-talk with adipocytes.

T cell migration and differentiation are also regulated in a rhythmic manner (Yu et al. 2013; Druzd et al. 2017). Deficiency of *Bmal1* in Tregs using *Foxp3-Cre* results in heightened activation and impaired oxidative metabolism in visceral WAT (Xiao et al. 2022). In lean mice, *Bmal1* in Tregs acts to suppress lipolysis. During HFD feeding, mice lacking *Bmal1* in Tregs have increased visceral WAT accumulation of CD11c$^+$ inflammatory macrophages and CD8$^+$ T cells. This reveals that the cell-intrinsic clock in Tregs regulates WAT lipolysis and inflammation.

Conclusions

Our understanding of the heterogeneity and functional plasticity of adipose tissue cell types has been transformed through advances in genetic analyses that have begun to converge with studies of the molecular clock. The finding that adipose tissue metabolic function exhibits robust 24-h variation in catabolic and anabolic transcriptional pathways and that overnutrition flattens the day/night variation in rhythmic feeding behavior and nutrient processing provides insight into how adipocytes adapt to changes in nutrient availability and processing in anticipation of daily changes in the light/dark environment. Environmental factors such as time of day, feeding time, macronutrient content of diet, and ambient temperature must all be considered to fully elucidate how adipose tissue contributes to energy homeostasis. A challenge will be to dissect the specific molecular roles of each individual component of the clock as well as their interacting partners within diverse adipose cell types. These effects may

also exhibit sexual dimorphism and display differences in adaptation to CR versus overnutrition. Incorporating circadian parameters into the investigation of adipose tissue biology will lead to a better understanding of how alignment of intrinsic circadian cycles with the light/dark cycle impacts adipogenesis, inflammation, and metabolism. Leveraging our knowledge of circadian control could be used to determine the optimal timing of feeding, exercise, and other therapies for metabolic disorders.

Competing interest statement

The authors declare no competing interests.

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