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Review

Circadian Metabolism: From Mechanisms to Metabolomics and Medicine

Steven A. Brown[1,*]

The circadian clock directs nearly all aspects of diurnal physiology, including metabolism. Current research identifies several major axes by which it exerts these effects, including systemic signals as well as direct control of cellular processes by local clocks. This redundant network can transmit metabolic and timing information bidirectionally for optimal synchrony of metabolic processes. Recent advances in cellular profiling and metabolomics technologies have yielded unprecedented insights into the mechanisms behind this control. They have also helped to illuminate individual variation in these mechanisms that could prove important in personalized therapy for metabolic disease. Finally, these technologies have provided platforms with which to screen for the first potential drugs affecting clock-modulated metabolic function.

Trends

Bidirectional molecular relationships link the circadian clock to energy homeostasis.

These links occur at both cellular and systemic levels.

Metabolomics, transcriptomics, and cellular assays have illuminated mechanisms and interindividual differences in this control.

Cellular circadian assays have provided screening platforms for clock-specific drugs that could be useful for metabolic disorders.

Circadian Metabolism: An Overview

Nearly all aspects of metabolism vary with time of day, at both cellular and systemic levels. These regular daily oscillations persist even in constant environmental conditions, making them 'circadian'. Food intake is circadian, digestion and detoxication are circadian, and cycles of breakdown and storage of fats and sugars are circadian. Even within cells, individual metabolic pathways are circadian. The first reports of circadian transcriptomics suggested that at least 10% of all transcripts are regulated in circadian fashion in most tissues [1,2], and for circadian metabolomics this fraction approaches 20% [3–5] (Box 1). Importantly, many of these rhythms are not merely a consequence of rhythmic food intake and behavior but persist even in constant conditions. Others appear 'driven' by feeding and fasting cycles [6] or by sleep and wake [7,8].

Metabolic Dysfunction from Clock Disruption

Since the publication of the observation that mice deficient for the circadian gene *CLOCK* have metabolic disorder [9], an explosion of research has occurred into the complex relationship between circadian dysfunction, obesity, diabetes, and **metabolic syndrome** (see Glossary). Numerous excellent and detailed reviews exist on this subject [10–12] and we introduce circadian clock mechanisms in Box 2. In brief, it is clear that model organisms with defects engineered into circadian clocks show many features of metabolic syndrome. These include obesity, diabetes, steatosis, cardiomyopathy, and atherosclerosis, not only in mammalian models [13] but also recently in flies [14,15].

These abnormalities emerge from various tissue-specific defects. For example, loss of circadian clock function in liver resulted in hypoglycemia during the fasting phase, implying a role for the circadian clock in buffering circulating glucose [16]. Loss of pancreatic clocks caused glucose intolerance [17,18]. Muscle-specific clock ablation resulted in increased oxidative fibers and muscle fibrosis [19] and is likely to be necessary for proper substrate utilization [20]. Arterial transplantation from clock-deficient to wild-type mice resulted in early atherosclerosis in transplanted pieces [21].

[1]Chronobiology and Sleep Research Group, Institute of Pharmacology and Toxicology, University of Zürich, 190 Winterthurerstrasse, 8057 Zürich, Switzerland

*Correspondence: Steven.brown@pharma.uzh.ch (S.A. Brown).

Box 1. The Power of Metabolomics

Metabolomics has furnished significant insights into the complexity of metabolic dysfunction. By profiling how sugars, FAs, simple carbohydrates, and hormones vary both in solid tissues and in circulation, it has been possible to characterize in great detail broad-spectrum metabolic disorders. Along with these observations, knowledge of human genetic variation has permitted the identification of novel factors leading to susceptibility to these disorders [122], and transcriptomics has provided glimpses into their underlying causes. The increased throughput and reduced cost of RNA profiling and mass spectrometry have recently made it possible to conduct metabolomics and transcriptomics studies using samples collected at many different times of day, in different matrices. However, the application of human circadian profiling in this fashion is a very young science and its clinical potential remains mostly unrealized.

Box 2. An Introduction to Basic Clock Mechanisms

Mammalian circadian physiology relies on a partly centralized and redundant network of circadian clocks throughout the body. A 'master clock' resides in the SCN of the hypothalamus, comprising 20,000 neurons and associated glia, each of which has a cell-autonomous circadian clock within. These independent clocks are coupled together via neuropeptidergic signaling, gap junctions, and standard synaptic connections, resulting in a clock network that is precise, robust, and flexible to light-entrained seasonal changes [123].

Peripheral circadian clocks of similar molecular mechanism exist in nearly all cells of the body and are kept synchronized via timing cues from the SCN. These timing cues include direct nervous signals from the autonomic nervous system (ANS), hormones, and indirect behavior-related signals derived from the timing of food and the daily fluctuation of body temperature. As a result, global circadian physiology in any given tissue is likely to be driven by a mixture of local and systemic signals. Under normal circumstances, direct signals from the SCN are in concordance with indirect behavior-driven signals like food timing. However, under duress these signals can become uncoupled; for example, repeated abnormal meal timing can resynchronize peripheral oscillators independently of SCN-driven timing signals [124].

At the cellular level, the molecular mechanism of the circadian clock in any cell is thought to rely primarily on coupled feedback loops of the transcription and translation of dedicated clock genes and proteins [125]. In one loop, the circadian transcriptional activators CLOCK and BMAL1 bind to *cis*-acting E-box elements to drive transcription of the repressors *Cry1/2*, *Per1/2/3*, and *Rev-Erbα/β*. Subsequently, CRY and PER proteins multimerize, return to the nucleus, and repress their own transcription. In a second linked loop, the activators RORα/β/γ compete with the REV-ERBα/β repressors at *cis*-acting RRE elements to drive circadian transcription of *Bmal1*. The proper function and timing of this network of transcription factors is governed by a wealth of post-translational modifications controlling their stability and/or targeting their degradation [126], as well as RNA-binding proteins and chromatin-modifying factors aiding in their transcriptional activities [127]. In addition, another redox-related circadian oscillation based entirely on post-translational mechanisms may also exist in most cells, completely independent of the 'canonical' transcription/translation-based clock circuitry [128,129].

Glossary

Breath metabolomics: sampling of metabolites from deep lung alveoli via real-time analysis of breath.
Caloric restriction: reduction of the total number of calories consumed during the day.
Chronopharmacology: the timing of medications for optimal efficacy and minimal side effects.
Lipidomics: metabolomics-based determination of lipid species.
Melatonin: circadian hormone released by the pineal gland with soporific and other effects.
Metabolic syndrome: according to the International Diabetes Federation, the cluster of physiological risk factors, like high blood pressure, obesity, and high blood sugar and cholesterol, that increases the risk of heart disease, stroke, and diabetes.
Time-restricted feeding: scheduled mealtimes that limit the intake of food to a particular period within the 24-h day without reducing its amount.

The mechanisms behind these phenotypes vary considerably. Generally, however, they can be classed into two categories: phenotypes resulting from circadian regulation of organ-specific functions necessary for metabolic homeostasis at the level of the whole organism; and circadian control of basic metabolic pathways at a cellular level. We consider these causes in further detail separately below.

Axes of Metabolic–Circadian Interaction: The Whole Body

Considering first the entire body, these signals are likely to represent a complex interplay between: (i) glucocorticoid hormones, which govern conversion of sugar, fat, and proteins into glucose; (ii) insulin, promoting the absorption of glucose from the blood and the storage of fat; and (iii) appetite hormones like leptin and ghrelin, governing food intake. At a macroscopic level, it has been postulated that the circadian clock contributes to metabolic homeostasis by acting as a type of rheostat [12], orchestrating shifts in metabolic patterns to accompany changes in activity and food consumption. The proper function of this rheostat depends at least in part on local circadian clocks to direct circadian control of individual metabolic hormones or the cellular response to them. For example, pancreatic beta-cell-specific clock function is necessary for the rhythmic secretion of insulin [17,18] and multiple aspects of metabolic homeostasis are dependent on circadian function of the adrenal gland, which secretes glucocorticoid hormones [22]. Complementing this control, tissue-specific action of glucocorticoids is dependent on local cooperation with cryptochrome proteins from the circadian clock [23].

Less well studied than liver and pancreas in a circadian context, adipocytes both secrete adipokine hormones and act as a rheostat for triglycerides in the bloodstream. Both functions are compromised in adipocyte-specific clock-deficient mice [24,25]. It has been suggested that multiple different aspects of adipocyte function in various types of adipose tissue might depend on circadian clocks [26]. Similarly, muscle secretes myokine hormones important for glucose homeostasis and here, too, circadian clocks may be implicated in their proper activity [27].

In reverse, the timing of food intake can directly shift circadian phase in peripheral tissues such as liver, lung, pancreas, kidney, and heart [28,29]. Recently, it has been suggested that this phase shift occurs via complex signaling cascades centered around changes in free fatty acids (FFAs) and glucose, which in turn affect PPARα and glucagon receptor-mediated signaling in the periphery [30]. In this context, glucocorticoid hormones act as a circadian 'brake' on feeding-induced signals, opposing and slowing feeding-induced peripheral clock re-entrainment [31]. The central clock in the suprachiasmatic nucleus (SCN) is thought to escape both of these controls because it lacks the relevant receptors [32,33].

An additional but mostly unexplored aspect of the circadian metabolic rheostat is supraspinal. Beyond the well-characterized effects of hormones like glucocorticoids and insulin on liver, fat, and muscle, an important feedback also occurs in brain: deletion of hypothalamic glucocorticoid receptors results in obesity through hypothalamic–pituitary–adrenal (HPA) axis dysregulation [34]. In reverse, circadian anticipation of food was recently shown to depend on liver-derived ketone bodies [35]. Thus, peripheral lipid storage and appetite are connected in a carefully regulated feedback loop and it is likely that circadian input to hypothalamic nuclei plays a key role.

Axes of Metabolic–Circadian Interaction: Cellular

A second important way in which the circadian clock controls metabolic homeostasis is at the cellular level, via direct influence on pathways of energy storage and energy utilization. These diverse interactions are summarized in Figure 1 (Key Figure).

The NAD^+–NADH Axis

One major axis occurs via levels of redox cofactors like NAD^+/NADH. The circadian clock controls the NAD salvage pathway via the enzyme NAMPT, catalyzing a key step in the synthesis of NAD [36]. This regulation, as well as mitochondrial activity – itself controlled by the circadian clock [37,38] – governs the NAD^+:NADH ratio. A specific family of proteins, the sirtuins, work as NAD^+-dependent deacetylases. SIRT1 regulates directly the expression of clock and clock-controlled genes (Figure 1A) via deacetylation of clock proteins and histones [39,40], creating a feedback loop between redox homeostasis and clock function. SIRT1 has also been proposed to act as a feedback signal of systemic nutrient levels via neurons of the ventromedial hypothalamus [41].

In addition to SIRT1, there exist several other sirtuin isoforms playing roles in various tissues and organelles. For example, SIRT6 cooperates with the metabolic transcription factor sterol response element-binding protein (SREBP1) (Figure 1E) to control circadian FA metabolism, interacting with a gene set distinct from that of its homolog SIRT1 [42]. Similarly, regulation of mitochondrial SIRT3 drives diurnal rhythms of acetylation of mitochondrial proteins to regulate oxidative phosphorylation [38]. The importance of circadian mitochondrial regulation has now been demonstrated in both liver and heart, where loss of BMAL1 results in reduced respiratory output, increased oxidative damage, and morphological changes [37,43].

The AMP-Dependent Protein Kinase (AMPK) Axis

Another complementary pathway has been characterized regulating ATP production. ATP levels are circadian in multiple tissues [44], probably corresponding to circadian regulation of

Key Figure

Connections Between Circadian Clocks and Metabolism.

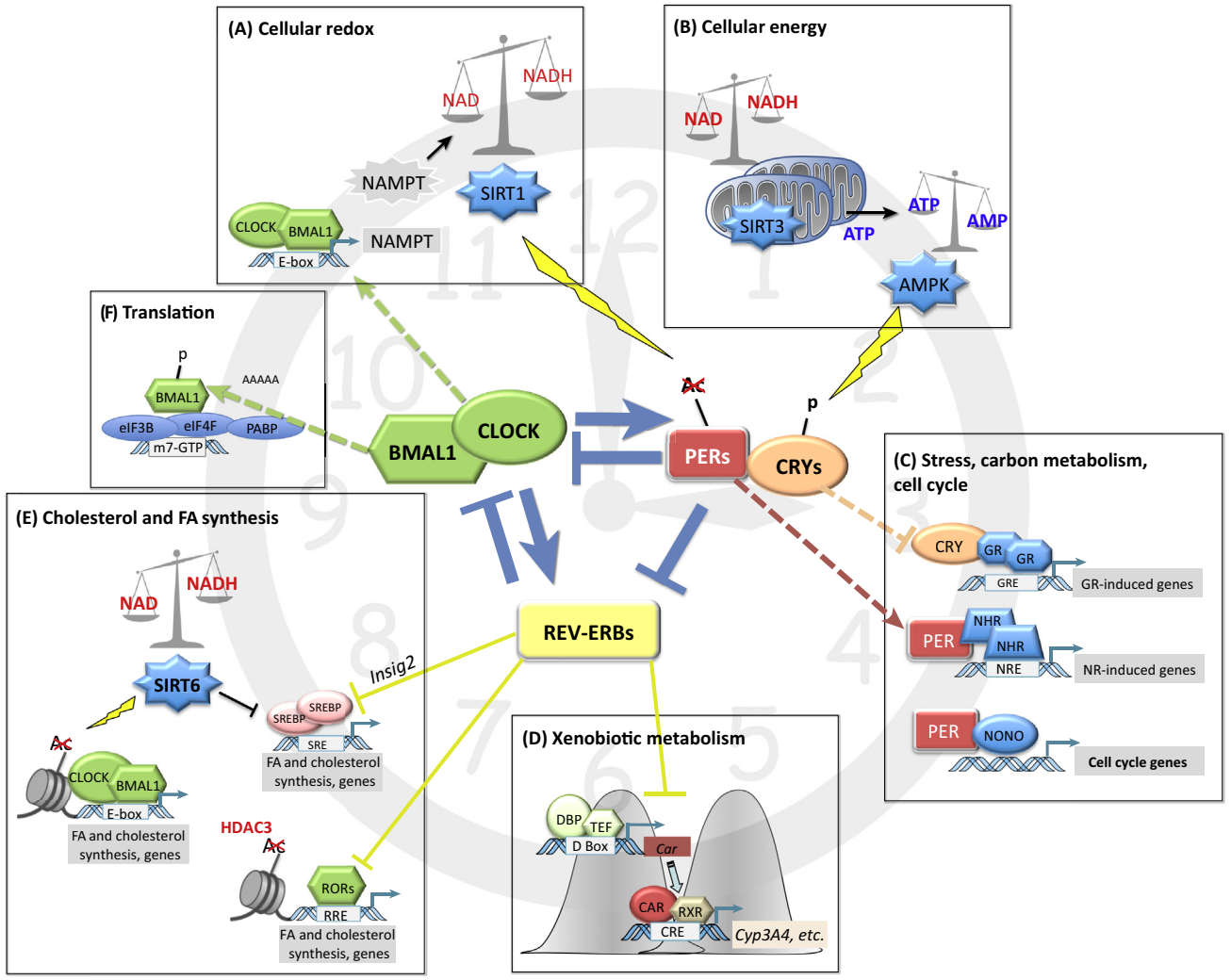

Figure 1. Core circadian clock factors like the PERs, CRYs, and REV-ERBs described in Box 1 (center) interact bidirectionally in diverse ways with the cellular metabolic machinery. (A,B) Circadian control of NAD production creates a redox-dependent feedback loop in which mitochondrial function is regulated. In turn, both NAD abundance and ATP availability post-translationally regulate circadian clock proteins. (C) Furthermore, clock proteins cooperate with diverse partners to act via distinct mechanisms at promoters of genes involved in stress, carbon metabolism, and the cell cycle. (D) They also stimulate a circadian transcription factor cascade to regulate xenobiotic metabolism and (E) via both direct and chromatin-mediated interactions control fatty acid (FA) and cholesterol synthesis. (F) Finally, BMAL1 binds to the mRNA cap to regulate translation.

mitochondrial function [38]. The balance of ATP and AMP in turn regulates AMPK, which phosphorylates the CRY proteins (Figure 1B) to regulate their degradation by the ubiquitin–proteasome complex [45]. It also phosphorylates casein kinase 1ε to influence PER protein stability [46]. Since AMPK regulates NAMPT expression, it is able to communicate with the NAD–sirtuin axis described previously [47].

Other Potential Metabolic Axes

It is likely that many more ties exist between redox homeostasis, metabolism, mitochondrial function, and the circadian clock. For example, tight coupling exists between the circadian clock and the cell cycle (Figure 1C) [48,49] and the cell cycle regulates mitochondrial function at both functional and morphological levels [50]. Therefore, we propose that circadian interactions with the cell cycle could also influence cellular metabolism. Other potential interactions might occur via post-transcriptional circadian oscillations in cellular redox couples and via circadian control of alternative carbon metabolism. The latter possibilities have been reviewed recently in this journal [11]. Finally, the clock protein BMAL1 directly modulates translation in a mammalian target of rapamycin (mTOR)-regulated fashion [51] (Figure 1F).

Control via Chromatin Modifications and Transcriptional Cascades

To achieve widespread and coordinated control of cellular metabolic pathways, a significant portion of circadian control is exerted via regulation of transcription. This can involve downstream cascades of transcription factors; for example, the PAR bZIP transcription factors DBP, TEF, and HLF play an important role in regulating xenobiotic metabolism via control of the nuclear constitutive androstane receptor (CAR) (Figure 1D) [52]. Similarly, REV-ERBα regulates SREBP activity via circadian transcription of *Insig2* [53] to regulate cholesterol and lipid metabolism (Figure 1E).

Like many transcription factors, clock proteins also act in conjunction with chromatin-modifying factors to achieve widespread cyclical activation and repression. These include histone acetylases and deacetylases, methyltransferases and demethylases, nucleosome remodeling complexes, and cofactors binding to long noncoding RNAs [54]. Some of these general factors appear to have functions specific to circadian metabolism. For example, the histone deacetylase HDAC3 cooperates with the clock REV-ERBα protein prominently at many genes involved in lipid metabolism and deletion of either can result in hepatic steatosis [55,56]. Another class is the sirtuins, discussed above.

Clock proteins also directly modulate the actions of nuclear hormones to regulate metabolism. For example, we have already alluded to glucocorticoid hormones, which regulate the conversion of complex energy substrates to glucose by driving transcription via *cis*-acting GRE elements. Interestingly, this activity is broadly opposed by cryptochrome clock proteins at the same promoters [23] (Figure 1F), and CRY protein levels are metabolically regulated by AMPK [45]. A similar dialog occurs between PER proteins and other nuclear hormone receptors. For example, PER2 interacts directly with PPARα to modify its activity at numerous target genes that play key roles in metabolism (e.g., *G6PC* encoding glucose-6-phosphatase [57] as well as directly competing with the activities of PPARγ to regulate lipid metabolism [58]). Moreover, PER2 expression is driven not only in circadian fashion by local clocks but also systemically via unknown circadian signaling cascades [59]. Another systemically driven circadian transcription factor is serum response factor (SRF), which ultimately activates RHO–ROCK signaling and the SRF–MRTF pathways [60]. This is likely to be one pathway by which systemic circadian signals can control cell division and therefore possibly cellular mitochondrial function.

From Broken Clocks to Human Disease

From the research described above, it is clear that destruction of circadian clocks – cellularly, tissue specifically, or globally – severely disrupts normal metabolism in animal models. In humans, while genetic disruption of circadian clocks remains unknown, several clock gene polymorphisms have been associated with altered metabolic parameters. For example, polymorphisms in *Cry2* have been associated with fasting glycemia and hepatic lipid content in large

Box 3. Time-Restricted Feeding

Time-restricted feeding has shown great promise in animal models to ameliorate or reverse aspects of metabolic syndrome and has been reviewed recently in this journal [13]. For example, it prevented metabolic disease in mice fed a high-fat diet [130] and completely reversed age-related cardiac dysfunction in flies [15]. So far, however, the impact of time-restricted feeding on humans remains unclear: although some meal-manipulation studies have shown promise, a full study testing time-restricted feeding as a treatment for obese individuals remains to be conducted. Time-restricted feeding should not be confused with **caloric restriction**, which has achieved recent media attention for potential antiaging benefits [131].

cohorts [61,62]. Numerous other associations with various aspects of metabolic syndrome have been found for polymorphisms in *Clock* and *Bmal1* [63,64], *Rev-Erbα* [65], and *Per2* [66].

Importantly, the relationship between clock function and metabolism is bidirectional. Thus, current evidence suggests: (i) that environmental disruption of the circadian cycle can lead to metabolic disease, while metabolic disease *per se* is associated with disrupted circadian rhythms; and (ii) that reinforcement of the circadian cycle can lead to measurable metabolic benefits. For example, disturbance of the circadian cycle via shift work is strongly associated with metabolic disease [67] and this association has been verified under laboratory settings in humans [68,69] and rodents [70]. In reverse, animal adiposity reduces the circadian amplitude of both gene expression and behavior [71]. Aging is another parameter that generally impairs both circadian function and metabolism [72]. Recently, reinforcing circadian metabolic function via **time-restricted feeding** has shown promise for mitigating some of this dysfunction, both from circadian disruption and from ageing (Box 3). Supporting these interrelationships, human longevity has been associated with both regular sleep and favorable lipid profiles [73].

Human Cellular Profiling and Metabolomics within the Circadian Field

Confronted with the bidirectional connection between metabolic regulation and circadian clock function, it is a clear health priority to explore the cellular factors that tie circadian clocks to metabolic syndrome in humans and to leverage these connections for diagnostic and potentially even therapeutic purposes. From humans, there are a limited number of tissues and matrices that are readily available: cells from solid tissues can be cultured to study their circadian properties *ex vivo* (e.g., period, phase, amplitude) or more readily available cells and matrices like blood or saliva can be serially sampled to examine circadian variation of substances therein. Both of these manipulations have informed us significantly about human physiology.

Lessons from Cell Cultures

The mechanistic study of circadian clock function in cell lines is well established. However, it is also possible to use primary cell cultures to study differences among clocks in groups of individuals: circadian clock properties in primary human fibroblasts can reflect the behavior of the individuals from which they were taken, whether assayed by questionnaire or by laboratory measurement [74,75]. Such methods have since been exploited to look at various circadian differences caused by age and disease [76,77]. The same types of studies could be conducted in other metabolically relevant human tissues: circadian clock function has been demonstrated in *ex vivo* cultures of human adipocytes [78] and myotubes [27] and in pancreatic alpha and beta cells [79], to name a few. Tissue-specific insulin sensitivity is preserved across various tissues *ex vivo*, as well as the ability of insulin to shift the circadian oscillator [80]. Similarly, disruption of circadian clocks in human islets results in defects in insulin secretion paralleling those observed in genetically modified mice [79]. Such investigations are likely to be only the beginning of numerous possible studies probing clock function in cultured human cells and tissues.

Circadian gene expression levels in humans can also be examined by serial sampling: suction blister epithelium [81], hair root follicles [82], adipose tissue [83], and buccal mucosa [84] have

each been used successfully; for example, to examine circadian perturbations in Smith–Magenis syndrome [85]. However, in solid tissues such experiments are rare. Using blood, serial measurements of circadian gene expression are more easily conducted but are hampered by lower circadian amplitude [86], possibly related to the heterogeneity of the cell types therein. Nevertheless, serial sampling of blood has proved a useful tool. PCR analyses of leucocyte clock gene expression in obese and diabetic individuals have reinforced the notion that obesity and diabetes may be linked to alterations in clock function [87,88]. Recently, human blood transcriptomics has been used to demonstrate vividly the effects of insufficient or mistimed sleep on circadian gene expression in this tissue: up to sixfold fewer circadian transcripts were observed [7].

Lessons from Metabolomics

With the advent of high-performance mass spectrometry, metabolites from these same matrices can be obtained. As expected, major metabolic pathways are under circadian control, whether one examines metabolites in bacterial cells [89], in mouse tissues like liver and muscle [4,90,91], or in human matrices like saliva and blood [3,92]. These include FA synthesis and degradation, amino acid biogenesis, energy- and redox-regulating substances, and carbohydrate and sugar metabolism. Integrated bioinformatics platforms have been made freely available to compare transcriptomics and metabolomics data from various sources [4,93]. Recent circadian studies using secondary electrospray ionization mass spectrometry (SESI-MS) have even succeeded in detecting over 1000 metabolites in a single human breath [94,95].

Beyond confirmation of circadian control of metabolic pathways, these studies have a further strong conclusion: both the sleep–wake cycle and feeding patterns may have strong effects on circadian metabolic oscillations. Circadian metabolism *per se* does not depend on diurnal patterns of sleep and food consumption: humans acutely deprived of sleep and fed hourly isocaloric meals showed robust circadian rhythmicity of metabolites in both blood serum and saliva [3]. However, chronic sleep restriction results in significant erosion of circadian transcriptional and metabolomics patterns [7,8] and circadian-fed versus fasted mice also show strong transcriptomic differences in liver [6].

Metabolome analyses will have multiple applications for human medicine. It is already clear that such techniques can directly detect drug pharmacokinetics in human breath [96]. Instead of serially sampling the hormone **melatonin** or cortisol [97,98], **breath metabolomics** can be used to predict the phase of the body's inner clock with a single breath [94], and analogous methods have been used based on transcriptome or blood metabolome sampling [5,99,100]. However, currently the precision of this method among many subjects is limited to 2–3 h because of interindividual variation in absolute metabolite levels. Large circadian metabolite databases from many subjects should permit the use of tailored standard curves to produce results of much higher accuracy.

Another particular benefit of this technology is that the metabolites being measured by breath metabolomics are derived from lung alveoli. Thus they represent a peripheral tissue directly, whereas melatonin production is stimulated by a direct supraspinal pathway and correlates closely with SCN phase. Theoretically it would be possible to compare circadian profiles of melatonin with other metabolites as an index of circadian desynchrony caused by lifestyle (e.g., shift work) or disease. Secondly, an easy assay for body time would permit the routine application of **chronopharmacology** to improve the efficiency of many medications [101].

Metabolome profiling might also diagnose potential mechanisms of circadian metabolic disturbance. In an experimental context in mice, metabolomics was used to show defects in circadian

lipid metabolism on deletion of SIRT6 [42]. **Lipidomics** has itself generated new insight into circadian control, showing, for example, that circadian clock function in cardiomyocytes specifically regulates triglyceride metabolism [102]. In humans, metabolome profiling has unearthed specific markers of sleep debt [3,8,103] and demonstrated the potent effects of synthetic glucocorticoids in disrupting peripheral circadian clocks [102].

A final recent trend related to metabolomics profiling is circadian analysis of the gut microbiome. Taxonomic analysis showed that about 15% of various bacterial taxa representing 60% of the total gut bacteria demonstrate circadian oscillations in their abundance, mostly dictated by diurnal rhythmicity in feeding [104,105]. These microbiota are essential for normal circadian rhythmicity of gene expression in gut epithelium. Such control is thought to be mediated by toll-like receptors detecting microbial metabolites [106]. Circadian control of gut microbiota could contribute directly to metabolic disease caused by chronic phase shifting (e.g., jetlag, shift work): whereas chronically shifted normal mice developed metabolic syndrome, chronically shifted mice whose gut flora had been eliminated with antibiotics did not [104].

Circadian Treatments: Chronopharmacology and Beyond

Mostly, the potential of the circadian clock in medicine is conceived in terms of chronopharmacology. Given that jejunal drug transporters, drug targets, and metabolic enzymes necessary for xenobiotic detoxication all vary in a circadian fashion, such potential is unsurprising, and several recent reviews have been written on this subject [101,107–109]. In particular, cancer therapy has received prominent attention for its chronopharmacological potential [108], and colorectal cancer survival has been linked to strong circadian function [110,111].

Recently, investigators have begun to seek drugs specifically targeting clock components, using clocks in cultured cells as a convenient high-throughput assay platform. In a recent review [112], these various studies were summarized as falling into two groups: (i) rational target-based studies, which have identified inhibitors of kinases important to clock function and inhibitors of clock-regulating nuclear receptors; and (ii) large-scale, phenotype-based chemical library screens through which further kinase inhibitors were identified as well as compounds blocking the degradation of circadian clock components like cryptochromes. Given the degree to which aspects of circadian metabolic function are preserved in primary human cells, we postulate that these screening strategies could also present opportunities for discovery in personalized medicine.

The development of lead compounds capable of altering clock function in a specific fashion represents an important step forward and immediately leads to the natural question of what to do with them, and in particular what to do with them in the case of clock-linked metabolic disorder. One obvious use would be as a phase-shifting agent; for example, the use of the hormone melatonin to ameliorate jetlag [113]. For longer-term habituation to shift work to minimize chronodisruption, one might manipulate circadian period length. The entrained phase is directly modified by circadian period (short periods lead to earlier phases and long periods to later ones), so modification of circadian period should change the preferred phase of behavior. Another obvious use would be to strengthen circadian clock amplitude. In one study [114], diverse small organic molecules were identified that acted as ‘clock-reinforcing’ compounds. The authors speculated that these could be used to counteract clock-associated pathophysiology, including defects leading to metabolic syndrome.

A second way to target metabolic syndrome via circadian means would be to specifically modulate the stability of clock proteins acting as metabolic regulators. A test case here was recently demonstrated in the case of a cryptochrome-stabilizing compound that suppressed

glucagon-mediated activation of glucose production [115]. Such molecules are likely to be the first of many: other recent studies report molecules that target the transcriptional activation activity of RORE elements or the transcriptional repressive ability of cryptochromes [116,117].

Finally, one might intentionally disrupt local circadian function to achieve particular physiological goals. Broken circadian clocks, as feedback loops, are 'jammed' entirely in one regulatory direction. For example, whereas disrupting the 'negative' limb of the circadian clock in mice by eliminating PER proteins increases cell proliferation and predisposes to cancer [118,119], disrupting the positive limb by eliminating BMAL1 increases stress-induced senescence [120]. Similarly, disrupting the negative limb increases the proliferation of skin cells at healing wounds while decreasing collagen and keratin secretion, and disrupting the positive limb increases their secretion while decreasing proliferation [121]. We postulate that drugs interfering with the circadian clock via different limbs will have analogously opposite effects on metabolism as well. Whether such clock disruptions can safely serve as adjuncts to other therapies is yet another question.

Outstanding Questions

What are the links between the circadian metabolic rheostat and brain control of sleep–wake and appetite?

What proportion of the complex phenotypes of clock-related metabolic disorder can be ameliorated with simple reinforcement of timing cues?

Can circadian metabolomics profiling in humans be used as a tool for personalized medicine?

Do clock-targeted compounds hold wider promise for treatment of non-circadian aspects of metabolic disease?

Concluding Remarks and Future Perspectives

Circadian clock mechanisms not only control metabolism but are also interwoven with them. We propose that an important first step in unraveling the complexity of these connections lies in the collection of circadian datasets across at-risk populations, using increasingly available cellular, transcriptomics, and metabolomics-based methods coupled with genetics (see Outstanding Questions). The results of these studies will allow the identification of specific clock interactions with important consequences for health. A second step would then be the potential application of clock-modifying compounds to exploit positive effects on metabolism, intentionally harnessing circadian mechanisms to influence diverse cellular signaling pathways. Together, such approaches hold considerable promise to improve health in increasingly busy lives.

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