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Keeping fat on time: Circadian control of adipose tissue

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ABSTRACT

Circadian clocks harmonize processes ranging from intracellular biochemistry to whole-body physiology in accordance with the Earth's 24 h rotation. These intrinsic oscillators are based on an interlocked transcriptional-translational feedback loop comprised from a set of core clock factors. In addition to maintaining rhythmicity in nearly every cell of the body, these clock factors also mediate tissue specific metabolic functions. In this review, we will explore how the molecular clock shapes the unique features of different adipose depots.

1. What makes us tick?

Countless mammalian behaviors and biological processes oscillate with respect to the 24-h rotation of the Earth. These rhythms are the result of circadian clocks that exist in almost every cell of the body. Clocks in peripheral tissues are kept in synchronicity by a master clock located in the hypothalamic suprachiasmatic nucleus (SCN) [1]. It receives light-induced signals from the retinal photoreceptors and orchestrates the 24-h oscillation pattern in organismal physiology through neural and humoral cues. In addition to light, the SCN and peripheral clocks are also influenced by physical activity, food intake [2] and environmental temperature [3,4]. Cumulatively, this biorhythmicity allows organisms to anticipate and prepare for daily changes or needs and thus provides an enormous selective advantage.

At the molecular level, the core circadian clock is a transcription-translation feedback loop. The positive arm is comprised of transcriptional activators, CLOCK and BMAL1, which heterodimerize to increase expression of *Ror* and the repressing arms, *Rev-erb*, *Per*, and *Cry* (Fig. 1). REV-ERB and ROR then feed back to suppress or activate *Bmal1* expression, respectively, and PER and CRY form a complex to inhibit CLOCK: BMAL1. This 24-h circuit is further shaped by post-translational modifications (e.g. AMPK-mediated phosphorylation) and nutrient status (e.g. NAD^+ levels).

In addition to maintaining the molecular rhythmicity, the core clock members also modulate tissue specific programs. This regulatory interface is critical for fine-tuned circadian control of peripheral metabolism. In adipose tissue, for example, circadian regulation of the master adipogenic factor, PPARγ, is a key link between the clock and fat metabolism. Notably, this is a bi-directional interaction where the clock and PPARγ impinge on each other [5–8] The role of PPARγ in circadian control has been reviewed extensively previously (see [9,10]) and, as a result, will not be focused on further here. In this review, we will specifically explore how peripheral clocks shape the unique biology of the different adipose depots.

2. Adipose Tissue: Three's Company

Adipose tissues are predominantly distinguished as white, brown and beige (or 'brite') adipose, each with unique metabolic characteristics. White adipose is the depot classically known for storing dietary energy as triacylglycerides (TAGs) in unilocular lipid droplets [11]. Brown adipose is known for its ability to convert chemical energy into heat, and for its multilocular lipid droplets and high mitochondrial density [12]. Beige adipocytes are harbored within white adipose depots and, upon stimulation, adopt thermogenic features similar to brown adipocytes [13,14]. In addition to the direct modulation of energy storage and expenditure the adipose organ also influences systemic metabolic homeostasis through production of hormones known as adipokines.

3. White adipose

White adipose tissue (WAT) is the most abundant type of adipose tissue in mammals. As mentioned, the traditional function attributed to this depot is long-term energy storage in the form of TAGs [15]. This energy is stored during postprandial periods where adipocytes take up dietary fats and carbohydrates from the circulation and convert them into TAGs through the process of lipogenesis [15]. Between meals, and in situations of caloric restriction and/or strenuous activity, the energy is released from WAT through lipolysis [15]. Lipolysis breaks down TAGs into free fatty acids (FFAs) and glycerol that are released into the circulation to meet the energy needs of other tissues [15].

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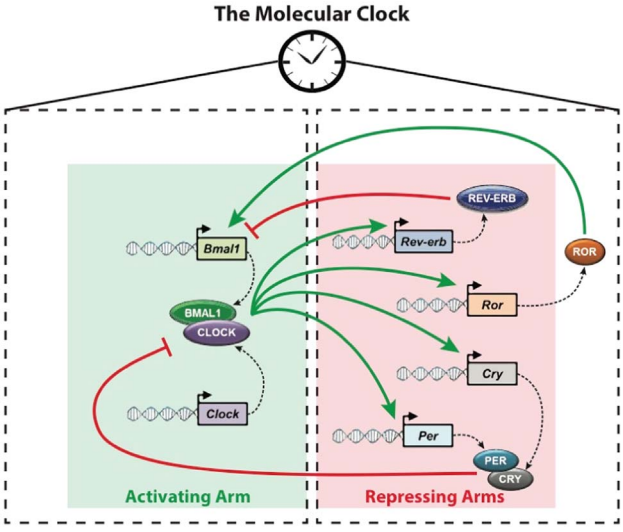

Fig. 1. Molecular architecture of the circadian clock. Circadian rhythms are generated by feedback loops of core circadian genes. In the activating arm, the heterodimeric transcription factors CLOCK and BMAL1 activate the transcription of *Rev-erb*, *Ror, Per,* and *Cry*. In the repressing arms, REV-ERB feeds back to suppress *Bmal1* expression, and the heterodimeric PER and CRY block CLOCK:BMAL1. ROR is a positive activator of *Bmal1* expression.

The balance of lipid storage and mobilization must be tightly regulated to ensure whole body energy homeostasis. Growing evidence indicates that the clock plays an important role in coordinating this control [16–18]. First, key lipid metabolism enzymes, adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL), and lipoprotein lipase (LPL), have been demonstrated to be under the transcriptional control of CLOCK and BMAL1 [16,17]. Second, mice with genetic disruption of *Bmal1* (*Bmal1*$^{-/-}$) [16,18] or a dominant negative mutation in *Clock* (*Clock*$^{\Delta 19}$) [16] showed an arrhythmic profile of FFAs and glycerol in serum compared to control mice. These clock-disrupted mice had decreased lipolysis and increased adiposity.

Interestingly, the increased adiposity observed with adipose-specific clock disruption did not seem to be attributable to adipose alone. These mice exhibited increased food intake during the day when rodents typically sleep [16,18]. Yet there was no change in total daily calorie intake [16,18] indicating that the time of consumption is as critical as the quantity [19]. One potential explanation for this phenomenon is circadian adipose-brain crosstalk. Adipose-specific Ad-*Bmal1*$^{-/-}$ (a.k.a. Ad-*Arntl*$^{-/-}$)mice had lower concentrations of polyunsaturated FFAs in the blood during the day, which seemed to influence signaling through the adipose-hypothalamic axis [18]. These mice also had reduced expression of carboxylesterase 1D (CES1D) that catalyzes the mobilization of TAG from adipose tissue and may be under transcriptional control by BMAL1 [18]. It is thus plausible that disruption of the clock in WAT leads to lower levels of CES1D and hence reduced release of polyunsaturated FFAs into the blood. The decrease in polyunsaturated FFAs is paralleled with an increase of neurotransmitters in the hypothalamic feeding centers that trigger food intake. This misalignment in feeding rhythmicity could create metabolic instabilities that promote obesity.

WAT is also an endocrine organ releasing a wide range of adipokines including leptin, adiponectin and resistin, all of which show robust circadian circulating levels [20–22]. The most studied adipokine is the “satiety hormone” leptin, which provides information to the hypothalamus about the levels of TAG storage in WAT. The blood concentration of leptin increases during the waking/feeding period and decreases during the sleeping/fasting period in both humans [21] and mice [22]. Leptin rhythmicity appears to be the net result of daily variations in food intake and circadian oscillation sensitive to the feeding related inputs [23,24]. In mice, the circadian regulation of leptin synthesis and release is, at least partly, intrinsic to adipocytes [25,26]. In accordance, circulating leptin levels were found to be dysregulated in both *Clock* $^{\Delta 19}$ and Ad-*Bmal1*$^{-/-}$ mice [18,22]. Adiponectin and resistin are also key players in the regulation of energy metabolism but the physiological relevance of their rhythmic expression profiles are currently not known [20]. The circadian pattern of white adipose physiology is depicted in Fig. 2.

4. Thermogenic adipose (brown and beige)

Brown adipose tissue (BAT) is a specialized thermogenic organ that converts chemical energy to heat and is protective against metabolic

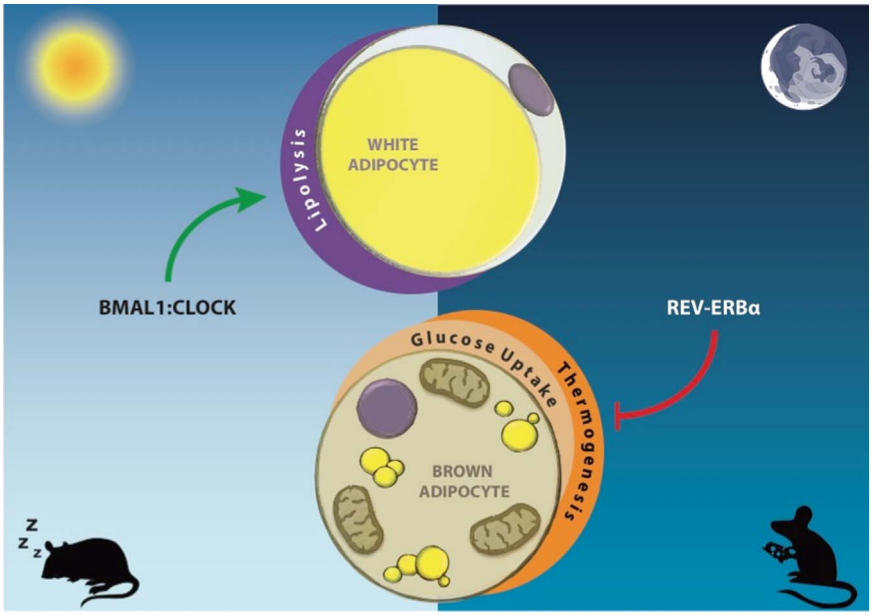

Fig. 2. Components of the circadian clock influence key processes in adipocytes. BMAL1 and CLOCK impinge on white adipose lipid mobilization by modulating the expression of key lipolytic enzymes, such as ATGL, HSL, and CES1D. The process of lipid storage also oscillates diurnally, but it is not known whether clock components impinge directly on this pathway or only indirectly through lipolysis. In brown adipose, REV-ERBα influences oscillations in glucose uptake and thermogenesis. Whether there is a rhythm to fatty acid uptake and how this influences thermogenesis remains unknown.