Chapter 21
Circadian Regulation of Metabolism in Health and Diseases

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Abstract The importance of circadian rhythm has been established through its evolutionary conservation and its connection to many health conditions. Circadian deregulation has emerged as an important risk factor for metabolic disruption and related chronic diseases. Chronic diseases are on the rise worldwide, and emerging evidence points toward restoration of circadian rhythms as a propitious approach to preventing and improving prognosis of many chronic disorders. This review will outline the evidence supporting the importance of the circadian system in metabolism by debriefing the molecular basis for the interaction between circadian timing and metabolic health and behavioral, genetic, and human epidemiological studies indicating the health implications of chrono-disruption.

21.1 Introduction

Metabolic homeostasis is central to a healthy lifespan. Traditionally, homeostasis is described as the property of a living system to establish and maintain a condition of equilibrium in its internal environment, even when faced with acute changes. As most plants, animals, and microbes in this planet have evolved to adopt to the predictable changes in light, temperature, humidity, and food availability, mechanisms maintaining metabolic homeostasis have also evolved to adapt to this diurnal changes. Accordingly, a major function of the circadian clock is to anticipate the daily cycle of nutrient intake and nutrient expenditure and maintain an internal equilibrium within a defined range of parameters. As a large number of metabolites and cellular contents are to be maintained at equilibrium in different organs, the circadian oscillator makes intricate connection with homeostatic mechanisms in a tissue-specific manner. Disruption to this homeostasis of a given metabolite can

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occur due to chronic changes in the availability, storage, or use of the metabolite or due to disruption to the circadian modulation of homeostatic regulation.

Metabolic disruption leads to many chronic diseases, including cardiovascular diseases, obesity, diabetes, and related comorbidities. As of 2012, it is estimated that half of adult population in the USA has one or more chronic health conditions [1], and 35 % suffer from metabolic syndrome [2]. To be diagnosed with metabolic syndrome, one should have at least three of the five metabolic risk factors: large waistline, high triglyceride level, low HDL cholesterol, high blood pressure, and high fasting glucose [3]. As of now, 60 % of deaths worldwide are due to noninfectious chronic diseases, and it is estimated that as much as 80 % of premature stroke, heart disease, and diabetes could be prevented [4]. An important approach to preventing these consequences involves restoring healthy lifestyle. As the daily rhythm in activity-sleep and feeding-fasting and the dependent rhythms in energy metabolism are integral part of lifestyle, it is becoming increasingly apparent that circadian regulation of metabolism has a central role in both prevention and prognosis of chronic metabolic diseases.

From an evolutionary perspective, for most part of human history, humans have lived with a robust daily rhythm in activity-rest and the associated rhythm in feeding-fasting. Early hunter-gatherer populations often ate sparingly mostly during the daytime, and, even today, these populations rarely suffer from cardiovascular diseases and other metabolic complications [5]. Accordingly humans and animals have developed adaptations for an intermittent food supply. This adaptive mechanism includes organs for *the uptake* and storage of rapidly mobilizable glucose and longer-lasting energy substrates, such as fatty acids in adipose tissue. Biochemical programs have also evolved to adapt to intermittent food intake: anabolic metabolism during feeding and catabolic metabolism during fasting. With the predictable nature of feeding-fasting cycle tied to the daily cycle of activity-rest, circadian oscillators have evolved to integrate ~24 h rhythms to anabolic and catabolic metabolism from single cell to the whole organism. Hence, organisms have evolved to go through the cycles of feeding, energy storage, use of stored energy during fasting, and trigger of hunger to reinitiate the cycle with a bout of feeding. As modern humans are untethered from the natural light-dark cycle and food is plentiful, chronic disruption of this genomic program of going through intermittent daily cycle of feeding and fasting is emerging as an important disruptor of metabolic homeostasis.

21.2 Organization of Circadian System in Mammals

21.2.1 Core Clock Players

The circadian clock circuitry acts to produce rhythms in behavior and physiology in anticipation of environmental changes. For metabolic functions, this can be

accomplished via rhythmic expression of genes encoding regulators and enzymes of important metabolic pathways. The core components of the circadian clock network include transcription factors CLOCK and BMAL1, which heterodimerize and bind to the E-box motifs on clock-controlled genes (CCGs) to drive their transcription. Two such CCGs include PER and CRY genes which, when activated throughout the day, cause accumulation of PER and CRY proteins by the night and form heterotypic complexes with additional proteins [6]. These complexes associate with CLOCK-BMAL1 heterodimers and repress their transcription activation potential. The autorepression of Per1/2 and Cry1/2 genes by their own PER/CRY proteins leads to a decrease in accumulation of these proteins, eventually allowing a new cycle of Per1/2 and Cry1/2 transcription to occur [6].

Similarly, another feedback loop of the clock system involves proteins REV-ERB, whose levels increase during the day and bind ROR-REVERB-response elements (RREs) within Bmal1 promoter, thus inhibiting Bmal1 transcription. At night, REV-ERBα protein amounts are low, allowing Bmal1 transcription to rise. The repressive effects of REV-ERB are in constant competition with the retinoic acid related orphan receptors (RORs) (a, b, g), which also bind to RRE on Bmal1 promoter to activate transcription [7, 8]. These transcriptional-translational regulatory loops operate in most tissues and control a notable fraction of the mammalian genome, with many of them also being implicated in metabolic pathways [9–11].

This modulation of transcription and subsequent feedback based on protein accumulation allows the circadian system to fine-tune its regulation based on internal and external factors. In addition to self-regulation via product accumulation, the clock system can be affected by feeding timing and other stimuli.

21.2.2 Local vs. Systemic Control

Every cell in an animal must adapt to the daily rhythm of food availability. At the same time, metabolic homeostasis involves tight coordination among several metabolic organs and neuroendocrine tissues. Furthermore, this complex coordination for energy homeostasis needs to be coupled to the organism's activity-rest cycle. The circadian regulatory system functions to temporally coordinate these complex mechanisms. The circadian system in mammals has a hierarchical architecture, with a central oscillator in the hypothalamic suprachiasmatic nucleus and peripheral clocks in almost all organs in the body. The SCN oscillator is entrained to the ambient light to be in sync with the 24 h light/dark cycle [12]. The cell-autonomous molecular circadian oscillator has nearly the same makeup in the SCN as well as in the periphery, but cells in the SCN are coupled via synaptic and paracrine signals. Coupling among the individual SCN neurons constitute a robust oscillator and makes it less susceptible to mild perturbations [13]. Peripheral oscillators do not appear to communicate with each other through tight local communications. Rather, they rely on systemic signals for phase coherence. Indeed, in SCN-lesioned mice, peripheral tissues gradually become out of phase with the

external light-dark cycle and with circadian oscillations in other tissues [14]. Similarly, synchronization is gradually lost in tissue explants and transiently synchronized fibroblasts [15, 16].

The SCN imparts temporal information to the peripheral clocks via systemic cues [14]. The peripheral oscillators are also sensitive to nutritional cues. In this way, circadian physiology gets modulated systemically from the central clock or locally via tissue-specific clocks in response to local metabolites. The discrimination between these two routes has been seen in mouse liver, in a circadian transcriptome analysis in mice with or without functional hepatocyte clocks [17, 18]. These studies have shown the liver circadian transcriptional rhythms are partly mediated by the cell-autonomous clock and "driven" by systemic rhythmic signals generated elsewhere in the body. Multiple systemic candidate signals have been suggested including steroid hormones and fluctuation in core body temperature [19]. In experiments relevant to circadian coordination of metabolism, daytime feeding of naturally nocturnal rodent can shift the phase of circadian gene expression in the liver, regardless of lighting conditions, leaving the phase of cyclic gene expression in the SCN unaffected. Thus, changes in feeding timing can uncouple peripheral oscillators from the central pacemaker [20–22].

Feeding modulation of peripheral clock suggests that time of eating is a strong zeitgeber (entraining cue) for peripheral clocks and might even supersede the synchronization cues received from the SCN clock. Under optimal conditions, the periphery and SCN are synchronized: the SCN imposes feeding rhythms by driving rest-activity cycles using food-dependent zeitgebers in addition to other cyclic cues (like body temperature or blood-borne signals) to set the phase in the periphery. However, alteration of metabolism, whether through food content or feeding timing, can lead to desynchronization of circadian clocks and a reciprocal disruption of metabolic pathways that seems to underlie the resulting adverse health effects to be termed as chrono-disruption. The mechanisms underlying this cross talk between food consumption and clock-driven metabolic control are complex [23] and require further elucidation; essential components of this system are discussed in the sections to follow.

21.3 Cross Talk: Molecular Basis of Circadian Oscillator-Metabolism Interface

There is extensive interplay between the clock system and metabolic inputs and outputs. Many clock target genes are directly involved in metabolic pathways, including lipid metabolism and the regulation of metabolic timing in peripheral tissues. One general example is the action of CLOCK/BMAL on the set PAR bZIP proteins DBP, HLF, and TEF, which causes these proteins to be transcribed with robust circadian rhythms in the SCN and peripheral tissues such as the liver. PARbZIP proteins have been found to target various enzymes involved in major

xenobiotic metabolic pathways [24]. Additionally, the metabolic states can be communicated to and modulated by glucose-sensing pathways, NAD+ sensing pathways, and nuclear receptor pathways.

21.3.1 Glucose Homeostasis

CLOCK/BMAL1 plays an integral role in glucose homeostasis. The clock circuitry can both act on and respond to glucose levels. BMAL1 itself promotes hepatic gluconeogenesis [25, 26], and there are many additional indirect pathways by which clock is involved in glucose regulation. As previously stated, CLOCK/BMAL1 activates CRY and PER genes, which are heavily implicated in glucose sensing and regulation, via interactions with AMPK, CREB, KLF10, and insulin pathways.

AMP-activated protein kinase (AMPK) serves as a sensor for the ratio of AMP/ATP within cells, putting it at the center of cellular energy homeostasis [27]. Increase in this ratio will lead to phosphorylation and activation of AMPK. AMPK activation leads to increased glucose uptake and decreased gluconeogenesis. It is also implicated in hepatic fatty acid oxidation, mitochondrial biogenesis, and insulin sensitivity. There is a consensus motif for phosphorylation of AMPK within CRY, so that phosphorylation of AMPK leads to phosphorylation and destabilization of CRY/PER complexes, disrupting their accumulation and thus derepression of the CLOCK complex [28]. This provides one pathway that communicates metabolic state to the CLOCK circuitry.

CREB also acts in glucose regulation via its action on gluconeogenesis. Fasting state leads to glucagon binding to its membrane receptor in hepatocytes, which activates a downstream G protein *Gs* and increases intracellular cAMP levels, leading to the phosphorylation of CREB [29]. pCREB shows robust rhythmic expression in the liver and acts to increase gluconeogenesis. CRY can also interact with Gs or adenylate cyclase to regulate temporal expression of pCREB and thus temporal regulation of gluconeogenesis [30]. In this way, clock genes provide important regulation in glucose homeostasis.

Conversely, there are also pathways by which glucose levels can affect clock action. The mRNA accumulation of KLF10, a negatively acting Zn^{2+} finger transcription factor, follows a diurnal rhythm in mouse liver that may be driven by intracellular glucose concentrations. Increased glucose upregulates KLF10, which binds to BMAL1 promoter and dampens BMAL transcription [31].

21.3.2 NAD^+ Sensing Pathways

NAD^+ levels fluctuate with metabolic state. Glycolysis oxidizes NADH to NAD+ during the reduction of pyruvate to lactate. Fatty acid synthesis also produces

higher NAD+ levels. NAD+ level shows daily oscillations in the liver, likely due to oscillations in Nampt transcription [32, 33]. NAD+ levels also vary in response to DNA damage, via damage-induced poly(ADP-ribose) polymerase-1 (PARP1), which acts to reduce NAD+ levels [34]. PARP normally has rhythmic accumulation in the liver. Additionally, in day-fed rodents, PARP oscillation is inverted, suggesting circadian patterns of PARP are food dependent. In the absence of PARP activity, the hepatic clock takes a long time to readjust to a shifted feeding window, suggesting PARP plays an important role in the phase entrainment of liver oscillators [35]. PARP activity directly impacts NAD+ levels, which can subsequently affect sirtuins, a family of NAD+-dependent deacetylases that impact a multitude of metabolic pathways and the circadian clock.

SIRT1 participates in cross talk with FoxO genes, providing a mechanism by which SIRT impacts metabolic pathways. During long-term fasting, NAD+ levels increase, which upregulates SIRT activity. This leads to deacetylation and activation of FoxO, which in turn impacts an array of metabolic pathways [36–38].

Additionally, NAD+-activated SIRT1 deacetylates and destabilizes sterol regulatory element-binding protein (SREBP), a transcription factor that upregulates synthesis of enzymes involved in sterol biosynthesis. The destabilization causes SREBP to build up and increase fatty acid and cholesterol synthesis [39]. The increase in sterols and fatty acids means an increase in ligands for nuclear receptors like PPAR and ROR (discussed in more detail in the next section). These can act on the CLOCK/BMAL system directly or via effects on PER/CRY accumulation, modulating the feedback pathway.

Worth noting, there are many other pathways by which SIRT can influence metabolism. Sirt1 can act on STAT3 in gluconeogenesis, NFKB in insulin secretion and sensitivity, LXR in lipid metabolism, CRTC2 in gluconeogenesis, and PGC1a in gluconeogenesis and fatty acid oxidation (reviewed in [40]). SIRT1 acts both as a sensor and regulator of metabolic pathways. The fact that it is regulated by circadian expression of NAD+, combined with its ability to influence pathways that regulate the core clock system, puts SIRT1 at an important node of the cross talk between metabolism and circadian circuitry [41].

21.3.3 Nuclear Receptor Pathways: PPAR, Reverb, ROR

In the mouse genome, 20 of the 49 nuclear receptor genes are expressed in a circadian manner, and nearly all of these nuclear hormone receptors implicated in metabolic processes [42]. Several of these are also implicated in clock machinery, either directly or indirectly. These include, among others, PPAR, REVERB and ROR.

As mentioned earlier, ROR proteins are activators of Bmal1 transcription. REVERB inhibits ROR function by competing for the same DNA binding site and thus inhibits Bmal1 transcription, thereby producing rhythmic levels of Bmal1 mRNA. Functionally active ROR-REVERB-response elements (RREs) are also

found to regulate the accurate temporal expression of additional circadian oscillator components [43–45].

Nuclear receptor corepressor (NCoR) and HDAC3 complex is recruited to enhance resident REVERB where it is tethered to DNA by tissue-specific transcription factors. Given the Reverb is strongly circadianly expressed, rhythmic repressive action of NCoR/HDAC3-REVERB exerts circadian regulation of tissue-specific outputs that are independent of ROR regulation [46].

Peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptors that function as transcription factors for numerous target genes, especially those encoding enzymes important for metabolic pathways. All three PPAR isoforms are expressed in a circadian manner in mouse tissue, and PPARα and PPARγ are direct regulators of core clock components, Bmal1 and Reverbα. Conversely, PPARα is also a direct Bmal1 target gene. PPAR lipid regulators continue to express with daily rhythms even when clock-disrupted mice are subject to daily eating-fasting rhythms [47, 48]. This data implicates rhythmic PPAR expression is supported by both the circadian clock and the feeding-fasting cycle.

21.4 Health Implications of Circadian Disruption

In the last century, the leading causes of morbidity and mortality in modern societies have shifted from infectious diseases to noninfectious chronic diseases including hypertension, hyperlipidemia, coronary artery disease, diabetes, cancer, and diseases of chronic inflammation [49]. Chronic disruption of circadian rhythm in experimental animals or as commonly experienced among shift workers raises the risk for many of these diseases. These correlative observations have catalyzed a rapidly expanding research into understanding the mechanism underlying the connection between circadian disruption and chronic diseases. We will discuss a few examples here and encourage the readers to explore these connections in several recently published excellent reviews [23, 41, 47, 50, 51].

21.4.1 Genetic Perturbations

Genetic perturbation of circadian oscillator in mice predisposes to metabolic pathologies. Such genetic perturbation models have been invaluable in understanding the mechanistic link between the circadian clock proteins and metabolic homeostasis. A comprehensive summary of metabolic diseases in circadian perturbation mouse models has been published recently [51]. Here we will cite a few examples. The simultaneous inactivation of Cry1 and Cry2 results in hyperglycemia [52], while liver-specific BMAL1 knockout mice show hypoglycemia [25]. Altered glucose homeostasis is a hallmark of insulin resistance and type 2 diabetes. $Clock^{\Delta 19}$ mutant mice exhibit greatly attenuated daily feeding rhythm,

with increased caloric intake during the day. These mice are obese and develop a metabolic syndrome [53]. Likewise, adipocyte-specific Bmal1-null mice exhibit increased food intake during the light phase and increased body weight [54]. These experiments provide a link between disruption of clock components and metabolic pathologies.

21.4.2 Inflammation

A common feature of a vast majority of chronic diseases is an increase in local or systemic inflammation marked by increased sensitivity of immune system to antigens and elevated levels of cytokines, lymphocytes, or macrophages in circulation or in specific tissue types. Circadian regulation in immune function is supported by observations in both animals and humans. Experimentally induced circadian disruption alters innate immune responses, with heightened release of pro-inflammatory cytokines in response to endotoxic shock in shifted mice vs. -non-shifted controls [55, 56]. In humans, dysregulation of the sleep/wake cycle affects the number of circulating lymphocytes [57]. These immune changes are likely mediators in many of the adverse health effects seen from circadian disruption, as inflammatory responses have been implicated as risk factors in multiple diseases including obesity, diabetes, IR, cancers, neurodegeneration, and cardiovascular complications.

Both immune cell proliferation (maturation, activation, trafficking) and immune cell function may be affected by circadian clock components. Proliferation and release of mature lymphocytes from bone marrow have been shown to be circadian [58]. Several trafficking factors including CXCL1 and SDF1 are also regulated in a circadian manner, and this regulation is likely mediated by JARID1a [59], which also modulates CLOCK/BMAL1 function [60]. Additionally, transcriptional regulators DEC1 and ROR-gamma have been implicated in both circadian regulation and lymphocyte maturation [61]. In differentiated cells, the function of the major immune regulator NF-kappaB is inhibited by CRY proteins, so that Cry-deficient mice show elevated levels of IL6 and succumb to LPS challenge [62]. Circadian regulation of immune function is not limited to a handful of genes. Genome-wide transcriptome studies have found hundreds of genes that are expressed in a circadian manner in mature macrophages [63].

21.4.3 Cardiovascular Complications

Similar to other systems, the cardiovascular system exhibits daily and seasonal rhythms. Heart rate, cardiac output, and blood pressure show daily rhythms [64]. Genes encoding core clock components and CCGs relevant to heart function show oscillations in heart tissues and in the aorta [65–69]. The SCN also stimulates

the pineal gland to produce melatonin at night. A relationship has been reported between melatonin and light/dark variations in inflammatory systemic markers [70]. Melatonin may act as a potent antioxidant, reducing myocardial damage induced by ischemia reperfusion. Patients with lower nocturnal concentrations of melatonin appear to have a higher chance of developing heart failure or cardiac death [71]. Circadian regulation of a key transcription factor KLF15 in mammalian heart sustains cardiac electrical properties. Disruption (either deficiency or overexpression) of KLF15 causes loss of rhythmic QT variation, abnormal repolarization, and enhanced susceptibility of arrhythmia [72].

21.4.4 Cancer

Epidemiological observations have linked circadian rhythms with cancer risk. Women with more hours per week and years working at night have a moderate increase in breast cancer. Shift work is also increasingly associated with elevated risk for cancer to a point that WHO has recognized shift work as a "potential carcinogen" [73]. After cancer diagnosis, prognosis is poorer in patients with disrupted circadian rhythms, while chronotherapy, where timing of drug administration is considered during treatment, has been shown to increase efficacy of some chemotherapeutic agents [74, 75]. These observations in humans support an intimate connection between circadian rhythm and cell cycle regulation; however, the mechanism appears to be more complex.

At least three different connections between circadian clock and cancer have been described: circadian regulation of cell cycle regulators, shared components between DNA damage response and circadian rhythm, and circadian regulation of metabolism. Circadian regulation of genes related to the cell cycle creates a compelling case for the involvement of circadian timing in cancer development. Circadian expression of WEE1 (G2/M transition) and MYC (G0/G1 transition) and cyclin D1 (G1/S transition) in mammals [76] can partly explain circadian gating of cell cycle regulation. Accordingly, partial hepatectomy-induced liver regeneration is slowed down in $Cry1^{-/-}$;$Cry2^{-/-}$ mice, indicating CRY is an important link between circadian physiology and cell proliferation [76]. PER1 and PER2 have been shown to be tumor suppressors in mice, and expression of all three PER genes is deregulated in breast cancer [77]. Some of the effects of PER proteins in cancer may relate to DNA damage response. PER1 protein can interact with DNA-damage response kinases ATM and CHK2 [78], which offer a direct link between clock component and cancer. Another immediate responder to DNA damage is PARP protein that rapidly synthesizes poly(ADP-ribose) polymers and thereby depletes cellular NAD store. The intimate connection between NAD, SIRT, and circadian clock and the demonstration that genetic perturbation of PARP or SIRT pathway affects both circadian clock and DNA damage response offer another link between cancer and clock. Recently, DNA damage response has been shown to alter the circadian clock via Hausp-dependent stabilization of CRY1 protein [79].

As cancer cells have an anabolic demand and are known to depend on glycolysis and autophagy to meet their energy requirement, circadian regulation of metabolism and autophagy underlies one connection. Cancer cells show an altered energy metabolism, using glycolysis rather than oxidative phosphorylation for energy, known as the Warburg effect [80]. In multiple tissue types, components of both mitochondrial oxidative phosphorylation and of glycolysis show daily rhythms [11]. Furthermore, diurnal rhythm in nutrient availability also modulates glycolytic pathway and mitochondria [22]. Therefore, for cancer cells to adapt a different energy metabolism strategy, the circadian rhythm regulation of metabolism must be disrupted. Similarly, some cancers become increasingly dependent on autophagy to recycle their cellular constituents, and many circadian clock components directly or indirectly regulate autophagy. Circadian expression of C/EBP beta [81], a potent activator of autophagy, and Rev-erb regulation of autophagy gene expression in the liver offer a temporal regulatory mechanism for autophagy [44]. Therefore, similar to energy metabolism, the cancer cells addiction to autophagy must override the circadian regulation of autophagy.

21.4.5 Obesity, Diabetes, Insulin Resistance

Association between disruption of circadian timekeeping and risk of metabolic syndrome, obesity, and type 2 diabetes has been extensively established [82]. Obesity, diabetes, and insulin resistance maintain close cause and effect relationships. BMAL knockout mice show reduced insulin and lack rhythmicity in insulin activity. When rhythmicity is rescued via expression of BMAL2, insulin action and activity is restored. Mice whose circadian function has been compromised – either via BMAL knockout or WT exposure to constant light – are more obese-prone when on a high-fat diet than normal controls [82]. In humans, circadian disruption may increase diabetes risk via inflammatory mechanisms independent of sleep loss, leading to decreased insulin sensitivity without compensatory increase in insulin secretion [83].

21.5 Relevance of Circadian-Eating Pattern Interactions

Given the extensive reciprocal interaction between circadian oscillator and metabolic regulators, simple change in daily eating pattern will likely perturb the temporal regulation of metabolic homeostasis and result in altered body composition, weight gain, and metabolic diseases. Experimental results in both animals and humans support this notion. Laboratory male mice typically consume a major portion of their daily diet during the dark phase of the light-dark cycle. However, mice housed in constant bright/dim light eat more than usual during the light phase [84]. Overall caloric intake and level of motor activity remain similar to that of

mice under standard conditions, but the shift of consumption to the light phase leads to weight gain and glucose intolerance [84]. In wild-type mice under nighttime-restricted feeding of regular chow, total caloric intake is unaffected, but hepatic triglyceride content decreases by 50 % [85].

Daily rhythm of feeding-fasting alone can drive rhythmic expression of hepatic mRNAs and metabolites in mice. Feeding rhythms could phase-entrain the accumulation of over 600 mRNAs and several metabolites in the liver of oscillator-deficient Cry1/Cry2 double-knockout mice [22]. These results indicate the powerful influence of feeding timing on circadian regulation of metabolism [86, 87]. The imposed daily eating-fasting rhythm may counteract some of the adverse metabolic effects of genetically perturbed circadian clock. In a Per1 phosphorylation mutant mouse, increased daytime eating predisposes to weight gain. However, this genetic predisposition to weight gain can be countered without reducing caloric intake by imposing a daily rhythm of feeding-fasting [88]. These results are highlighting that the circadian regulation of metabolic homeostasis is a synergistic product of direct cell-autonomous regulation and indirect feeding-fasting regulated processes.

Moreover, few human studies have indicated managing the daily pattern of eating-fasting might be a new approach to controlling obesity and metabolic disease. In a weight loss study in Spain, subjects who ate earlier lunch showed higher weight loss than those who ate later – even when both groups were controlled for total caloric intake and physical activity [89]. In an evidence-based eating time survey, majority of adult non-shift workers were found to spread their daily nutrient intake $>$14 h. A small subset of volunteers, after adopting a 10–11 h eating schedule, reported sustained weight loss of ~4 % over a year with associated improvement in subjective measure of energy level during the day and sleep quality at night [90]. The impact of daily eating-fasting rhythm on health may be pleiotropic. In a retrospective analyses of self-reported 24 h food recall, a correlation between overnight fasting of $\geq$13 h and reduction in blood biomarkers of cancer risk has been found [91]. This raises the possibility that daily pattern of feeding-fasting even under the modern lifestyle of extended illumination can provide protection against several chronic diseases.

21.6 Conclusions and Future Directions

The importance of circadian timing has been demonstrated both through evolutionary relevance and observed health implications of disrupted clock systems. While the pathways that lie in the clock-metabolism interface are extensive and require further elucidation, molecular connections between the two are well established and provide an important gateway by which circadian disruption contributes to metabolic syndrome and related pathologies.

Promising studies have shown alteration in circadian rhythm to have therapeutic effects. The timing of feeding and fasting, irrespective of total caloric intake, can reduce weight gain and improve metabolic parameters. It has been shown that time-

restricted feeding can have long-term effects against preexisting obesity even when applied only 5 days per week [86]. These results underlie the importance of further study of the function and regulation of internal clocks and the integral role these mechanisms play in human health and behavior.

References

1. Ward BW, Schiller JS, Goodman RA (2014) Multiple chronic conditions among US adults: a 2012 update. Prev Chronic Dis 11:E62
2. Reynolds K, He J (2005) Epidemiology of the metabolic syndrome. Am J Med Sci 330:273–279
3. Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C (2004) Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Arterioscler Thromb Vasc Biol 24:e13–e18
4. Vita-Finzi L (2005) Preventing chronic diseases: a vital investment. World Health Organization, Geneva
5. Cordain L, Eaton S, Brand Miller J, Mann N, Hill K (2002) Original communications-the paradoxical nature of hunter-gatherer diets: meat-based, yet non-atherogenic. Eur J Clin Nutr 56:S42
6. Hardin PE, Panda S (2013) Circadian timekeeping and output mechanisms in animals. Curr Opin Neurobiol 23:724–731
7. Preitner N, Damiola F, Lopez-Molina L, Zakany J, Duboule D, Albrecht U, Schibler U (2002) The orphan nuclear receptor REV-ERBalpha controls circadian transcription within the positive limb of the mammalian circadian oscillator. Cell 110:251–260
8. Sato TK, Panda S, Miraglia LJ, Reyes TM, Rudic RD, McNamara P, Naik KA, FitzGerald GA, Kay SA, Hogenesch JB (2004) A functional genomics strategy reveals Rora as a component of the mammalian circadian clock. Neuron 43:527–537
9. Hughes M, Deharo L, Pulivarthy SR, Gu J, Hayes K, Panda S, Hogenesch JB (2007) High-resolution time course analysis of gene expression from pituitary. Cold Spring Harb Symp Quant Biol 72:381–386
10. Panda S, Antoch MP, Miller BH, Su AI, Schook AB, Straume M, Schultz PG, Kay SA, Takahashi JS, Hogenesch JB (2002) Coordinated transcription of key pathways in the mouse by the circadian clock. Cell 109:307–320
11. Zhang R, Lahens NF, Ballance HI, Hughes ME, Hogenesch JB (2014) A circadian gene expression atlas in mammals: implications for biology and medicine. Proc Natl Acad Sci U S A 111:16219–16224
12. Nayak SK, Jegla T, Panda S (2007) Role of a novel photopigment, melanopsin, in behavioral adaptation to light. Cell Mol Life Sci 64:144–154
13. Aton SJ, Herzog ED (2005) Come together, right. . .now: synchronization of rhythms in a mammalian circadian clock. Neuron 48:531–534
14. Mohawk JA, Green CB, Takahashi JS (2012) Central and peripheral circadian clocks in mammals. Annu Rev Neurosci 35:445–462
15. Welsh DK, Yoo SH, Liu AC, Takahashi JS, Kay SA (2004) Bioluminescence imaging of individual fibroblasts reveals persistent, independently phased circadian rhythms of clock gene expression. Curr Biol 14:2289–2295
16. Yamazaki S, Numano R, Abe M, Hida A, Takahashi R, Ueda M, Block GD, Sakaki Y, Menaker M, Tei H (2000) Resetting central and peripheral circadian oscillators in transgenic rats. Science 288:682–685

17. Hughes ME, Hong HK, Chong JL, Indacochea AA, Lee SS, Han M, Takahashi JS, Hogenesch JB (2012) Brain-specific rescue of clock reveals system-driven transcriptional rhythms in peripheral tissue. PLoS Genet 8:e1002835
18. Kornmann B, Schaad O, Bujard H, Takahashi JS, Schibler U (2007a) System-driven and oscillator-dependent circadian transcription in mice with a conditionally active liver clock. PLoS Biol 5:e34
19. Kornmann B, Schaad O, Reinke H, Saini C, Schibler U (2007b) Regulation of circadian gene expression in liver by systemic signals and hepatocyte oscillators. In: Cold Spring Harbor symposia on quantitative biology. Cold Spring Harbor Laboratory Press, pp 319–330
20. Balsalobre A, Brown SA, Marcacci L, Tronche F, Kellendonk C, Reichardt HM, Schutz G, Schibler U (2000) Resetting of circadian time in peripheral tissues by glucocorticoid signaling. Science 289:2344–2347
21. Damiola F, Le Minh N, Preitner N, Kornmann B, Fleury-Olela F, Schibler U (2000) Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. Genes Dev 14:2950–2961
22. Vollmers C, Gill S, DiTacchio L, Pulivarthy SR, Le HD, Panda S (2009) Time of feeding and the intrinsic circadian clock drive rhythms in hepatic gene expression. Proc Natl Acad Sci U S A 106:21453–21458
23. Asher G, Sassone-Corsi P (2015) Time for food: the intimate interplay between nutrition, metabolism, and the circadian clock. Cell 161:84–92
24. Gachon F, Olela FF, Schaad O, Descombes P, Schibler U (2006) The circadian PAR-domain basic leucine zipper transcription factors DBP, TEF, and HLF modulate basal and inducible xenobiotic detoxification. Cell Metab 4:25–36
25. Lamia KA, Storch KF, Weitz CJ (2008) Physiological significance of a peripheral tissue circadian clock. Proc Natl Acad Sci U S A 105:15172–15177
26. Rudic RD, McNamara P, Curtis AM, Boston RC, Panda S, Hogenesch JB, Fitzgerald GA (2004) BMAL1 and CLOCK, two essential components of the circadian clock, are involved in glucose homeostasis. PLoS Biol 2:e377
27. Inoki K, Kim J, Guan KL (2011) AMPK and mTOR in cellular energy homeostasis and drug targets. Annu Rev Pharmacol Toxicol 52(1):381–400
28. Lamia KA, Sachdeva UM, DiTacchio L, Williams EC, Alvarez JG, Egan DF, Vasquez DS, Juguilon H, Panda S, Shaw RJ, Thompson CB, Evans RM (2009) AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. Science 326:437–440
29. Altarejos JY, Montminy M (2011) CREB and the CRTC co-activators: sensors for hormonal and metabolic signals. Nat Rev Mol Cell Biol 12:141–151
30. Hatori M, Panda S (2010) CRY links the circadian clock and CREB-mediated gluconeogenesis. Cell Res 20:1285–1288
31. Guillaumond F, Gréchez-Cassiau A, Subramaniam M, Brangolo S, Peteri-Brünback B, Staels B, Fiévet C, Spelsberg TC, Delaunay F, Teboul M (2010) Krüppel-like factor KLF10 is a link between the circadian clock and metabolism in liver. Mol Cell Biol 30:3059–3070
32. Nakahata Y, Sahar S, Astarita G, Kaluzova M, Sassone-Corsi P (2009) Circadian control of the NAD+ salvage pathway by CLOCK-SIRT1. Science 324:654–657
33. Ramsey KM, Yoshino J, Brace CS, Abrassart D, Kobayashi Y, Marcheva B, Hong HK, Chong JL, Buhr ED, Lee C, Takahashi JS, Imai S, Bass J (2009) Circadian clock feedback cycle through NAMPT-mediated NAD+ biosynthesis. Science 324:651–654
34. Burkle A (2001) Physiology and pathophysiology of poly(ADP-ribosyl)ation. Bioessays 23:795–806
35. Asher G, Reinke H, Altmeyer M, Gutierrez-Arcelus M, Hottiger MO, Schibler U (2010) Poly (ADP-ribose) polymerase 1 participates in the phase entrainment of circadian clocks to feeding. Cell 142:943–953
36. Dentin R, Liu Y, Koo SH, Hedrick S, Vargas T, Heredia J, Yates J 3rd, Montminy M (2007) Insulin modulates gluconeogenesis by inhibition of the coactivator TORC2. Nature 449:366–369

37. Puigserver P, Rhee J, Donovan J, Walkey CJ, Yoon JC, Oriente F, Kitamura Y, Altomonte J, Dong H, Accili D, Spiegelman BM (2003) Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1alpha interaction. Nature 423:550–555
38. Zhang W, Patil S, Chauhan B, Guo S, Powell DR, Le J, Klotsas A, Matika R, Xiao X, Franks R, Heidenreich KA, Sajan MP, Farese RV, Stolz DB, Tso P, Koo SH, Montminy M, Unterman TG (2006) FoxO1 regulates multiple metabolic pathways in the liver: effects on gluconeogenic, glycolytic, and lipogenic gene expression. J Biol Chem 281:10105–10117
39. Espenshade PJ, Hughes AL (2007) Regulation of sterol synthesis in eukaryotes. Annu Rev Genet 41:401–427
40. Bheda P, Jing H, Wolberger C, Lin H (2016) The substrate specificity of sirtuins. Ann Rev Biochem 85:405–429
41. Sahar S, Sassone-Corsi P (2009) Metabolism and cancer: the circadian clock connection. Nat Rev Cancer 9:886–896
42. Yang X, Downes M, Yu RT, Bookout AL, He W, Straume M, Mangelsdorf DJ, Evans RM (2006) Nuclear receptor expression links the circadian clock to metabolism. Cell 126:801–810
43. Bugge A, Feng D, Everett LJ, Briggs ER, Mullican SE, Wang F, Jager J, Lazar MA (2012) Rev-erbalpha and Rev-erbbeta coordinately protect the circadian clock and normal metabolic function. Genes Dev 26:657–667
44. Cho H, Zhao X, Hatori M, Yu RT, Barish GD, Lam MT, Chong LW, DiTacchio L, Atkins AR, Glass CK, Liddle C, Auwerx J, Downes M, Panda S, Evans RM (2012) Regulation of circadian behaviour and metabolism by REV-ERB-alpha and REV-ERB-beta. Nature 485:123–127
45. Gerhart-Hines Z, Feng D, Emmett MJ, Everett LJ, Loro E, Briggs ER, Bugge A, Hou C, Ferrara C, Seale P, Pryma DA, Khurana TS, Lazar MA (2013) The nuclear receptor Rev-erbalpha controls circadian thermogenic plasticity. Nature 503:410–413
46. Fang B, Lazar MA (2015) Dissecting the Rev-erbalpha cistrome and the mechanisms controlling circadian transcription in liver. Cold Spring Harb Symp Quant Biol 80:233–238
47. Eckel-Mahan K, Sassone-Corsi P (2013) Metabolism and the circadian clock converge. Physiol Rev 93:107–135
48. Eckel-Mahan KL, Patel VR, de Mateo S, Orozco-Solis R, Ceglia NJ, Sahar S, Dilag-Penilla SA, Dyar KA, Baldi P, Sassone-Corsi P (2013) Reprogramming of the circadian clock by nutritional challenge. Cell 155:1464–1478
49. Mokdad AH, Marks JS, Stroup DF, Gerberding JL (2004) Actual causes of death in the United States, 2000. JAMA 291:1238–1245
50. Bass J, Takahashi JS (2010) Circadian integration of metabolism and energetics. Science 330:1349–1354
51. Zarrinpar A, Chaix A, Panda S (2016) Daily eating patterns and their impact on health and disease. Trends Endocrinol Metab 27:69–83
52. Lamia KA, Papp SJ, Yu RT, Barish GD, Uhlenhaut NH, Jonker JW, Downes M, Evans RM (2011) Cryptochromes mediate rhythmic repression of the glucocorticoid receptor. Nature 480:552–556
53. Turek FW, Joshu C, Kohsaka A, Lin E, Ivanova G, McDearmon E, Laposky A, Losee-Olson S, Easton A, Jensen DR, Eckel RH, Takahashi JS, Bass J (2005) Obesity and metabolic syndrome in circadian clock mutant mice. Science 308:1043–1045
54. Paschos GK, Ibrahim S, Song WL, Kunieda T, Grant G, Reyes TM, Bradfield CA, Vaughan CH, Eiden M, Masoodi M, Griffin JL, Wang F, Lawson JA, Fitzgerald GA (2012) Obesity in mice with adipocyte-specific deletion of clock component Arntl. Nat Med 18:1768–1777
55. Castanon-Cervantes O, Wu M, Ehlen JC, Paul K, Gamble KL, Johnson RL, Besing RC, Menaker M, Gewirtz AT, Davidson AJ (2010) Dysregulation of inflammatory responses by chronic circadian disruption. J Immunol 185:5796–5805
56. Marpegán L, Bekinschtein TA, Costas MA, Golombek DA (2005) Circadian responses to endotoxin treatment in mice. J Neuroimmunol 160:102–109

57. Redwine L, Hauger RL, Gillin JC, Irwin M (2000) Effects of sleep and sleep deprivation on interleukin-6, growth hormone, cortisol, and melatonin levels in Humans 1. J Clin Endocrinol Metabol 85:3597–3603
58. Boucher H, Vanneaux V, Domet T, Parouchev A, Larghero J (2016) Circadian clock genes modulate human bone marrow mesenchymal stem cell differentiation, migration and cell cycle. PLoS One 11:e0146674
59. Christensen J, Agger K, Cloos PA, Pasini D, Rose S, Sennels L, Rappsilber J, Hansen KH, Salcini AE, Helin K (2007) RBP2 belongs to a family of demethylases, specific for tri-and dimethylated lysine 4 on histone 3. Cell 128:1063–1076
60. DiTacchio L, Le HD, Vollmers C, Hatori M, Witcher M, Secombe J, Panda S (2011) Histone lysine demethylase JARID1a activates CLOCK-BMAL1 and influences the circadian clock. Science 333:1881–1885
61. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ, Littman DR (2006) The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell 126:1121–1133
62. Narasimamurthy R, Hatori M, Nayak SK, Liu F, Panda S, Verma IM (2012) Circadian clock protein cryptochrome regulates the expression of proinflammatory cytokines. Proc Natl Acad Sci U S A 109:12662–12667
63. Keller M, Mazuch J, Abraham U, Eom GD, Herzog ED, Volk H-D, Kramer A, Maier B (2009) A circadian clock in macrophages controls inflammatory immune responses. Proc Natl Acad Sci 106:21407–21412
64. Durgan DJ, Young ME (2010) The cardiomyocyte circadian clock: emerging roles in health and disease. Circ Res 106:647–658
65. Bray MS, Shaw CA, Moore MW, Garcia RA, Zanquetta MM, Durgan DJ, Jeong WJ, Tsai JY, Bugger H, Zhang D, Rohrwasser A, Rennison JH, Dyck JR, Litwin SE, Hardin PE, Chow CW, Chandler MP, Abel ED, Young ME (2008) Disruption of the circadian clock within the cardiomyocyte influences myocardial contractile function, metabolism, and gene expression. Am J Physiol Heart Circ Physiol 294:H1036–H1047
66. Tsai JY, Kienesberger PC, Pulinilkunnil T, Sailors MH, Durgan DJ, Villegas-Montoya C, Jahoor A, Gonzalez R, Garvey ME, Boland B, Blasier Z, McElfresh TA, Nannegari V, Chow CW, Heird WC, Chandler MP, Dyck JR, Bray MS, Young ME (2010) Direct regulation of myocardial triglyceride metabolism by the cardiomyocyte circadian clock. J Biol Chem 285:2918–2929
67. Young ME, Brewer RA, Peliciari-Garcia RA, Collins HE, He L, Birky TL, Peden BW, Thompson EG, Ammons BJ, Bray MS, Chatham JC, Wende AR, Yang Q, Chow CW, Martino TA, Gamble KL (2014) Cardiomyocyte-specific BMAL1 plays critical roles in metabolism, signaling, and maintenance of contractile function of the heart. J Biol Rhythms 29:257–276
68. Young ME, Razeghi P, Cedars AM, Guthrie PH, Taegtmeyer H (2001) Intrinsic diurnal variations in cardiac metabolism and contractile function. Circ Res 89:1199–1208
69. Young ME, Razeghi P, Taegtmeyer H (2001) Clock genes in the heart: characterization and attenuation with hypertrophy. Circ Res 88:1142–1150
70. Reiter RJ, Calvo JR, Karbownik M, Qi W, Tan DX (2000) Melatonin and its relation to the immune system and inflammation. Ann N Y Acad Sci 917:376–386
71. Sahna E, Parlakpinar H, Turkoz Y, Acet A (2005) Protective effects of melatonin on myocardial ischemia-reperfusion induced infarct size and oxidative changes. Physiol Res 54:491
72. Jeyaraj D, Haldar SM, Wan X, McCauley MD, Ripperger JA, Hu K, Lu Y, Eapen BL, Sharma N, Ficker E, Cutler MJ, Gulick J, Sanbe A, Robbins J, Demolombe S, Kondratov RV, Shea SA, Albrecht U, Wehrens XH, Rosenbaum DS, Jain MK (2012) Circadian rhythms govern cardiac repolarization and arrhythmogenesis. Nature 483:96–99
73. Stevens RG, Hansen J, Costa G, Haus E, Kauppinen T, Aronson KJ, Castaño-Vinyals G, Davis S, Frings-Dresen MH, Fritschi L, Kogevinas M, Kogi K, Lie JA, Lowden A, Peplonska B, Pesch B, Pukkala E, Schernhammer E, Travis RC, Vermeulen R, Zheng T,

Cogliano V, Straif K (2011) Considerations of circadian impact for defining 'shift work' in cancer studies: IARC working group report. Occup Environ Med 68:154–162
74. Fu L, Lee CC (2003) The circadian clock: pacemaker and tumour suppressor. Nat Rev Cancer 3:350–361
75. Savvidis C, Koutsilieris M (2012) Circadian rhythm disruption in cancer biology. Mol Med 18:1249–1260
76. Matsuo T, Yamaguchi S, Mitsui S, Emi A, Shimoda F, Okamura H (2003) Control mechanism of the circadian clock for timing of cell division in vivo. Science 302:255–259, Epub 2003 Aug 2021
77. Chen S-T, Choo K-B, Hou M-F, Yeh K-T, Kuo S-J, Chang J-G (2005) Deregulated expression of the PER1, PER2 and PER3 genes in breast cancers. Carcinogenesis 26:1241–1246
78. Gery S, Komatsu N, Baldjyan L, Yu A, Koo D, Koeffler HP (2006) The circadian gene per1 plays an important role in cell growth and DNA damage control in human cancer cells. Mol Cell 22:375–382
79. Papp SJ, Huber A-L, Jordan SD, Kriebs A, Nguyen M, Moresco JJ, Yates JR, Lamia KA (2015) DNA damage shifts circadian clock time via Hausp-dependent Cry1 stabilization. Elife 4:e04883
80. Vander Heiden MG, Cantley LC, Thompson CB (2009) Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 324:1029–1033
81. Ma D, Panda S, Lin JD (2011) Temporal orchestration of circadian autophagy rhythm by C/EBPbeta. EMBO J 30:4642–4651
82. Arble DM, Bass J, Behn CD, Butler MP, Challet E, Czeisler C, Depner CM, Elmquist J, Franken P, Grandner MA, Hanlon EC, Keene AC, Joyner MJ, Karatsoreos I, Kern PA, Klein S, Morris CJ, Pack AI, Panda S, Ptacek LJ, Punjabi NM, Sassone-Corsi P, Scheer FA, Saxena R, Seaquest ER, Thimgan MS, Van Cauter E, Wright KP (2015) Impact of sleep and circadian disruption on energy balance and diabetes: a summary of workshop discussions. Sleep 38:1849–1860
83. Qian J, Scheer FA (2016) Circadian system and glucose metabolism: implications for physiology and disease. Trends Endocrinol Metab 27:282–293
84. Fonken LK, Workman JL, Walton JC, Weil ZM, Morris JS, Haim A, Nelson RJ (2010) Light at night increases body mass by shifting the time of food intake. Proc Natl Acad Sci U S A 107:18664–18669
85. Adamovich Y, Rousso-Noori L, Zwighaft Z, Neufeld-Cohen A, Golik M, Kraut-Cohen J, Wang M, Han X, Asher G (2014) Circadian clocks and feeding time regulate the oscillations and levels of hepatic triglycerides. Cell Metab 19:319–330
86. Chaix A, Zarrinpar A, Miu P, Panda S (2014) Time-restricted feeding is a preventative and therapeutic intervention against diverse nutritional challenges. Cell Metab 20:991–1005
87. Hatori M, Vollmers C, Zarrinpar A, Ditacchio L, Bushong EA, Gill S, Leblanc M, Chaix A, Joens M, Fitzpatrick JA, Ellisman MH, Panda S (2012) Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. Cell Metab 15:848–860.
88. Liu Z, Huang M, Wu X, Shi G, Xing L, Dong Z, Qu Z, Yan J, Yang L, Panda S, Xu Y (2014) PER1 phosphorylation specifies feeding rhythm in mice. Cell Rep 7:1509–1520
89. Garaulet M, Gomez-Abellan P, Alburquerque-Bejar JJ, Lee YC, Ordovas JM, Scheer FA (2013) Timing of food intake predicts weight loss effectiveness. Int J Obes (Lond) 37:604–611
90. Gill S, Panda S (2015) A smartphone app reveals erratic diurnal eating patterns in humans that can be modulated for health benefits. Cell Metab 22:789–798
91. Marinac CR, Natarajan L, Sears DD, Gallo LC, Hartman SJ, Arredondo E, Patterson RE (2015) Prolonged nightly fasting and breast cancer risk: findings from NHANES (2009–2010). Cancer Epidemiol Biomarkers Prev: Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol 24:783–789