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BBA - Molecular and Cell Biology of Lipids 1866 (2021) 158961

Contents lists available at ScienceDirect

BBA - Molecular and Cell Biology of Lipids

journal homepage: www.elsevier.com/locate/bbalip

Review

Circadian control of brown adipose tissue

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ARTICLE INFO

Keywords:
Brown adipocytes
Cardiometabolic health
Circadian rhythm
Lipids
Shift work

ABSTRACT

Disruption of circadian (~24 h) rhythms is associated with an increased risk of cardiometabolic diseases. Therefore, unravelling how circadian rhythms are regulated in different metabolic tissues has become a prominent research focus. Of particular interest is brown adipose tissue (BAT), which combusts triglyceride-derived fatty acids and glucose into heat and displays a circannual and diurnal rhythm in its thermogenic activity. In this review, the genetic, neuronal and endocrine generation of these rhythms in BAT is discussed. In addition, the potential risks of disruption or attenuation of these rhythms in BAT, and possible factors influencing these rhythms, are addressed.

1. Introduction

Organisms have dealt with daily and seasonal fluctuations in their environment ever since the earth has been habited. To synchronize with the daily variations of the environment, an evolutionary conserved endogenous time-keeping system exists. This system orchestrates an approximate 24-hour oscillatory pattern in intracellular and behavioral processes, which is referred to as the circadian rhythm (*circa* = approximately, *dies* = day). The importance of the circadian rhythm in energy metabolism in mammals is illustrated by numerous studies showing that disruption of the circadian rhythm through genetic, environmental or behavioral factors can contribute to increased risk of developing cardiometabolic diseases. For example, shift work is associated with metabolic syndrome, type 2 diabetes mellitus and cardiovascular diseases [1–4]. Therefore, unravelling how circadian rhythms are regulated in different metabolic tissues has become a major research focus. An important metabolic organ is brown adipose tissue (BAT), which can combust triglyceride (TG)-derived fatty acids (FAs) and glucose into heat. BAT exhibits both circannual (seasonal) and circadian (daily) rhythms in its thermogenic activity, which we will discuss in the next sections of this review. In addition, we will discuss the potential risks of circadian disruptions or loss of amplitude in circadian rhythms of BAT as occurs for instance during aging. Finally, we will describe the potential implications of rhythms in BAT activity as a therapeutic means to promote thermogenic activity, which is relevant in the battle against cardiometabolic diseases.

2. Generation of biological rhythms

Circadian rhythms are present in almost every cell of the body including neurons in the brain and adipocytes in the various adipose tissue depots, across all species. Generation of circadian rhythms is a complex process. In principle, these rhythms are self-sustained molecular systems, however, they are also entrained to the environment *via* external cues called 'zeitgebers' (literally 'time-givers'), such as light exposure and food intake, the latter being especially relevant in our current society. In addition, they are subject to a hierarchical architecture, with neurons in the suprachiasmatic nucleus (SCN) receiving photic information serving as the master synchronizer of the peripheral circadian clocks [5,6]. A more detailed description of the various processes involved is outlined below.

Two key processes determine the generation of the endogenous cellular circadian rhythms: 1) activation and repression of key clock components entrenched in the transcriptional-translational feedback loop (TTFL), and 2) post-translational modifications of proteins involved in the biological clock [5,7,8]. In mammals, the TTFL mainly consists of two interconnected feedback loops that collectively determine and regulate the near-24-hour-rhythm, namely the core loop and the stabilizing loop (Fig. 1). In the core loop, the two transcription factors BMAL1 and CLOCK dimerize and translocate to the nucleus. This BMAL1:CLOCK complex initiates the transcription of repressor genes: two cryptochrome members (*CRY1* and *CRY2*) and three periods (*PER1, PER2* and *PER3*). The corresponding proteins repress BMAL1 and CLOCK, thereby stopping their own gene transcription. BMAL1 and CLOCK remain

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https://doi.org/10.1016/j.bbalip.2021.158961
Received 1 February 2021; Received in revised form 22 April 2021; Accepted 26 April 2021
Available online 30 April 2021

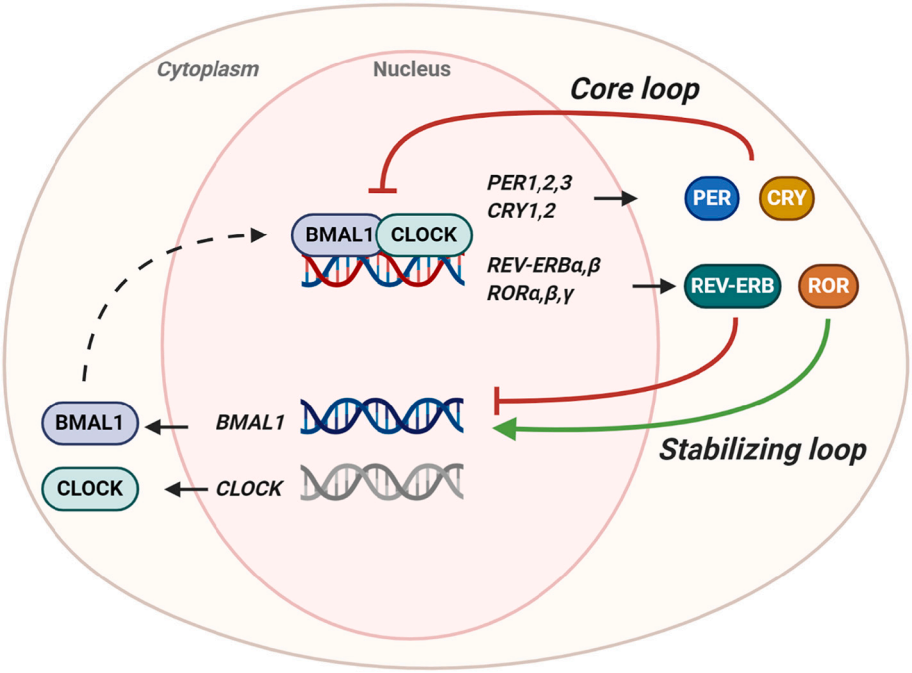

Fig. 1. The molecular clock. After dimerizing and translocating into the nucleus, the BMAL1:CLOCK complex initiates transcription of three periods (PER1, PER2 and PER3) and two cryptochrome members (CRY1 and CRY2). PER and CRY proteins in turn repress BMAL1 and CLOCK, thereby negatively regulating their own gene transcription. In addition, the BMAL1:CLOCK complex drives the expression of nuclear receptors (REV-ERBα and REV-ERBβ) and retinoid-related orphan receptors (RORα, RORβ and RORγ). REV-ERB proteins repress transcription of BMAL1, RORs promote the expression of BMAL1. The clock proteins in turn initiate transcription of tissue-specific target genes.

suppressed until CRY and PER are degraded, after which the cycle starts again. In the stabilizing loop, the BMAL1:CLOCK complex drives the expression of nuclear receptors (*REV-ERBα* and *REV-ERBβ*) and retinoid-related orphan receptors (*RORα*, *RORβ* and *RORγ*), of which the proteins control the expression of *BMAL1*. While REV-ERB proteins repress transcription of *BMAL1*, RORs promote the expression of *BMAL1*. In addition to the TTFL, post-translational modifications of core clock proteins determine the robustness of the circadian rhythm [9–12]. Together, the clock genes and clock proteins form the molecular clock, which cycles around in an approximate 24-hour pattern. The clock proteins can regulate transcription of tissue-specific target genes, resulting in endogenous circadian rhythms in many tissue-specific genes and proteins.

Although this molecular clock machinery is self-sustaining, vertebrates show a hierarchical composition in the time-keeping network. That is, they accommodate a clock-regulating center in the brain [6]. This so-called 'master clock' is located in the neurons of the SCN, a small structure within the anterior hypothalamus. The SCN receives photic information from the retina about the external light/dark cycle and communicates this information to distinct brain areas and peripheral tissues through synaptic and non-synaptic mechanisms, such as paracrine and endocrine signaling. Thereby, the SCN synchronizes peripheral circadian clocks to external environmental information [6]. In addition, the SCN also receives and integrates information about the internal environment to fine-tune its output. The importance of the SCN is illustrated by studies showing that SCN-ablated rodents do not exhibit circadian rhythmicity in endocrine aspects, such as the adrenal corticosterone rhythm, [13] and drinking behavior [14], suggesting that the SCN reigns over circadian physiology and behavior. In addition, neural grafts from the SCN region restore circadian rhythms in arrhythmic hamsters whose own SCN had been ablated. Moreover, experiments in which the SCN was transplanted from mutated short-period hamsters to long-period hamsters showed that the restored rhythms always adopt the period of the donor [15]. The importance of the SCN has been further demonstrated in humans. For instance, patients treated for nonfunctioning pituitary macroadenomas often suffer from a disturbed sleep-wake rhythm, probably due to dysfunction of the adjacent located SCN [16,17].

The photoperiod (*i.e.*, the amount of daylight perceived) serves as the primary zeitgeber for the SCN. However, to adapt to other environmental fluctuations, endogenous molecular clocks respond to several other zeitgebers that can foster chronobiological homeostasis by transiently extending or reducing intracellular oscillations. A rhythm that is synchronized with the day/night cycle by zeitgebers is formally called a diurnal rhythm and may or may not be a circadian rhythm. Next to the photoperiod, zeitgebers include temperature, physical activity, and eating/drinking patterns. The rhythm of feeding behavior, for example, acts as a powerful synchronizer for peripheral clocks in the liver, muscle, and adipose tissue, while leaving the SCN in most cases unaffected [18,19]. The result is that many organs exhibit a unique set of genes that are expressed in a circadian fashion, ranging from 3% of genes in the hypothalamus up to 16% in the liver. In mice, in total, 43% of all known genes exhibit a circadian profile anywhere in the body [20]. To date, there is no consensus on how the circadian clock exactly induces tissue-specific rhythmic gene expression, but there are suggestions that clock genes rely on tissue-specific transcription factors [21,22] as well as tissue-specific enhancer-enhancer interactions to facilitate DNA binding [23].

Together, the central and peripheral clocks have profound impact on biological processes. Notably, many aspects of metabolism are under stringent circadian control in order to maintain homeostatic balance during day and night and therefore promote survival [5,24]. Timing of zeitgeber information is thus crucial for optimal clock responses and therewith metabolic health, but is also challenged in our modern society. A significant proportion of the population is involved in shift work to fulfil the demands of our 24/7 economy, resulting in misalignment of rhythms in energy metabolism and the sleep-wake cycle. In addition, technological developments such as electrical lights, computers and smartphones have made it possible for humans to do virtually anything they want whenever they want. However, the extensive use of these devices in the evening or night often results in an extension of the photoperiod affecting sleep quality [25]. Exposure to dim light at night is already sufficient to shift behavioral and metabolic rhythms, induce weight gain, and disturb the rhythm in body temperature [26]. Another example is that late-night-snacking profoundly disrupts peripheral oscillators [27] and is associated with adverse metabolic and cardiovascular outcomes [28,29]. Knowledge on how the circadian clock is regulated in specific tissues and what the implications are on health

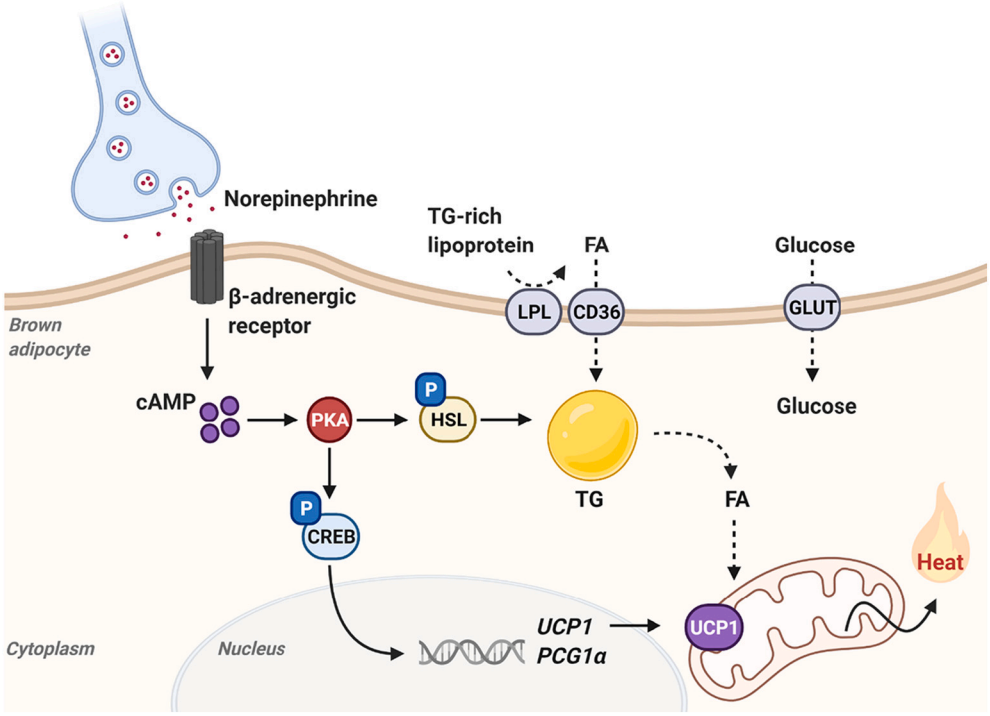

Fig. 2. Regulation of thermogenesis in brown adipocytes. Norepinephrine binds to the β-adrenergic receptor. As a consequence, cyclic adenosine monophosphate (cAMP) is produced intracellularly, which activates protein kinase A (PKA). PKA phosphorylates the cAMP response element-binding protein (CREB) to activate the thermogenic gene program. In addition, PKA phosphorylates hormone sensitive lipase (HSL) to lipolyze intracellularly stored triglycerides (TG). The released fatty acids can either activate UCP1 or be used as a substrate for oxidation. In addition, brown adipocytes can take up fatty acids, from TG-rich lipoproteins, and glucose from the bloodstream, both for use as substrate for oxidation.

outcomes is been recognized to be of major importance for the prevention and optimal treatment of disease. In the next sections we will specifically focus on the circadian mechanisms regulating the activity of BAT.

3. Brown adipose tissue

Adipose tissue functions as an endocrine organ and plays a central role in energy metabolism. Most adipocytes are classified as white adipocytes, residing in white adipose tissue (WAT) depots located at various locations throughout our body. These adipocytes are characterized by large unilocular lipid droplets that contain TG to provide fuel to other organs during fasting. In contrast, brown adipocytes within BAT and beige/brite adipocytes scattered throughout WAT are characterized by multiple small lipid droplets and abundant mitochondria that uniquely contain uncoupling protein 1 (UCP1). In the presence of UCP1, brown adipocytes can convert chemical energy into heat, a process referred to as non-shivering thermogenesis.

The most potent activator of thermogenesis in BAT is cold exposure. Cold leads to activation of the sympathetic nervous system (Fig. 2). In mice, norepinephrine (NE) activates BAT *via* the β3-adrenergic receptor [30]. The regulation of human BAT is less clear although recent data indicate a crucial involvement of β2-adrenergic receptor [31]. In both cases, cyclic adenosine monophosphate (cAMP) is produced intracellularly that activates protein kinase A (PKA). PKA phosphorylates the cAMP response element-binding protein (CREB) to activate the thermogenic gene program and furthermore phosphorylates hormone sensitive lipase (HSL) to liberate FA from intracellularly stored TG. The released FA can either allosterically activate UCP1 or be used as a substrate for oxidation. Oxidation of reducing equivalents, mainly nicotinamide adenine dinucleotide (NADH), using the terminal electron acceptor oxygen in the inner mitochondrial membrane, produces a proton motive force. In the presence of activated UCP1 within the inner mitochondrial membrane, protons run back along the gradient, resulting in heat production instead of ATP generation [30,32].

Besides its key role in thermoregulation, some studies suggest that BAT contributes to the preservation of the energy balance. This phenomenon is called 'diet-induced thermogenesis' and is most likely an adaptive response to overfeeding to prevent excessive levels of circulating glucose and lipids [33,34]. The fact that BAT takes up large amounts of glucose and TG-derived FA upon cold- or diet-induced thermogenesis, can be used to visualize and quantify metabolic BAT activity. For mice, we [35,36] and others [37] have used radiolabeled [^{14}C]deoxyglucose (^{14}C-DG) and glycerol tri[^{3}H]oleate (^{3}H-TO), to quantify glucose and TG-derived FA uptake, respectively. In humans, the presence of active BAT was originally identified by the use of [^{18}F]fluorodeoxyglucose (^{18}F-FDG) and detection by position emission tomography combined with X-ray computed tomography (PET-CT) [38–41]. Magnetic resonance imaging (MRI) has been used as an alternative technique to assess BAT volume and activity based on changes in TG content of the tissue [42], and infrared thermography as alternative technique to non-invasively monitor temperature of the neck and suprascapular area [43]. All these techniques have pros and cons, also depending on body composition and insulin sensitivity status of the subject. Most likely a combination of different techniques including future lipid-based tracers is needed to measure true metabolic BAT activity in humans.

The promotion of BAT function and the browning of WAT have emerged as promising therapeutic targets to increase energy expenditure and counteract weight gain. Human BAT volume and activity as determined by ^{18}F-FDG uptake after cold exposure inversely correlate with fat mass [40] Furthermore, repeated or prolonged cold exposure reduces fat mass in humans [44]. In addition, the presence of active BAT also inversely associates with diabetes incidence and fasting glucose levels [45,46], whereas cold exposure has been shown to improve glucose tolerance and insulin sensitivity [47]. A recent study analyzed 134,529 ^{18}F-FDG PET-CT scans in thermoneutral condition and showed that individuals with detectable BAT suffered significantly less from cardiovascular diseases than individuals without detectable BAT [48]. Moreover, in mice the uptake of TG-derived FA by activated BAT is accompanied by enhanced hepatic TG-rich lipoprotein (TRL) remnant clearance and promotion of reverse cholesterol transport through increased formation and functionality of high-density lipoproteins (HDL). The combined effect is reversal of dyslipidemia and attenuation

of atherosclerosis development [35,49,50]. Questions remain, however, to what extent classic BAT can be activated in humans and whether research should focus on browning of WAT. WAT browning is an intriguing concept given the large amount of WAT present in (obese) individuals. In addition, a clear distinction between classic brown and beige/brite fat likely does not exist in humans [38,51]. Nonetheless, irrespective of what the appearance of the thermogenic tissues, accumulating evidence suggests that thermogenic adipocytes in human adipose depots can be recruited and/or (trans)differentiated in response to specific stimuli [52].

Thus, BAT is a thermogenic organ with roles in temperature regulation and energy metabolism in mammals. Since outdoor temperatures and the need for energy fluctuate throughout the year and even on a daily basis it is not surprising that BAT displays a circannual and diurnal rhythm, on which we will focus in the following paragraphs.

4. Circannual and diurnal rhythms in BAT

4.1. Circannual rhythms in BAT

Preparations for the cold winter in small mammals presumably starts in fall with enhanced lipid accumulation in WAT and BAT [53]. This is followed by an increase in the thermogenic capacity through elevation in the number of mitochondria in BAT during winter [54]. Such adaptations of adipose tissue to the environment are likely critical for the survival of small mammals, nonetheless, also in humans the prevalence of metabolically active (*i.e.*, ^{18}F-FDG-positive) BAT is higher in winter as compared to the summer months [38,48]. Indeed, ten days of cold exposure recruits BAT in humans [55]. However, not only daytime outdoor temperature, but also day length of the day preceding ^{18}F-FDG PET-CT scanning are found to be predictive for the glucose uptake by BAT [56]. Interesting enough, it has even been reported that a stronger correlation exists between the presence of active BAT and photoperiod than with outdoor temperature [57]. Based on these data it is tempting to speculate that seasonal changes in photoperiod may be a way to prepare the body for upcoming changes in temperature. In line with this notion, experimental exposure to a short photoperiod of 8 h prevents weight gain in field voles [58] and stimulates BAT growth and thermogenic potential in hamsters [59,60]. Conversely, exposing mice to a prolonged photoperiod diminishes metabolic BAT activity, as evidenced by strongly reduced uptake of TG-derived FA and glucose by BAT, resulting in increased lipid deposition in WAT [61].

Changes in temperature and photoperiod may thus both be responsible for the initiation of seasonal adaptive changes in BAT morphology, and this also seems to have implications for cold-induced thermogenic activity, which is markedly increased in humans during wintertime [39]. Collectively, this suggests that when BAT is considered as a therapeutic target, seasonal changes in the basal state of BAT may be an important determinant of the treatment outcome.

4.2. Diurnal rhythms in BAT

In addition to seasonal changes, also diurnal rhythms are present in BAT. Murine BAT is characterized by circadian gene expression of clock proteins as well as of proteins involved in adipose function and thermogenesis [62]. Using RNA-sequencing, about 8% of all protein coding genes in BAT were found to fluctuate in a twenty-four hour cycle, twice as many as in WAT [20]. These rhythms in gene expression most likely translate to tissue function, as ^{18}F-FDG uptake by murine BAT also exhibits a rhythm within the course of the light-dark cycle, peaking 3 h before the start of the dark/active phase of mice [63]. In line with these data, it was recently reported that primary human brown adipocytes in synchronized cell cultures display a diurnal rhythm in insulin-stimulated glucose uptake [64]. This rhythm in glucose uptake is superimposed by a rhythm in the expression of glucose transporter protein 4 (*GLUT4*) and *UCP1*. Interestingly, these circadian expressions are also maintained in cultured human BAT explants, irrespective of the co-incubation with adrenergic or glucocorticoid antagonists, hinting to cell-autonomous mechanisms being involved.

In addition to glucose, the uptake of TG-derived FA by BAT displays a high-amplitude diurnal rhythm, at least in mice [65]. BAT shows the highest uptake of TG-derived FA at the onset of the active period, which coincides with high expression of lipoprotein lipase (LPL) and low expression of angiopoietin-like 4 (ANGPTL4), which is a potent LPL inhibitor. The diurnal rhythm in BAT activity determines the rate at which lipids can be cleared from the circulation, thereby regulating the daily rhythm in plasma lipid concentrations. In mice as well as humans, postprandial lipid excursions are nearly absent at waking which may at least partly be due to higher BAT activity at this time of day.

Collectively, these studies show that BAT is more active near the start of the active period in rodents and likely also in humans. Again, it is tempting to speculate about the reasons for this pattern. BAT activity likely relates to body temperature, which reaches its lowest point in the middle of the inactive phase [66]. At that point non-shivering thermogenesis might be required to increase body temperature until wakening. In the next section we will discuss how diurnal rhythms in BAT are generated.

5. Circadian mechanisms regulating BAT activity

5.1. Clock genes and BAT function

Many clock gene-mutant mouse strains show changes in BAT morphology and/or function, underscoring the importance of the circadian clock in BAT. Whole-body *Clock* mutant mice are hyperphagic and obese when fed a high fat diet. Additionally, they have many features of metabolic syndrome, including hyperlipidemia [67], and their BAT is characterized by excessive lipid accumulation and loss of its multilocular appearance. Global and adipose-specific *Bmal1* knockout typically potentiate diet-induced obesity [68]. Brown adipocytes in these animals are characterized by enlarged lipid droplets [69], but also by increased expression of thermogenic genes [69,70]. The net effect on thermogenesis is likely context dependent with one study reporting decreased cold-tolerance [70], while others reported no [69,71] or even improved cold-tolerance [72]. Mechanistically, BMAL1 suppresses brown adipogenesis *via* direct transcriptional control of key components of the transforming growth factor beta (TGF-β) pathway [72]. BMAL1 is also known to facilitate transcription of PER2, but PER2 actually stimulates BAT function through interaction with peroxisome proliferator-activated receptor alpha (PPARα) [73].

BAT function is improved in mice lacking REV-ERBα, related to the repressing activity of REV-ERBα on *Ucp1* gene expression. Consequently, UCP1 expression and cold-tolerance exhibit a diurnal rhythm, both of which are in antiphase with REV-ERBα [74]. More recently, it was shown that BAT-specific REV-ERBα deficiency was sufficient to elevate UCP1 levels [75]. REV-ERBα shares nuclear targets with RORα, with opposite regulation of *Bmal1* expression, while both REV-ERBα and RORα show repressive effects on *Ucp1* expression. Homozygous staggerer (sg/sg) mice, featuring a global RORα dysfunction, are lean and protected from diet-induced obesity explained by a remarkable increase in the thermogenic gene program including *Ucp1* expression in BAT and WAT [76,77].

5.2. Circadian sympathetic innervation of BAT

The sympathetic nervous system is the dominant driver of thermogenic activity in BAT. Interestingly, the autonomic nervous system itself follows a circadian pattern as well. SCN neurons connect with neurons regulating sympathetic activity in BAT of Siberian hamsters [78] and injection of glutamate into the SCN increases BAT thermogenesis in rats [79]. To what extent these connections translate into actual circadian release of NE in BAT is, to the best of our knowledge, not known.

Nevertheless, circadian disruption upon continuous light exposure was found to reduce sympathetic outflow towards BAT [61] and sympathetic denervation abolishes rhythmic TG-derived FA uptake by BAT [65], although this latter observation should be interpreted with caution as denervation by itself has quite a dramatic effect on BAT activity.

Interestingly, chronic cold exposure (4 °C) was reported to strongly increase the amplitude of circadian rhythm in genes controlling *de novo* lipogenesis in BAT of mice, including *Srebf1* and *Mlxipl*, encoding SREBP-1 and ChREBP respectively [75,80]. *De novo* lipogenesis in BAT is required for the adaptation to chronic cold exposure [75]. On the other hand, during thermoneutrality (30 °C), ChREBP-dependent lipogenesis was shown to cause lipid accumulation in inactive BAT [80]. Depletion of SREBP-1 hinders maintenance of body temperature specifically during the light phase when mice were housed at 4 °C [75]. The question remains, however, to what extent this is a direct effect of (circadian) sympathetic nervous system activity. In addition, it is not clear why *Srebp1* and *Mlxipl* expression is specifically stimulated in the dark phase. Possibly, nutrient availability during the dark phase, combined with *de novo* lipogenesis helps the mouse to prepare for the resting phase when BAT activity is needed to maintain body temperature.

Thus, circadian disruption negatively affects BAT function, possibly *via* the sympathetic nervous system, and sympathetic activity increases the amplitude of genes involved in BAT functioning. On the other hand, it seems that adrenergic input is dispensable for circadian BAT activity since mice lacking β-ARs (β-less mice) have low expression of *Ucp1* at room temperature, but the circadian rhythmicity of *Ucp1* and clock genes in BAT is maintained [81]. Similarly, housing mice at thermoneutrality, coinciding with minimal to no sympathetic outflow to BAT, leaves circadian rhythmic heat production intact [74]. The latter indicates that apart from circadian sympathetic outflow other factors must be involved in the generation of the diurnal rhythm in BAT.

5.3. Circadian release of hormones and BAT function

Many hormones exhibit circadian or diurnal rhythms and quite a few of them have been implicated in BAT function, either direct or indirect *via* modulating sympathetic outflow [82]. In this review, we will focus on two (types of) hormones that are well-known for their contribution to the circadian control of metabolism, namely glucocorticoids (GC) and melatonin.

5.3.1. Glucocorticoids

The release of GCs, mainly represented by corticosterone in mice and cortisol in humans, is controlled through the hypothalamus-pituitary-adrenal (HPA) axis and shows a robust rhythm peaking at the onset of the active phase. The promotor regions of the clock genes *PER1* and *PER2* [83], as well as those of many genes involved in BAT function [84], contain glucocorticoid receptor (GR)- and mineralocorticoid receptor (MR)-responsive elements. Clock proteins can physically interact with GR to regulate transcription in peripheral tissues, as CLOCK represses GR action [85], CRY trans-activates GRs [86], and the recently newly identified BMAL:CLOCK inhibitor CHRONO represses GR expression [87].

In BAT, GCs typically promote adipogenesis including uptake of TG-derived FA and glucose [88] but simultaneously repress the thermogenic gene program [89], resulting in increased nutrient storage. Surprisingly, exogenous adrenocorticotropic hormone (ACTH), which promotes GC release as part of the HPA axis, was found to directly promote BAT activity [90], although the question remains whether endogenous ACTH reaches levels that are sufficiently high to elicit this effect. A recent study also suggested species-specific differences in the regulation of UCP1 expression by GCs, with repression of transcriptional activity in brown adipocytes of mice and stimulation in human cells. Despite these data it is very unlikely that GCs will ever be used to promote BAT activity in humans, because long term treatment was found to suppress BAT activity in both mice and humans [91], possibly due to a strong overflow in nutrient influx and the necessity to store these. In addition, GCs are well-known to cause multiple negative metabolic side effects [92,93].

Adrenalectomy increases BAT activity in mice, while rhythmic activity is maintained [65], indicating that at least the peak at wakening is not required for circadian BAT activity. On the other hand, continuous high dose corticosterone treatment not only reduces BAT activity but also decreases the amplitude of circadian activity. However, in this type of experiments it is not possible to discriminate between effects caused by absence of rhythmicity or by changes in GC levels. We [94] and others [95] have therefore implanted low-dose corticosterone-containing pellets. These pellets increase circulating GC levels just above the threshold at which the negative feedback on the HPA axis kicks in shutting off endogenous GC production, resulting in flattened corticosterone levels without exceeding the normal levels. Flattening of corticosterone rhythms resulted in lipid accumulation in BAT, while TG-derived FA uptake was decreased, due to alterations in rhythmic LPL expression [94]. Interestingly, these effects were independent of GR expression by the brown adipocyte and rather explained by reduced sympathetic outflow at waking [94].

5.3.2. Melatonin

The SCN controls timing of melatonin production by the pineal gland and it thus directly transfers information about circadian cycles. Accordingly, melatonin shows a robust circadian rhythm with peak levels during night time. On exposure to (day)light melatonin levels rapidly drop to almost undetectable levels [96]. In addition, melatonin secretion varies over the year with prolonged secretion during long dark winter nights (*i.e.* short photoperiods) as compared to short summer nights (*i.e.* long photoperiods) [97]. Siberian hamsters, transferred from a long photoperiod to a short photoperiod, exhibit a naturally-occurring reversal in obesity. This is mediated by the duration of melatonin secretion and subsequent activation of the sympathetic nervous system [98]. Consequently, pinealectomized rats show reduced UCP1 levels [99] and an impaired BAT response to either HFD or cold [100], phenotypes that could be reversed by exogenous melatonin administration.

Melatonin may promote BAT activity through different ways. Besides stimulating sympathetic activity, it also inhibits ACTH-mediated GC secretion [101]. In addition, melatonin receptors are not only expressed in the brain but also in metabolic organs and cells including adipocytes [102]. An early study already showed that melatonin can promote BAT growth in hamsters [103]. A more recent study showed comparable effects in patients with melatonin deficiency, who show increased BAT volume and activity as measured with PET-MRI after 3-months oral melatonin administration [104]. Of note, most inbred mouse strains, including the widely used C57BL/6 mice, are naturally deficient for melatonin because serotonin is not acetylated by serotonin *N*-acetyltransferase (arylalkylamine *N*-acetyltransferase, AANAT) to generate melatonin [105]. Nevertheless, as we have repeatedly demonstrated that BAT activity has a circadian rhythm in C57BL/6 J mice [61,65], we may conclude that although melatonin is able to modulate circadian BAT activity, it is dispensable.

6. Relevance of BAT rhythms: when are interventions useful and most efficient?

It should be clear by now that circadian disruptions affect BAT activity and that BAT is characterized by pronounced circannual and diurnal rhythms with respect to thermogenic capacity and activity. This raises at least three questions: 1) What factors attenuate or disturb the circadian rhythm in BAT? 2) Can we prevent metabolic problems associated with attenuated or disturbed circadian rhythms by stimulating BAT? 3) Should we consider circadian cycles in BAT during therapy, otherwise known as chronotherapy? For example, when pharmacologically stimulating BAT, we could question whether the time of the day is a crucial determinant. Below we will discuss these questions over a few examples.

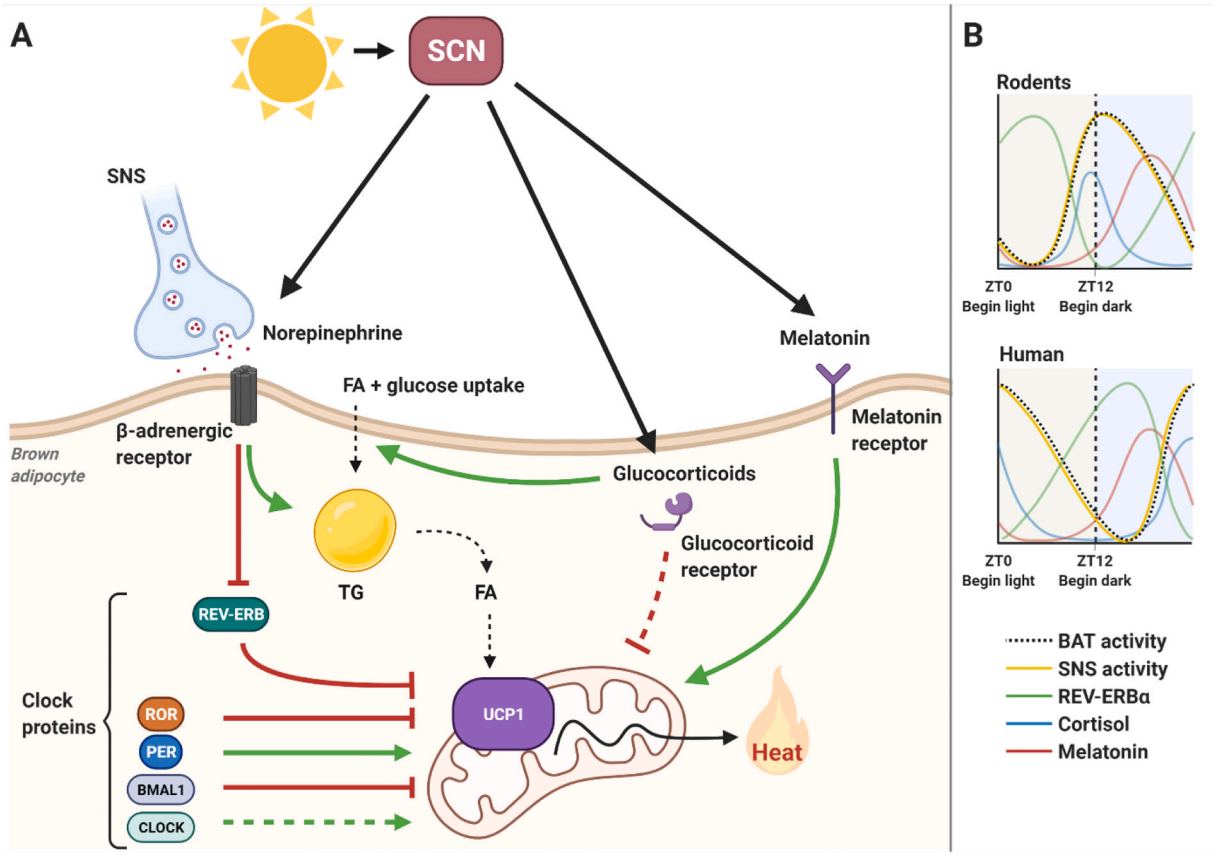

Fig. 3. Proposed model of how several circadian factors influence diurnal rhythms in BAT. A) The SCN contains a direct innervation pathway to BAT *via* the sympathetic nervous system (SNS), releasing norepinephrine locally that binds to β-adrenergic receptors. In addition, rhythmic secretion of glucocorticoids stimulates fatty acids and glucose uptake by the brown adipocyte but conversely inhibits thermogenesis. The SCN also promotes synthesis of melatonin by the pineal gland, which signals through melatonin receptors and enhances thermogenesis in brown adipocytes. Clock genes are also involved. Sympathetic innervation represses REV-ERB in brown adipocytes, which inhibits thermogenesis. ROR and BMAL1 suppress thermogenesis, while PER and CLOCK probably stimulate thermogenesis. B) The circadian rhythm in BAT thermogenesis coincides with the rhythm of SNS activity and is in anti-phase with the activity of REV-ERBα. Cortisol peaks at the beginning of the active phase, while melatonin peaks during the inactive phase.

6.1. Aging

During aging, there is an increased chance of developing pathologies, including cardiovascular diseases, type 2 diabetes mellitus, and cancer. Aging is associated with deterioration of the biological clock, including a reduced amplitude in the rhythms of hormones, body temperature, and processes that regulate the sleep-wake cycle.

During aging in both humans and mice BAT mass and activity are reduced, and browning of white fat is decreased [38,106]. Transcriptome analysis covering a 24-hour period of BAT in aged mice revealed 1021 differentially expressed genes including elevated amplitude of *Cry1* and *Rev-erbα* expression [107]. Comparison of the rhythmic lipidome of BAT in young and old mice shows that lipids tend to accumulate with age, yet the number of oscillating lipids reduces and the time of the day at which lipids reach their peak shifts [108]. Mechanistically there are many possibilities by which age can affect BAT function, including reduced sympathetic outflow and dysfunction of mitochondria, and therefore it will probably be challenging to disentangle the various contributors from that of altered circadian rhythmicity. Nevertheless, it is tempting to speculate that restoring rhythmic BAT activity may promote healthy aging. Accordingly, several studies have described improved BAT activity in long-lived animals [109–112] and that transplantation of BAT from young to old mice reverses the aging phenotype [112].

6.2. Timing of food intake

The time of the day is an important determinant for the fate of consumed nutrients. Interesting enough, diet-induced thermogenesis (*i.e.* the postprandial increase in energy expenditure) is known to exhibit a day-night rhythm, with the highest response in the early morning [113–115]. This may help explain why meal timing affects body weight regulation and why shift work is a risk factor for obesity [116–118]. The role of BAT in diet-induced thermogenesis is somewhat controversial, however, the response is higher in persons who have detectable ^{18}F-FDG uptake by BAT compared to those without [119]. Following a meal, ^{18}F-FDG uptake [120], oxygen consumption, and blood flow in human BAT acutely increase [121]. In mice, the time of the day also determines to what extent TG-derived FA are taken up by BAT, which is reflected in postprandial TG levels [65].

Regardless of the contribution of BAT to diet-induced thermogenesis, the diurnal rhythm in diet-induced thermogenesis provides the scientific basis for the concept of time-restricted feeding. Mice fed a high fat diet only during the dark phase show reduced weight gain compared to *ad libitum* fed mice, an effect accompanied by increased and rhythmic UCP1 expression and whole body energy expenditure [122–124]. In human studies without instructions on caloric intake, time-restricted eating (*i.e.* only eating during an either self- or by the researchers selected 6–9 h window) reduces body weight and other risk factors of cardiovascular disease and type 2 diabetes in obese individuals with/without metabolic syndrome [125–127]. Independent of weight loss, early time-restricted food intake with a feeding period between 9 a.m. and 3 p.m. was also

found to improve insulin sensitivity in men with prediabetes [128]. Surprisingly, a very recent randomized controlled trial did not find any metabolic benefit of late time-restricted eating between 12 p.m. and 8 p.m. [129], indicating that more research is needed to identify who may or may not benefit from such intervention and what time-window would be, if at all, effective.

6.3. Timing of cold exposure and therapeutic interventions

Chronotherapy is a relatively new field of research that is based on the premise that administering medications or other interventions at specific times of the circadian cycle will minimize side effects and/or maximize therapeutic effects. As mentioned before, cold exposure is the dominant driver of BAT activity and mice tolerate cold considerably better at the end of the dark phase than at the end of the light phase [74,130]. To the best of our knowledge, no study has addressed this issue in humans thus far. Nonetheless, night time cold acclimation was demonstrated to result in a pronounced increase in BAT abundance accompanied by improved insulin sensitivity [131]. Similarly, the question whether the effects of pharmacological interventions targeting BAT are time-dependent has not been addressed in humans nor in mice. With respect to circannual rhythms, cold-induced thermogenic activity is markedly increased in humans during wintertime [39,56,57], indicating that the basal state is important.

Very interestingly, over the last years various small molecules have been developed to interfere with key clock proteins. Two agonistic compounds, SR9011 and SR9009, targeting REV-ERBα were found to improve energy homeostasis in diet-induced obese mice [132]. Treatment with SR1555, an inverse agonist of RORγ, induces UCP1 expression in BAT of diet-induced obese mice [133]. To what extent these molecules promote thermogenic activity in BAT and can be applied to reverse circadian disturbances in humans is yet to be discovered.

7. Conclusions and future directions

The discovery of metabolically active BAT in human adults a decade ago led to interest in BAT as a therapeutic target to combat metabolic and cardiovascular diseases. While animal studies show great promise, it is unclear as yet to what extent BAT or browned WAT contributes to energy expenditure in humans. Nonetheless, UCP1 positive adipocytes are present in human adults and are expected to have, even in small amounts, an impact on energy homeostasis in the long term. Accumulating evidence suggests that rodent and human BAT possesses clear circannual and diurnal rhythms, regulated through processes summarized in Fig. 3. These rhythms should be considered when measuring BAT function and possibly when therapeutically targeting BAT, although more research is needed to confirm this. On the other hand, disruption of circadian rhythms through genetic clock deficiency in mice, prolonged light exposure, (mimicking) shift work, and other ways have been associated with metabolic and cardiovascular problems, often accompanied by deteriorated BAT function. The exact contribution of BAT dysfunction to these phenotypes is not known and probably difficult to address, but unravelling the affected mechanistic pathways may lead to the discovery of novel therapeutic targets to combat cardiometabolic diseases.

Funding

This work was supported by the Netherlands CardioVascular Research Initiative of the Dutch Heart Foundation, Dutch Federation of University Medical Centers, the Netherlands Organization for Health Research and Development and the Royal Netherlands Academy of Sciences [CVON2017-20 GENIUS-II] to PCNR (Patrick C.N. Rensen). MRB (Mariëtte R. Boon) is supported by the Dutch Diabetes Research Foundation Fellowship [grant number 2015.81.1808] and SK (Sander Kooijman) by the Dutch Heart Foundation (2017T016).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Illustrations were created with BioRender.com.

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