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Circadian Disruption and Metabolic Disease: Findings from Animal Models

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Abstract

Social opportunities and work demands have caused humans to become increasingly active during the late evening hours, leading to a shift from the predominantly diurnal lifestyle of our ancestors to a more nocturnal one. This voluntarily decision to stay awake long into the evening hours leads to circadian disruption at the system, tissue, and cellular levels. These derangements are in turn associated with clinical impairments in metabolic processes and physiology. The use of animal models for circadian disruption provides an important opportunity to determine mechanisms by which disorganization in the circadian system can lead to metabolic dysfunction in response to genetic, environmental, and behavioral perturbations. Here we review recent key animal studies involving circadian disruption and discuss the possible translational implications of these studies for human health and particularly for the development of metabolic disease.

Keywords

Circadian Rhythms; Energy Metabolism; Cardio-metabolic Disease; Shift-work; Restricted Feeding

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Conflicts of interest
The authors have no conflict with people or organizations that inappropriately influence the content of this article.

INTRODUCTION

Circadian rhythms allow organisms to anticipate, rather than react to, daily changes in the external environment and to synchronize their behavioral and physiological processes to predictable environmental changes in order to optimize energy utilization, reproduction, and survival. While some animals evolved to be active during the night (*e.g.* mouse, rat) over the course of evolution, humans have developed to be active predominately during the day. However, unlike other animals, humans are unique in that they often voluntarily shift their activity period to an abnormal time of day, effectively forcing a misalignment between their activity period and their internal circadian clock. Behavioral modification, such as late evening activities, shift work, or jet lag from traveling rapidly across time zones can cause external and internal circadian rhythm disruption which in turn has been linked to metabolic disturbances including, under chronic conditions, obesity, metabolic syndrome, and diabetes as well as other physical and mental disorders [1–4].

Approximately 15% of Americans are employed as shift workers [5] and are forced to adopt a work-rest schedule that does not match the 24 hour solar day, resulting in "circadian misalignment". Shift work has been associated with an increased risk for obesity and its cardio-metabolic consequences [6–11]. Furthermore, the number of people experiencing circadian disruption on a daily or weekly basis is expected to increase as the trend for a non-stop 24 hour society spreads and more and more people voluntarily shift to more nocturnal activity. The use of animal models to uncover genetic and environmental links between circadian disruption and metabolic disease is essential for our understanding of the underlying mechanisms and for the development of therapeutic strategies.

In this review, we summarize key findings from animal studies which reveal the relationship between circadian disruption and metabolic disease. We begin by briefly reviewing circadian rhythms and their fundamental role in life processes. Next, we discuss how altering the timing of light cues, such as during shift work, can lead to detrimental effects on metabolism. We then focus on how disruption of the feeding/fasting rhythm may contribute to metabolic dysfunction independent of light cues. As the interaction between the circadian system and metabolism is not unidirectional, we then present evidence demonstrating that metabolic elements are capable of modifying circadian rhythms and gene expression. Indeed, the discovery of key genes and gene networks that are linked to both circadian and metabolic systems, and the use of animal models of genetic circadian disruption has begun to elucidate key mechanisms linking circadian physiology and metabolic function and dysfunction. The final section of this review discusses future implications of utilizing animal models to address important questions concerning circadian disruption and metabolic disease.

A. CIRCADIAN RHYTHMS: IN THE BRAIN AND PERIPHERY

In mammals, the master *circadian* (from Latin for "about a day") clock has been identified in the bilaterally paired suprachiasmatic nuclei (SCN) located in the anterior hypothalamus of the brain. The SCN is synchronized to the 24 hour day by light signals that stimulate melanopsin-containing retinal ganglion cells [12], which, in turn, relay light-dark information to the SCN via the retinohypothalamic tract [13]. In this way, the rising and setting of the sun dictates and entrains much of our daily rhythms via the SCN, which then relays time-of-day information to the rest of the brain as well as peripheral organs, leading to coordinated rhythms and behaviors. Sleep/wake, feeding/fasting and body temperature rhythms are examples of rhythms controlled by the circadian clock and thus synchronized to the 24 hour solar day.

In addition to feeding/fasting cycles, metabolically relevant circadian fluctuations involve glucose [14] and insulin. Oral glucose tolerance is impaired in the evening compared to the morning, an effect believed to be due to a combination of both decreased insulin secretion and altered insulin sensitivity in the evening [15]. The 'dawn phenomenon' refers to an elevation in blood glucose levels prior to the onset of the active period that has been well-documented in patients with diabetes and has been also reported in a few studies in normal volunteers [16–17].

Under normal conditions, molecular and behavioral rhythms remain synchronized by the SCN. As the SCN uses light as a *Zeitgeber* (German for "time giver"), light stimuli given at different circadian times has differential shifting effects on the SCN clock. These responses can be replicated and predictably mapped on a Phase Response Curve (PRC). For example, light occurring early during the circadian day (*i.e.* near sunrise) will advance the phase of the SCN clock as well as the phase of downstream molecular and behavioral rhythms [18]. In contrast, light occurring late in the day (*i.e.* near sunset) will phase delay the SCN clock and downstream molecular and behavioral rhythms. If no time cues, such as light, are received, the SCN will autonomously cycle with a period of about 24 hours (*e.g.* 23.5 – 24.5 hours) in animals. Lesioning the SCN causes arrhythmic locomotor behavior [19] and abolishes the diurnal variation in glucose metabolism in rats [20] as well as in most if not all endogenous 24 hour rhythms.

1. Peripheral Clocks

Under entrained light:dark (LD) conditions, the SCN orchestrates the cyclic rhythms of many behaviors, tissues, hormones, genes, and other physiological processes. While it was initially believed that the SCN was the sole clock regulating rhythms throughout the body, studies over the last decade have demonstrated that the molecular clock mechanism operates in a cell autonomous fashion in most, if not all, peripheral tissues and extra-SCN regions of the brain [21–23]. [Figure 1] For example, blood leukocytes, heart, lung, adipose, liver, and pancreatic tissues show persistent circadian rhythms *in vitro*.

While peripheral clocks can express self-sustained rhythms under normal conditions, they are synchronized to the 24 hour day by the SCN. Without the SCN, as in SCN-lesioned mice, peripheral tissues continue to cycle but gradually become out of phase with both the external LD cycle and with circadian oscillations in other tissues, a condition referred to as internal desynchronization or internal misalignment [23]. The amount of time it takes for a tissue to become misaligned is dependent on the tissue. It is also possible that in addition to desynchronization at the tissue to tissue level, individual cells within a specific tissue may become desynchronized from each other, leading to another level of internal desynchronization.

Peripheral clocks appear to be regulating rhythmicity of at least 10% of the expressed genes within each tissue, and their discovery has revolutionized our understanding of how circadian and metabolic networks overlap to regulate physiologic processes in the whole organism. Internal desynchronization likely has both direct and indirect effects on a wide range of human disorders and diseases. For example, cardiovascular risk factors have been linked to alternations in peripheral (and in some cases central) circadian clocks and rhythms (for review [1]). However, a major question is left unanswered: do desynchronized rhythms lead to cardiometabolic disease, or are they a consequence of it? Research with animal models offers a unique and powerful means to elucidate relationships, causes, and mechanisms linking metabolic disorders and circadian disruption.

2. Evolutionary Hypothesis

Circadian rhythms may have developed early in evolution as a survival mechanism to optimally synchronize the organism to the predictive changes in the diurnal, light/dark environment. When behavioral rhythms (*e.g.* the sleep/wake cycle) are entrained to the external LD cycle, specific phases (*e.g.* peaks and troughs) of rhythms in physiology and behavioral rhythms occur at the optimal time of day or night. This synchronization of internal and external rhythms is presumed to be beneficial for the organism. Often, however, it is not until our circadian rhythms are disturbed that we notice their importance for our function and well-being.

One can imagine that a nocturnal animal venturing out during the day light hours may become a meal to the predator it evolved to avoid. Although few field-based studies have been carried out, one study found that SCN-lesioned diurnal ground squirrels were more likely to be killed by an evening predator than non-lesioned controls, possibly due to abnormally high nighttime activity among the lesioned squirrels [24]. A second study found that SCN-lesioned chipmunks were more likely to be eaten by weasels, again possibly due to abnormally high activity levels during the day compared to controls [25].

While the presence of an intact circadian clock system is not essential for the survival of the organism (*i.e.* animals do not become sick and die following SCN lesion), the lack of clear health effects may be due to the inadequately challenging environment where laboratory animals reside. Rodents housed in a pathogen-free, predator-free, comfortable temperature environment with controlled LD cycles and constant food and water availability will encounter few stressors in their daily lives. However, a number of recent studies have found that when an animal is further challenged, as with genetic disposition to disease or with a disease-inducing agent, the importance of having a well-entrained circadian system becomes clear (see following section).

B. SHIFTING LIGHT: THE PRIMARY TIMING CUE OF THE SCN

To investigate circadian disruption, three types of abnormal relationship of behavioral rhythms with the LD cycle may be distinguished: 1) ‘jet lag’, in which an advance or delay in the timing of light leads to a temporary circadian disruption followed by re-entrainment to the new LD cycle, 2) ‘shift work,’ in which normally occurring diurnal behavioral and physiological rhythms are voluntarily or experimentally shifted (often repeatedly) to abnormal phases of the LD cycle, resulting in a misalignment between internal behavioral/physiological rhythms as well as a misalignment between internal rhythms and the external day/night cycle, and 3) ‘non 24 hour days,’ in which a substantial period mismatch occurs between the external LD cycle and the endogenous circadian clock that causes a lack of entrainment and continuous disruption with phases of alignment and misalignment between the internal clock and the external day. Other forms of circadian disruption can occur due to gene mutations or forced behavioral modification (*e.g.* abnormal sleep/wake, feeding/fasting cycles). While this review will focus on animal models linking circadian and metabolic dysfunction, other reviews have focused on the link between circadian clocks and immune function [2], cancer [3], and neurobehavioral disorders and dysfunctions [4].

1. ‘Jet Lag’ Models

‘Jet lag’ refers to rapid travel across time zones and in humans has been associated with gastrointestinal problems and mental dysfunction. While it has been known for some time that jet lag can cause a shift in behavioral, physiological, and hormonal rhythms in humans [26], the use of animal models has provided an explanation for the underlying mechanisms. Jet lag is of particular interest to circadian researchers due to the quick misalignment and need for subsequent re-entrainment of the circadian system. This can be mimicked in animal

models by phase shifting the LD cycle (*e.g.* by 6–14 hours), thus requiring the re-entrainment of the internal clock to the new LD cycle, a process which can take many days (*e.g.* 5–10 days). Acute single shifts and chronic multiple shifts in the LD cycle have been used in animal models for some time and have been paired with additional stressors to reveal the importance of the circadian system in overall fitness. A single 6 hour phase advance, for example, can lead to differential tissue re-entrainment time among peripheral tissues. Notably the SCN's core clock gene, *Per1*, entrains to the new light cycle time very quickly (*e.g.* within 1 day) while other tissues like the lung, muscle, or liver take 6 or more days [22]. Tissue-dependent resetting times are seen during phase delays as well, indicating that some tissues are able to re-entrain to jet lag more quickly than others. The differential re-entrainment times can therefore lead to periods where some tissues are entrained while others are not.

2. 'Shift-Work' Models

As mentioned previously, few studies have indicated that the circadian clock by itself is essential for life of the organism. If you remove the heart, the animal dies. But, if you remove the circadian clock, the animal appears to function just fine. While in normal healthy animals, acute or chronic phase shifts cause little or no adverse effects, if the animal is sufficiently challenged, the adverse effects of circadian disruption (*e.g.* in animal models of shift-work) become more apparent. For example, aged mice given repeated 6 hour phase advances have increased mortality compared to control mice or mice exposed to repeated 6 hour phase delays [27]. Chronic weekly reversal of a 12 hour light, 12 hour dark cycle in cardiomyopathic hamsters results in a significant reduction in survival time and accelerated cardiomyopathy [28]. If a colitis-inducing drug (DSS) is added to the drinking water, chronic shifts in the LD cycle cause more severe body weight loss and overall poor health compared to non-shifted animals [29]. Repeated and chronic phase shifts in the LD cycle have also been reported to promote cancer growth, a further indication that a consistently desynchronized circadian rhythm is detrimental to health [30].

In the phase shift studies mentioned above, animals are shifted and are re-entraining constantly to a new LD cycle. While these studies demonstrate the importance of internal synchrony, they do not really mimic shift work as experienced by most humans. Recent, high fidelity animal studies better mimic human shift workers by using a protocol which maintains rats on a stable LD cycle but forces the rats to be active during the light (rest) phase. In such studies, rats are placed in a very slowly rotating wheel 8 hours a day during 'work' hours while having free access to food and water. This 'work' during the day is sufficient to cause a shift in some circadian rhythms, including activity and feeding. In addition, the shift work protocol leads to metabolic disturbances such as the loss of glucose rhythmicity, an inverted triglyceride rhythm, and an increase in body weight [31]. Of note, this animal shift work protocol allowed 'weekends off' during which the rats were not forced to work and could choose at which time they were active. This parallels the human shift worker who may work the night shift during the 5 day work week, but attempt to be active during the day on the weekends for social engagements.

It is important to note that not all human shift workers try to revert to a normal diurnal rhythm during the weekends. Some essentially remain permanent shift workers while others may shift on irregular and unpredictable schedules. This added component of shifting schedules could potentially be leading to or exaggerating metabolic impairments from the already present circadian disturbance during shift work. In other words, in addition to disruption due to misalignment (*e.g.* being desynchronized), shift workers are also repeatedly shifting (*e.g.* resynchronizing), causing the circadian system a double stress: one from the working hours occurring during the 'wrong' circadian phase (as well as sleep at the

'wrong' circadian phase) and the other from having to constantly re-synchronize to the weekend or to the next work schedule.

3. 'Non 24 hour days'

As the period of the internal circadian clock is close to 24 hours, placing an animal or a human on a lengthened or shortened day challenges the circadian system on a constant basis, thus forcing abnormal or failed entrainment to the LD cycle. For example, when healthy, wild type mice are fed a high fat diet and placed on a 6 hour day (3L:3D), body weight and blood glucose levels are significantly elevated compared to high fat fed control mice on a 12L:12D cycle [32], suggesting that a mismatch between the external LD cycle and the internal clock may induce and/or exaggerate metabolic impairments.

The converse is also true in that a mutant animal with a non-24 hour internal clock displays metabolic improvement when placed on a LD cycle close to its internal period. The heterozygous *tau* mutant (*tau*/+) hamster is characterized by a spontaneous mutation which shortens the animal's circadian period from 24 to 22 hours. Early studies indicated that the *tau*/+ mutant has a reduced lifespan compared to controls [33] and develops severe renal and cardiac disease [34] when maintained under normal 24 hour LD cycles. However, when *tau*/+ mutant hamsters were housed under a 22 hour light cycle (to match their endogenous circadian period), the adverse renal and cardiac phenotypes failed to develop, thus indicating the importance of matching the internal period with the external LD cycle. What makes the finding of such profound cardiovascular and renal disease in *tau*/+ hamsters entrained to a 24 hour (but not 22 hour) LD cycle particularly noteworthy is that this was the first demonstration of circadian disorganization as a causal risk factor for organ disease [34]. Interestingly, homozygous *tau* mutant (*tau*/*tau*) hamsters do not show the cardiorenal pathology during exposure to a 24 hour day, presumably because their extremely short circadian period (about 20 hours) does not allow hamsters to readily synchronize to a 24 hour LD cycle [35]; instead the homozygous *tau* mutant animal is in a free-running circadian state. This suggests that the cardiorenal pathology observed in the *tau*/+ hamster is due to circadian misalignment rather than a direct gene effect. Furthermore, ablating the SCN within young *tau*/+ mutant hamsters prevented the development of renal and cardiac disease, indicating that, at least in a safe laboratory environment, "no clock is preferable to an incorrect clock".

Non-24 hour "forced desynchrony" protocols in humans have supported the hypothesis that circadian misalignment can lead to metabolic dysfunction. Placing humans on a 28 hour day, for example, prevents entrainment to the LD cycle and, over the period of several days, causes subjects to experience varying degrees of misalignment of their sleep/wake cycle from their endogenous circadian rhythms [36]. During this misalignment protocol, the subjects ate and slept according to the experimental 28 hour day, while their internal circadian clock continued to cycle at approximately 24 hours causing a misalignment during most days on the protocol between the behavioral sleep/wake and LD cycles and internal rhythms. Most strikingly, when behavioral rhythms of feeding and sleeping were 12 hours out of phase with the endogenous circadian rhythm as assessed by the 24 hour rhythm of melatonin levels, subjects exhibited the most profound alterations in metabolism, including increased glucose and insulin levels, decreased leptin levels and elevated mean arterial pressure [36]. Furthermore, a 12 hour misalignment was associated with a reversal of the cortisol rhythm and reduced sleep efficiency.

4. Shifting Light: Other Variables to Consider

Jet lag, shift work, and non-24 hour days all typically allow the laboratory animal free access to food, *i.e.* the animals are feed *ad libitum*. While humans typically have free access

to unlimited amounts of food, often cultural or work-related schedules promote eating at specific times. For example, the night-shift worker will eat meals at night during scheduled breaks. During jet lag, social events based around meals encourage eating at specific times, even if those meal times do not align with habitual feeding times and appetite rhythms. For this reason, many circadian disruption protocols implemented in animals fall short of mimicking important circumstances of circadian disruption in humans. Since the feeding/fasting cycle plays such a major role in metabolism, mimicking shifts in feeding times may be especially important in developing an adequate animal model for shift work and metabolic effects. Another confounding factor in studies of circadian disruption is sleep restriction and/or deprivation. Recently, pivotal studies have indicated that sleep restriction alone can lead to drastic changes in metabolism [37], and since some amount of sleep loss usually occurs during circadian disruption, it remains important to determine which aspects of metabolic dysfunction are due to sleep restriction and which are the result of circadian disruption independent of sleep restriction.

C. SHIFTING FOOD: THE CUE FOR THE FOOD ENTRAINABLE OSCILLATOR (FEO)

For some time, there has been speculation about another internal oscillator specifically responsive to feeding cues, termed the “Food Entrainable Oscillator” (FEO). Early experiments observed an increase in locomotor activity 1–2 hours prior to predictable food presentation, termed Food Anticipatory Activity (FAA), when food availability was restricted to a small window in the light phase in nocturnal animals, a protocol referred to as “restricted feeding” (RF). Recently, a network of brain regions within the hypothalamus, instead of a single region, has been suggested to be acting as the FEO [38–39] in which feeding cues received during the ‘wrong’ circadian phase cause the FEO to relieve or inhibit the normal inhibition of locomotor activity exerted by the SCN during the light phase. This process of “inhibiting the inhibitor” allows nocturnal animals to become temporary active during the light phase and leads to FAA.

1. An Entrained Disconnect Between the SCN and the FEO

During RF, some behavioral and molecular rhythms entrain to the timing of food presentation and persist even when the animal is fasted. Naturally, feeding rhythms will entrain to the feeding time, but peripheral clock expression in metabolic tissues (*e.g*. the liver) and hormonal rhythms (*e.g.* leptin) will also shift their peak time to concur with feeding. The expression of circadian clock genes (*e.g. period* genes) in metabolic peripheral tissues continue to peak with the onset of food availability despite removal of the food cue. While FAA will continue at the original food presentation time for at least three days of fasting, suggesting the presence of a true, sustained circadian rhythm, it is important to note that overall activity remains entrained to the SCN and LD cycle and does not invert or become arrhythmic (for review see Mistlberger [40]). The continuous cycling of gene expression, hormone peaks, and FAA, even in the absence of food cues, indicates that the animal is both predicting and synchronizing to the food cue and that there is another oscillator capable of entraining at least some rhythms to the timing of food availability. This equates to two separate circadian components under RF, one driven by the SCN that remains synchronized and entrained to the LD cycle (*e.g*. activity) and another driven by food and synchronized to the food presentation time (*e.g.* metabolic peripheral tissues).

By using a RF protocol, it is possible to cause a disconnect and misalignment between the light (SCN) and food-entrainable oscillators (FEO). Since mice and rats are naturally nocturnal feeders, limiting food access to the light phase causes some behaviors, such as feeding dependent hormonal rhythms, and the expression of circadian clock genes and clock

controlled genes within metabolic tissues (*e.g.* the liver) to become entrained to the novel feeding time [41]. The rodent liver, an important metabolic organ with well-characterized rhythms of circadian clock gene and clock controlled gene expression, tends to show peak circadian expression (*e.g. period* genes) during the dark (active) phase and troughs during the light (inactive) phase. Studies have found that the peak in clock gene expression in the liver is highly correlated to circadian feeding rhythms [41] and that the entrainment to food cues can occur quickly – in a day or less [42]. Consequently, these feeding entrained rhythms become misaligned from the SCN which remains synchronized to light cues. Therefore, RF creates a disconnect between the light-entrained rhythms and the food-entrained rhythms, whereas under *ad libitum* conditions, the food-entrained rhythms remain synchronized and controlled by the SCN.

The observation of a FEO affecting some rhythms while others remain entrained to the SCN [41] has enabled researchers to examine a unique challenge to the circadian system in which the feeding-dependent and light-dependent rhythms are out of phase with one another. In other words, during RF, feeding and some metabolic rhythms become entrained to the food presentation time, while other rhythms, behaviors, and tissues remain synchronized to the SCN. Thus, several organs, tissues, and behaviors are entrained by food availability, and temporally disconnected from the SCN. Utilizing this FEO/SCN dissociation offers an exciting window through which new insights about the link between circadian disruption and metabolism can be explored.

Recent association studies in humans suggest that mistimed feeding is linked to weight gain, obesity, and metabolic syndrome, creating a demand for further research using animal models. For example, patients with ‘Night-Eating Syndrome’ (NES) consume a high proportion of their calories during the evening, including awakenings each night to consume additional calories. The NES patient’s shift in feeding rhythms may cause a maladaptive alignment between metabolic signals and the LD cycle which could lead to predisposition to metabolic syndrome [43]. Furthermore, non-breakfast eaters, including subjects with NES, show a shift in feeding patterns toward the evening and are more likely to be overweight than individuals eating breakfast [44–46]. It is possible that the circadian disruption resulting from consuming calories during the ‘wrong’ circadian time may lead to weight gain, indicating that meal timing may be a key factor contributing to metabolic impairment seen with circadian disruption.

2. The Timing of Feeding and Metabolism

Previously, the focus of RF protocols has been to examine the entrainment and re-entrainment of behavioral rhythms as well as gene expression in various brain regions and peripheral tissues, with only a small emphasis on the metabolically relevant tissues. More recently, RF protocols are being used to examine a new theme – how feeding during the ‘wrong’ time of day can affect metabolism. We have recently obtained the first causal evidence indicating that feeding at the ‘wrong’ circadian time can lead to weight gain. In this experiment, mice were fed a high fat diet during either the 12 hour light phase or the 12 hour dark phase. After 6 weeks of abnormally timed feeding, mice fed during the light phase gained 2.5 times more weight than those eating during their normal nocturnal hours [47] [Figure 2]. Although locomotor activity (measured by infra-red beam break) and caloric intake were not statistically significantly different between the light and dark-fed conditions, the light-fed mice were on average consistently less active than the dark-fed group over time, indicating a decrease in energy expenditure. Furthermore, the light-fed group also consumed slightly more calories than the dark-fed group. The impact of reduced energy expenditure and increased energy intake is likely to be additive and contribute to differences in body weights between the two groups. Although the mechanism behind light-fed weight gain in rodents is unknown, alterations in body temperature regulation, circulating levels of

satiety hormones and sleep could be contributing to the phenotype. Light phase feeding has been noted to cause a decrease in dark phase body temperature [41] which could contribute to increased weight gain. Circulating satiety hormones, such as leptin, may have a circadian variation independent of meal timing and be contributing to food intake levels as seen with humans on a circadian misaligned schedule [36]. Sleep restriction or poor sleep quality could also be leading to weight gain [37], although our preliminary data [48] indicate no overall sleep differences between light and dark-fed mice [Figure 3].

The results from other studies have supported the hypothesis that the timing of feeding is an important factor in weight gain. For example, when rats on a shift work protocol are forced to work during the day, but are restricted to eating only during the night, excess weight gain, usually gained from abnormally timed feeding, is prevented, thus supporting a role for feeding time as an important factor in circadian disruption and metabolism [49].

While numerous association studies have observed the relationship between abnormally timed feeding (*e.g.* in shift workers, non-breakfast eaters, patients with NES) and increased BMI in humans, no human circadian study has systematically tested the causal effect of abnormally timed feeding on body weight in a controlled laboratory setting where total caloric intake is held constant. For example, how do normal, healthy subjects who eat from, for example, 8am – 8pm daily compare in body weight and metabolic characteristics to those who are restricted to eating earlier in the day (*e.g.* 5am – 5pm) or very late in the day (*e.g.* 2pm – 2am). While some diet books and old adages (*e.g.* “Eat breakfast like a king, lunch like a prince, and dinner like a pauper.”) claim that concentrating your calories toward the early day can be beneficial, a proper controlled clinical circadian feeding study has yet to be carried out.

D. HOW METABOLISM CAN SHAPE RHYTHMS

While disrupted rhythms can lead to metabolic impairments, the reverse is also true – disrupting metabolism can alter circadian rhythms. Feeding mice a high fat diet, for example, has been shown to cause three main changes in circadian rhythms: lengthened period, blunted feeding rhythm, and alterations in the expression of circadian clock genes [50]. When placed into constant conditions, mice fed a high fat diet display a lengthening of free-running period compared to low fat fed controls. This may be due in part to altered synchronization to light cues [51]. Interestingly, the time at which mice consume the high fat food is also altered with a substantial increase in calories during the light phase, effectively ‘blunting’ the feeding rhythm (*e.g.* causing a decrease in the day – night difference). Furthermore, these additional light phase calories can account for the observed body weight increase, supporting the hypothesis that animals eating during the ‘wrong’ time have an increased likelihood of weight gain. In addition to a change in the feeding rhythm, the rhythmicity and protein transcript levels are attenuated in canonical clock genes, hormone receptors that regulate the clock, and clock-controlled genes involved in fuel utilization in the hypothalamus and peripheral tissues when mice are fed a high fat diet [50].

While the metabolic environment can lead to changes in rhythms, so can changes in the expression of metabolic genes. Mutations in the leptin gene, a satiety hormone released by the adipose tissue, can lead to obesity and also to an abnormal circadian phenotype. Leptin-deficient *ob/ob* mice contain a mutation in the leptin hormone rendering leptin unable to bind to receptors, and mice become obese, hyperphagic, and develop the metabolic syndrome. This metabolic mutation also causes disturbed sleep, attenuated diurnal and overall decreased locomotor activity, and attenuated feeding rhythm behavior [52], indicating a deterioration in sleep and circadian homeostasis. Within the *db/db* mouse, the long form of the leptin receptor is altered rendering leptin signaling ineffective. The *db/db*

mouse consumes more calories throughout the day, typically by consuming larger meals [53]. In general, *ob/ob* and *db/db* mice are similar phenotypically, both developing type 2 diabetes around 3–4 weeks of age, are hyperinsulinemic and later become hypoinsulinemic at 2–3 months of age. Both also have attenuated eating patterns, consuming a significantly greater amount of calories during the light phase compared to wild type animals.

E. INTERPLAY BETWEEN CORE CLOCK GENES AND METABOLISM

Genetic polymorphisms within the core clock genes *Clock* and *Bmal1* are also associated with metabolic phenotypes, including obesity, hypertension, and type 2 diabetes [54–56]. A series of transcription/translation feedback loops comprise the genetic underpinnings of the core molecular clock that drives the molecular and physiological circadian rhythms in mammals (for review see [57–60]). Briefly, the core clock genes CLOCK and BMAL1 form a heterodimer that activates transcription of the *period* (*Per1*, *Per2*, and *Per3*) and *crypotochrome* (*Cry1* and *Cry 2*) genes. The PERs and CRYs subsequently heterodimerize, translocate back to the nucleus, and inhibit the transcriptional activity of CLOCK:BMAL1, closing a transcriptional/translational feedback loop that represents a central core of the molecular circadian clock.

1. Clock-Controlled Target Genes and Circadian Control of Metabolism

While the core clock components have been well defined, much less well understood is how the array of downstream clock controlled target genes collectively act to regulate circadian rhythms on a tissue-specific and physiologic level. Microarray analyses have revealed that 3–20% of the entire transcriptome displays 24 hour oscillations of gene expression in various tissues, many of which are key rate-limiting enzymes involved in metabolic processes such as lipid metabolism, gluconeogenesis, and oxidative phosphorylation [61–62]. Of note, there is a high degree of tissue-specificity in terms of oscillation of target genes – only a small subset of genes show similar patterns of gene expression between liver and heart. A major area of current research therefore involves investigation of how tissue-specific rhythmic gene expression allows each cell/tissue to function optimally at the appropriate time within the LD cycle. We are just beginning to appreciate the implications of coordination of the timing of gene expression of key metabolic genes within different tissues with the fasting/sleep and feeding/wake cycles. For example, expression of genes involved in glucose production, glycogen breakdown, and fatty acid oxidation are elevated within liver to enable the organism to maintain glucose homeostasis in the fasted state, while gastrointestinal tract enzymes critical for appropriate nutrient absorption are elevated during the fed state [63]. Much remains to be learned, however, regarding how the circadian patterns of metabolic gene expression may facilitate the switch between the daily cycles of fasting and feeding in a tissue-specific manner.

2. Nutrient Sensors at the Intersection of Clock and Metabolic Pathways

A critical question that has emerged from the above studies concerns the nature of the molecular link between the circadian and metabolic pathways. Is there a molecular ‘sensor’ that is common to both pathways that can act to synchronize the external nutritional status of the organism with the core circadian network in order to optimize fuel harvesting and utilization with the daily cycles of fasting and feeding? Here, we briefly describe two families of proteins that have recently been identified as being able to “sense” or receive nutrient information from the environment and in turn translate this information to the clock.

First, several nuclear hormone receptor proteins, which are activated by the binding of dietary lipids and fat-soluble hormones, are not only regulated by the clock, but also directly regulate core clock components themselves as well as downstream metabolic targets, thus a

complex feedback loop links nutrient status with the core clock network and metabolic outputs. For example, REV-ERBa3 a direct target of CLOCK:BMAL1, represses *Bmal1* transcription in addition to its role as a regulator of hepatic gluconeogenesis, adipocyte differentiation, and lipid metabolism [64–66]. Similarly, RORa and PPAR, both positive regulators of *Bmal1* transcription, are involved in lipogenesis and lipid and glucose metabolism [67–68].

A second family of proteins to emerge at the intersection of the circadian and metabolic pathways are those involved with NAD biosynthesis and the NAD-dependent deacetylase SIRT1. CLOCK:BMAL1 directly regulate the expression of the gene encoding the rate-limiting enzyme in NAD biosynthesis, nicotinamide phosphoribosyltransferase (NAMPT), in peripheral tissues [69–70]. Not only is NAD critical for cellular redox reactions, but it also serves as a substrate for the NAD-dependent and nutrient responsive deacetylase SIRT1, which negatively regulates the core molecular clock machinery [71–72]. Similar to the nuclear hormone receptor family of proteins, both NAMPT and SIRT1 are regulated not only by the molecular circadian clock, but also by the nutritional status of the organism. For example, decreased glucose levels in skeletal muscle elevate *Nampt* expression in an AMPK-dependent manner [73–74], and fasting/caloric restriction elevates SIRT1 levels in multiple tissues [75–78]. Furthermore, both NAD and SIRT1, in addition to their regulation of the core clock machinery, regulate a host of downstream metabolic processes, including glucose-stimulated insulin secretion, adipocyte differentiation, and gluconeogenesis [79]. The identification of these “nutrient sensors” at the intersection of the circadian and metabolic pathways likely holds the key to further understanding how individual tissues coordinate daily cycles of feeding, fuel utilization, sleep, and activity.

F. GENETIC MODELS FOR EXAMINING THE EFFECTS OF DISRUPTED RHYTHMS AND DISEASE

Cloning the *Clock* gene and elucidating its effects on metabolism, coupled with the discovery that core clock genes interact with energy regulating orphan receptor genes [80], has opened up a new era in the field of circadian rhythms and metabolism. A mutation in the key circadian gene, *Clock*, within a mouse with a C57BL/6J background provided one of the first genetic links between the circadian and metabolic systems [81]. The *Clock* mutant mouse has a lengthened free-running period, is hyperphagic and obese, and develops some signs of metabolic syndrome (*i.e.* hyperlipidemia and hyperglycemia) by 6 months of age [82]. *Clock* mice also have attenuated day – night differences in activity and feeding. In fact, the excess calories consumed during the light phase appear to account for the increase weight gain in *Clock* mutant mice [82], a finding which supports the hypothesis that feeding at the ‘wrong’ time of day can lead to excess weight gain.

Following the metabolic studies in the *Clock* mutant animal, other circadian core genes and clock controlled genes were examined for their effects on metabolism. *Bmal1* knockout mice display a complete loss of rhythmicity in constant darkness [83] and have similar metabolic defects as the *Clock* mutant, including decreased gluconeogenesis, loss of the normal diurnal oscillations of glucose and triglycerides, increased whole body insulin sensitivity, and impaired glucose-stimulated insulin secretion [84–85]. BMAL1 also plays an important role in the regulation of adipocyte differentiation and lipogenesis [86]. *Per2* mutant mice have increased bone mass and lack glucocorticoid rhythms [87–88]. Mice lacking both *Cry1* and *Cry2* display altered patterns of circulating growth hormone (*e.g.* less non-secreting episodes resulting in overall higher circulating growth hormone levels) and sexually-dimorphic metabolic genes in liver [89], and transgenic mice overexpressing mutant CRY1 develop symptoms of the metabolic syndrome, including polydipsia, polyuria, and hyperglycemia [90].

A question that arises from these animal genetic models concerns whether the metabolic phenotypes are due to the disruption of the core clock mechanism itself, or whether they are secondary to altered feeding patterns present in these mice. [Figure 4.] Recent evidence suggests that the metabolic phenotypes are indeed due to disruptions in the core clock machinery, as mice with selective ablation of *Bmal1* in the liver still displayed fasting hypoglycemia and altered glucose clearance despite having normal locomotor activity and feeding patterns [91–92]. Furthermore, selective ablation of *Bmal1* within pancreatic β–cells revealed a cell autonomous role of the pancreatic clock in regulation of glucose-stimulated insulin secretion [85]. Future studies with additional tissue-specific manipulations of the core clock components are expected to shed light on the role of individual tissue oscillators and on the molecular mechanisms underlying the metabolic disease phenotypes observed in circadian mutant mice. Further research is needed to clearly define the relative role of genes, behavior, and environment on circadian disruption and metabolism.

Summary

A substantial portion of the population is affected by some form of circadian disruption (*e.g.* shift work, jet lag), and as the trend for a ‘24 hour’ society continues, more and more people are becoming increasingly active during the nocturnal hours as opposed to the diurnal hours when our human ancestors evolved to be active during the light portion of the 24 hour day. While the association between circadian disruption and metabolism has been observed for some time in humans (*e.g.* shift workers), the underlying mechanisms that lead to the development of metabolic disease, obesity, diabetes, and cardiometabolic disorders remain poorly understood, leading to the need for animal models to elucidate how circadian dysfunction can lead to various pathologies. Animal models of circadian disruption have primarily focused on shifting the LD cycle to imitate human jet lag or shift work. These light shifting protocols have merit in examining the negative effects of shifting the circadian clock; however they do not fully mirror what is happening in the human who often also experiences a shift in feeding rhythms in order to accommodate work or social settings. Restricted feeding protocols in animals allow examination of the effects of abnormal alignment between the LD cycle and the feeding rhythm. Recent research has supported the hypothesis that the timing of feeding alone may have an important impact on metabolism. It is important to note that just as rhythms can shape metabolism, metabolism can also shape rhythms as seen when environmental and genetic changes to metabolism cause rhythm abnormalities. The discovery that the circadian clock and metabolic systems are interrelated at the molecular and genetic level in various animal models has opened up new avenues of discovery into the mechanisms behind circadian disruption and metabolic disease in humans.

Practice Points

- Animal studies indicate that the increased weight gain observed within the shift work population is caused, at least in part, by eating during the ‘wrong’ time of day.
- While controlled, laboratory studies in rodents find few health impairments following acute or chronic phase shifts in the circadian clock (*e.g.* during jet-lag), additional stressors such as increased age, or genetic or environmental stressors reveal detriments to health associated with chronic shifts in the LD cycle.

Research Agenda

- What specific aspects of circadian disruption lead to metabolic disease? For example, is there a difference in metabolic outcomes between circadian misalignment and repeated re-entrainment conditions?
- How can we create animal models that better mirror circadian disruption that occurs in the human population?
- Is it possible to alter circadian rhythms in such a way as to obtain beneficial effects on metabolism?
- What are the underlying molecular/cellular mechanisms which lead to metabolic disease from circadian disruption? Do these differ under different circadian disruption conditions (e.g. misalignment vs. re-entrainment; acute vs. chronic disruption)?
- If disruption of central and peripheral rhythms can result in metabolic disease, are the effects due to central and peripheral disruption together or can central or peripheral disruption independently lead to metabolic impairment?
- What other variables may contribute to metabolic disturbance from circadian disruption (e.g. aging, stress, hormones)?

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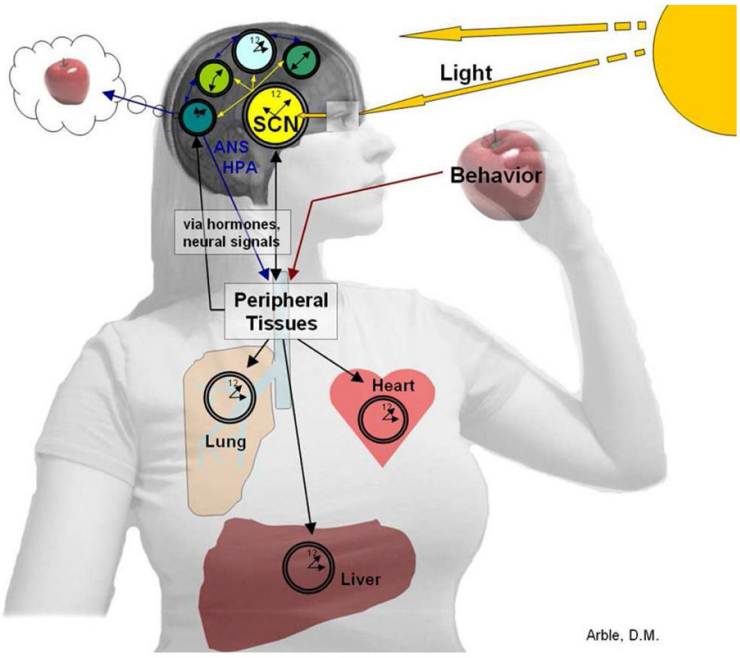

Figure 1.

Light entering the retina stimulates specialized photoreceptors and send signals to the suprachiasmatic nuclei (SCN) via the retinohypothalamic tract. The SCN then orchestrates the timing of other brain regions. These brain regions can then influence one other, cause behavior changes and send timing cues to peripheral tissues using hormones and neural signals through, for example, the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS). Behaviors such as feeding can also directly influence the expression of circadian clock and clock controlled genes within peripheral tissues. Hormones and neural signals originating from the periphery can then feedback to the SCN and other brain regions to influence circadian rhythms and genes.

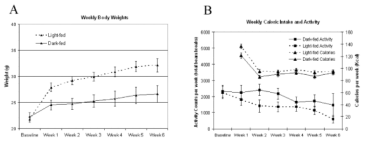

Figure 2.

The effect of circadian feeding on body weight, caloric intake, and activity. (**a**) The effect of light or dark phase feeding on body weight. Body weight (mean ± s.e.m.) of C5BL/6J mice fed 60% high fat only during the 12-h light phase (dashed line) or only during the 12-h dark phase (solid line). Body weights were taken at the end of the 12-h feeding phase in all animals. Within 2 weeks of maintenance on the high fat diet, the light-fed animals weighed significantly more than the dark-fed animals (*$p < 0.05$ light-fed vs. dark-fed) and remained significantly heavier over the next 4 weeks. (**b**) Weekly activity counts and caloric intake. Total weekly activity counts (squares) and calories (kcal, triangles) are depicted for both light-fed (dashed line) and dark-fed (solid line) groups (mean ± s.e.m.). Note that while over the 6-week period neither activity nor caloric intake differed significantly ($p > 0.10$), the light-fed group appears to be consuming more calories and moving less than the dark-fed group. This raised the possibility that the additive effect of a small increase in caloric intake and a small decrease in activity can together contribute to specific differences in body weight.

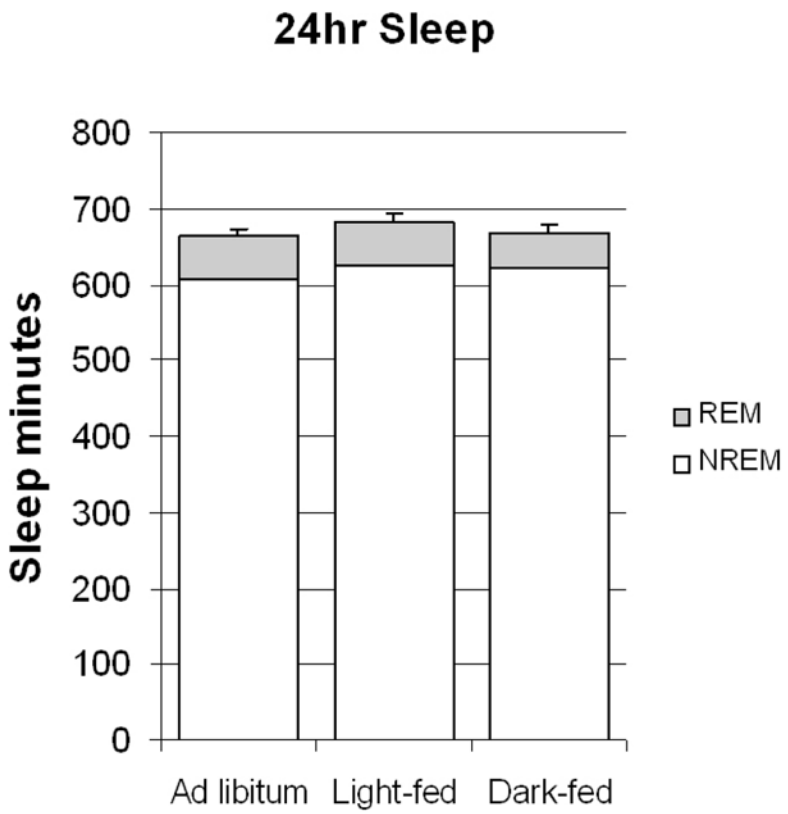

Figure 3.
Sleep During Phase Restricted Feeding. Male, 6-week old C5BL/6J mice were fed a standard diet either *ad libitum* (Ad libitum), only during the 12 hour light phase (Light-fed), or only during the 12 hour dark phase (Dark-fed). Sleep recording from week 5 of restricted feeding indicates no differences among the groups in NREM (white bars), REM (gray bars), or total sleep time over the 24 hour day.

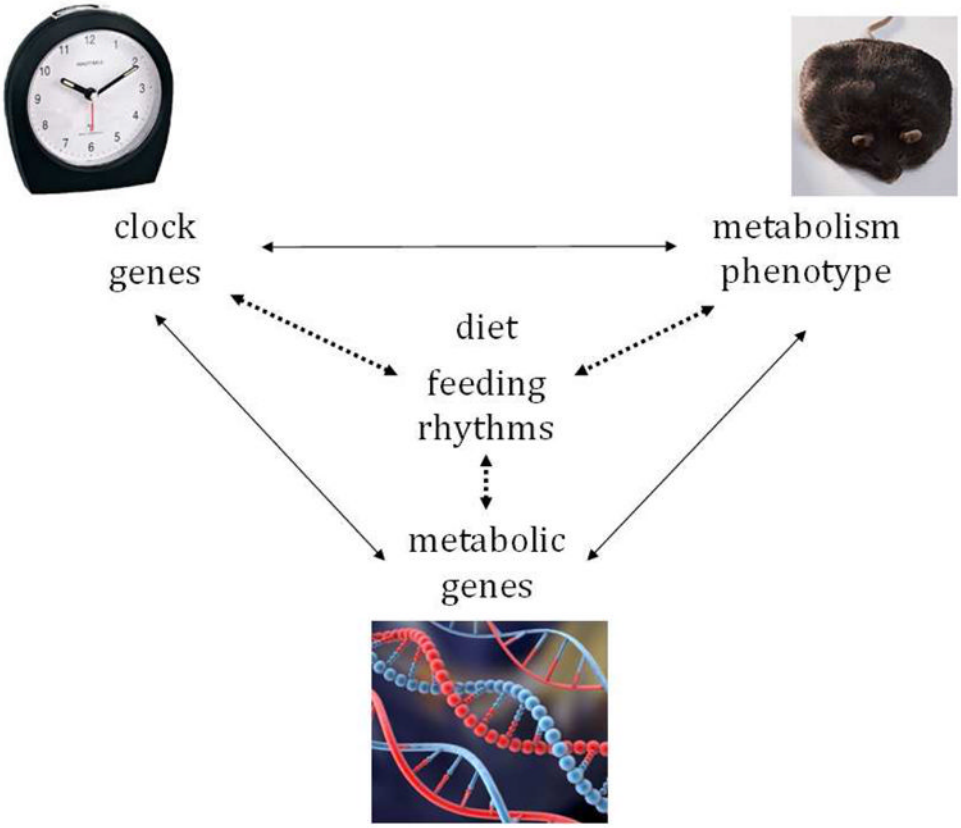

Figure 4.

Clock and metabolic genes interact with each other and can independently influence the metabolic phenotype seen in rodent models. Circadian rhythms, such as the feeding rhythm, and environment (*e.g.* diet), may also feedback and influence gene expression and metabolism characteristics.