

## Plasticity of circadian clocks and consequences for metabolism

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The increased prevalence of metabolic disorders and obesity in modern society, together with the widespread use of artificial light at night, have led researchers to investigate whether altered patterns of light exposure contribute to metabolic disorders. This article discusses the experimental evidence that perturbed environmental cycles induce rhythm disorders in the circadian system, thus leading to metabolic disorders. This notion is generally supported by animal studies. Distorted environmental cycles, including continuous exposure to light, affect the neuronal organization of the central circadian pacemaker in the suprachiasmatic nucleus (SCN), its waveform and amplitude of the rhythm in electrical activity. Moreover, repeated exposure to a shifted light cycle or the application of dim light at night are environmental cues that cause a change in SCN function. The effects on the SCN waveform are the result of changes in synchronization among the SCN's neuronal cell population, which lead consistently to metabolic disturbances. Furthermore, we discuss the effects of sleep deprivation and the time of feeding on metabolism, as these factors are associated with exposure to disturbed environmental cycles. Finally, we suggest that these experimental studies reveal a causal relationship between the rhythm disorders and the metabolic disorders observed in epidemiological studies performed in humans.

**Keywords:** circadian, desynchronization, suprachiasmatic nucleus, metabolic disorders, light

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### Environmental Effects on the Rhythm of the SCN

Perturbations in the circadian rhythm can develop under a variety of conditions, including ageing, neurodegenerative diseases and metabolic disorders. In order to effectively treat these diseases, it is important to elucidate the causal relationships behind these associations. Towards that end, several animal models have been used to specifically address the relationship between circadian disorders and metabolic disturbances. These models include transgenic mouse models with specific mutations in clock genes, as well as animals in which the function of the suprachiasmatic nucleus (SCN) has been altered by manipulating environmental cues. Transgenic models have been particularly valuable for elucidating the signalling pathways involved in the pathogenesis of metabolic disorders such as diabetes and obesity [1]. Moreover, performing complementary studies in wild-type animals and studying the effect of the environment on SCN functions are valuable as well, given the growing body of evidence suggesting that improper circadian timing interferes with health. For example, sleep disruption in humans is correlated with an increased risk of developing diabetes, and shift work has been associated with increased body mass index (BMI), altered plasma lipids and impaired insulin sensitivity [2–4] (Table 1). These studies indicate a potentially large influence of stable, robust environmental light–dark cycles on the maintenance of health.

### Environmental Manipulations in Animal Models

Several studies manipulated the environmental light conditions and examined the resulting effects on metabolic parameters (Table 2). These manipulations include repeated shifts in the light–dark cycle, thereby interfering with ongoing light–dark cycles (for example, to mimic shift work) [5]. Other studies have explored the effect of continuous exposure to light on metabolism, thereby examining the role of desynchronized SCN pacemaker activity on disease [6,7]. This paradigm reduces the rhythm amplitude (as in ageing), rather than inducing a continuously disrupted rhythm (as in shift work). A third approach has been to expose animals to a standard light–dark cycle, but with the introduction of dim light at night (dLAN) [8]. This protocol mimics the widespread increase in the use of artificial lighting at night in modern society. The results of these studies consistently show the robust effects of environmental conditions on metabolism. Therefore, these results indicate that environmental light conditions play a causal role in the pathogenesis of metabolic disorders by interfering with the central clock in the SCN. Thus, understanding the organization of this central clock is extremely relevant to both health and disease.

### The SCN Neuronal Network

The SCN is a bilaterally organized structure that contains approximately 20 000 neurones in rodents. Each neurone generates a circadian rhythm in electrical activity, with high ensemble neuronal activity in the middle of the subjective day [9]. Molecular and cytosolic clocks interact to produce a circadian rhythm in cellular physiology and neuronal excitability [10,11]. The intracellular clock controls ion conductances,

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thus leading to a rhythm in neuronal activity [12]. Individual neurones in the SCN are electrically active for surprisingly short periods (approximately 5 h) within the 24-h cycle, and their individual rhythms are not entirely in phase [13,14]. This relative desynchronization is an indication of a network that is both under pressure (also referred to as a ‘frustrated network’) and highly plastic. Under the influence of environmental conditions, synchronization can either increase or decrease, leading to a more compressed or decompressed waveform of the neuronal population.

Neurotransmitters [e.g. vasoactive intestinal peptide (VIP) and  $\gamma$ -aminobutyric acid (GABA)] and gap junctions play an important role in the regulation of phase synchrony [15]. When an animal is entrained to a short photoperiod, neuronal synchrony increases. At the behavioural level, this is reflected by a short resting period and a long active period in nocturnal animals such as mice. When entrained to a long

photoperiod, neurones become desynchronized, and the animal’s active period shortens accordingly. Desynchronization among neurones causes a relative flattening of the SCN’s ensemble rhythm, as relatively fewer neurones are active at the same time. An extreme case of desynchronization occurs under continuous light, in which neurones desynchronize even further, leading to extremely low amplitude rhythms and – in some cases – arrhythmia [16]. Continuous exposure to light eventually leads to metabolic disorders such as insulin resistance and obesity [6,7].

“Circadian disruption results in desynchronization among neurons of the SCN, which can decrease the circadian rhythm amplitude and eventually lead to metabolic disorders such as obesity and insulin resistance.”

**Table 1.** Metabolic consequences of circadian disruption.

Circadian disruption	Species	Metabolic consequences	Reference
Continuous light	Rats	Decreased daily food and water intake and increased visceral adiposity (not body weight)	[48]
		Increased plasma cholesterol levels	[46]
		Wild type rats: no effect on insulin secretion or insulin sensitivity	[49]
		Diabetes-prone HIP rats: increased plasma glucose levels and reduced insulin secretion	[49]
Dim light at night	Mice	Increased body weight	[6,7]
	Mice	Loss of circadian rhythmicity in energy metabolism and insulin sensitivity	[6]
10 h light–10 h dark 3 h light–3 h dark	Rats	Suppressed melatonin night-surge	[52]
	Mice	Decreased rhythmic expression of clock genes in the liver	[8]
Jet lag protocol	Mice	Increased body weight, reduced glucose tolerance and altered circadian pattern in food intake	[54]
		Increased body weight and insulin and leptin levels	[55]
Shift work	Mice	High-fat diet: increased body weight, but decreased food intake	[57]
		High-fat/high-sucrose diet: hypercholesterolaemia, hyperglycaemia and glucose intolerance, but no effect on body weight or food intake	[58]
Sleep deprivation	Mice	No effect on body mass or glucose levels	[56]
		High-fat/high-sucrose diet: altered intestinal microbiota	[59]
Eating during the resting phase	Rats	Increased body weight, increased abdominal fat, arrhythmic blood glucose levels and reduced glucose tolerance	[5]
	Humans	Increased risk of developing obesity and/or type 2 diabetes	[2–4]
Sleep deprivation	Rats	No effect on body weight	[66–69]
		Increased food intake and adiposity	[70]
Eating during the resting phase	Humans	Increased risk for developing obesity and insulin resistance on a high-fat diet	[71]
		Increased risk of high BMI, obesity and/or type 2 diabetes	[61–65]
Eating during the resting phase	Mice	Increased body weight	[91]
	Humans	Increased risk of developing obesity	[87,89]

BMI, body mass index; HIP, human islet amyloid polypeptide transgenic.

**Table 2.** Studies in animals and humans on environmental conditions affecting the circadian clock and peripheral parameters.

	SCN	Peripheral
Animal studies	6,8,54,130,131,16,132,133,134,135	6,48,49,50,7,52,8,53,54,55,56,57,58,59,60,5,130,133,135,136,137,138
Human experimental studies	x	94,139,140,141,142,143,128

To study the effect of environmental conditions on the circadian clock and peripheral parameters, the animals have been exposed to constant light, dim light at night, aberrant light cycles or shift-work paradigms. The human studies referred to in this table only included studies in which experimental interventions were conducted. Cohort studies have been excluded from this overview. SCN, suprachiasmatic nucleus.

## Light's Effects on the SCN

Neurotransmitter function in the central nervous system can change under long days [17]. For example, exposure to a long photoperiod increases the level of GABAergic excitation [18]. Although GABA is normally an inhibitory transmitter (and therefore potentially synchronizing), its action becomes excitatory in animals exposed to a 16-h photoperiod [18]. GABA is a major transmitter in the brain and plays a strong role in regulating mood and sleep. The correlation between the increased incidence of diabetes, sleep disorders and depression in modern society – together with the increase in artificial light exposure and the altered action of GABA under long photoperiods – is intriguing and clearly warrants further investigation.

Light is perceived by retinal photoreceptors, and the information is conveyed to the SCN via the retinohypothalamic tract (RHT). The RHT projects to VIP-containing neurones in the ventral ('core') part of the SCN; these projections are distributed throughout the SCN's neuronal network, including the dorsal ('shell') part of the SCN, which contains arginine vasopressin (AVP)-expressing neurones [19]. In a simplified model, the core SCN neurones are first reset by light input and predominantly establish the phase of the dorsal SCN. The two most important neurotransmitters in terms of phase adjustment or synchronization in the SCN are VIP [20] and GABA [21]. For example, after a rapid change in the light–dark cycle (the so-called jet-lag protocol), GABAergic communication between the shell and the core is essential for synchronization of the SCN neurones and the eventual shift in the SCN's neuronal network to match the new light–dark cycle [22].

The effect of light on the phase of the central circadian clock depends both on the time of day in which the light is perceived and on the state of the network. For example, animals entrained to a long photoperiod (e.g. 16 h of light) have a wider phase distribution of the peaks in single neuronal activity than animals entrained to a shorter photoperiod (e.g. 8 h of light) [23]. Thus, a light pulse delivered during the subjective night to an animal entrained to a short photoperiod will be more effective at shifting the phase of the SCN's neuronal ensemble, as the sensitive phase of all neurones will be better aligned. The neurones will then have a consistent phase shift, resulting in a large shift in the ensemble. In contrast, when neurones have a more scattered phase distribution (i.e. when entrained to a long photoperiod), their phase-shift response will be less synchronized, resulting in a smaller shift in the ensemble [24].

## The Influence of Photoperiod

The mechanism by which the photoperiod influences the phase-shift adjustment is not entirely understood. Nevertheless, VIP seems to play an essential role in photoperiod encoding, as VIP-deficient animals have no 'memory' of photoperiod after they are placed in continuous darkness; in wild-type animals, this memory is an essential feature of photoperiod encoding [25]. Compelling evidence also suggests that GABAergic signalling plays a role [18,26]. For example, GABAergic transmission in the SCN can cause both inhibition and excitation of neuronal activity [27]. Therefore, one possible mechanism by which GABA can contribute to changes

in the SCN's network properties is the photoperiod-induced change in the balance between neuronal excitation and inhibition. Consistent with this notion, exposing animals to a long photoperiod was recently reported to increase the level of GABAergic excitation in SCN neurones [18]. An important question that arises from this work is whether exposure to a long photoperiod changes GABAergic function in other brain areas as well. GABAergic inhibition is a major regulator of sleep and mood, and decreased GABAergic inhibition has been associated with depression and sleep disorders.

The use of artificial lighting in our modern society is increasing, and recent studies have provided evidence that even low-intensity light can affect the circadian system in humans [28,29]. The effects of light on the circadian system are determined primarily by photoreceptors in the eye, which transmit light information to the SCN. Three classes of retinal photoreceptors detect environmental light: the rods and cones in the outer retina, and photosensitive retinal ganglion cells (pRGCs), which utilize the photopigment melanopsin. The role of melanopsin in circadian photoentrainment was first identified in 2002 [30–32] and has since become well established. Melanopsin is maximally sensitive to 480 nm (i.e. blue) light. A growing body of evidence now indicates that rods and cones also transmit light information to the SCN [33–35]. Together, these compelling findings may be highly relevant to humans, as rods and cones are more sensitive to low-intensity light than melanopsin; thus, together these photoreceptors greatly increase the circadian system's range of light intensities. The accumulating knowledge can lead to recommendations for the wavelength composition and intensity of light appliances used during the day, for example in nursing homes. Rather than focussing on a blue light-enriched environment, the entire visible spectrum may be just as effective. On the other hand, these findings underscore the risk of using moderate-intensity light in the evening, as such artificial lighting may affect the circadian system when delivered at a lower intensity and over a wider spectral range than previously believed.

“Light exposure in the evening can affect the circadian system at a lower intensity and over a wider spectral range than previously believed.”

## Disruption of the Light–Dark Cycle and the Effect on Metabolism

The widespread use of artificial lighting at night in modern society coincides with the rise in the prevalence of metabolic disorders in the past century. In recent years, the global incidence of overweight and obese individuals has increased dramatically; more than 1.6 billion people are estimated to be currently overweight and/or obese [36]. Obesity is associated with many adverse health conditions, including cardiovascular disease, hyperlipidaemia, type 2 diabetes, osteoarthritis, sleep apnoea, depression and cancer. Therefore, identifying factors, such as light pollution, that might contribute to the obesity epidemic has important implications with respect to public health.

Environmental light pollution and changes in light exposure can disrupt the normal circadian rhythm originating from the

SCN. The levels of many hormones follow circadian oscillations, including insulin, glucagon, adiponectin, corticosterone, leptin and ghrelin. In addition, the circadian clock regulates metabolism and energy homeostasis in the liver and other peripheral tissues by mediating the expression and/or activity of metabolic enzymes and transport systems involved in cholesterol metabolism, amino acid regulation, drug and toxin metabolism, the citric acid cycle and glycogen and glucose metabolism [37–39]. The rhythmic expression and activity of these metabolic pathways arises primarily from the coordinated expression of clock genes such as *Clock*, *Bmal1*, *Per1*, *Per2*, *Per3*, *Cry1* and *Cry2* [40]. The SCN transmits its rhythmic signal to peripheral oscillators via hormones and via the autonomic nervous system [41–43], thereby influencing nearly all aspects of physiology and behaviour, including the sleep–wake cycle, cardiovascular activity, the endocrine system, body temperature, renal activity, gastrointestinal tract physiology and liver metabolism [44]. By phenotyping transgenic mice with mutant and/or deleted clock genes, a direct link was made between the circadian clock and the metabolic disorders. Moreover, disrupting the circadian system – either by repetitive jet lag [45] or by light exposure at night [46,47] – has been shown to increase mortality in ageing mice.

### Continuous Light Exposure

Disrupting the circadian system by chronically exposing a nocturnal animal to light during the night immediately disrupts the animal's circadian locomotor activity; specifically, the circadian period lengthens, and rhythmicity is strongly reduced. *In vivo* recordings in the SCN of C57BL/6 mice exposed continuously to light revealed that the rhythmicity of the SCN's electrical activity is reduced [6]. During the first few days of continuous light exposure, the amplitude gradually declined, reaching a plateau amplitude of 44% (compared to the amplitude in the SCN during a normal light–dark regime) on the third day. This seemingly moderate decrease in the SCN's rhythm amplitude as a result of continuous light exposure has been shown to result in metabolic disorders as discussed below.

In rats, continuous light exposure induces a decrease in the daily intake of both food and water; in contrast, visceral adiposity increases [48]. In addition, plasma cholesterol levels increase in rats housed in continuous light [46]. Another study reported that disrupting the circadian rhythm either by continuous light exposure or by advancing the light cycle by 6 h every 3 days did not alter glucose-stimulated insulin secretion or insulin sensitivity in wild-type rats. In contrast, this same disruption accelerated the development of diabetes in a diabetes-prone rat model, the human islet amyloid polypeptide transgenic (HIP) rat [49]. Disrupting the circadian rhythm in diabetes-prone HIP rats increased the animals' fasting blood glucose levels and reduced glucose-stimulated insulin secretion, whereas insulin sensitivity was unaffected. The accelerated development of diabetes in HIP rats upon continuous light exposure or phase shift was due to a decrease in the number and function of pancreatic  $\beta$ -cells. As the circadian clock in pancreatic islets has been linked to  $\beta$ -cell survival, and because continuous light exposure has been shown to disturb this clock, the decrease in  $\beta$ -cell number and

function was likely the result of a disruption in the circadian clock in pancreatic islets [50].

Although exposing rats to continuous light increases their visceral adiposity, it does not appear to affect body weight gain. In contrast, exposing mice to continuous light increases their body weight [6,7]. We previously reported that exposure to continuous light completely abolished the peripheral circadian rhythms in both energy metabolism and insulin sensitivity [6]. Disrupting the circadian rhythm by exposure to continuous light had a direct effect on energy metabolism. Continuous exposure to light triggered an immediate increase in body weight gain as a result of reduced activity of brown adipose tissue (BAT) [6,51]. This finding suggests that short-term changes in circadian rhythm – for example, due to jet lag or disrupted sleep patterns in humans – can have immediate effects on homeostasis, thereby contributing to the development of secondary metabolic pathophysiology.

“Even short-term changes in circadian rhythmicity, for instance due to jet lag or temporarily diminished sleep, can have immediate effects on homeostasis, thereby contributing to the development of secondary metabolic pathophysiology.”

### Dim Light at Night

Even exposure to a relatively low level of light (e.g. 0.08  $\mu\text{W}/\text{cm}^2$  or 0.20 lux) at night can suppress the night-time surge in melatonin levels [52]. Reducing the amplitude of PER1 and PER2 levels in the SCN by exposing mice to dLAN (5 lux, which is similar to light pollution in urban areas) resulted in a decrease in the rhythmic expression of clock genes in the liver [8]. Selective denervation of the liver revealed that light can affect gene expression in the liver via both hormonal and autonomic pathways [53]. Interestingly, exposing mice to dLAN increased body mass to a similar extent as exposing mice to continuous light [54]. Moreover, glucose tolerance was reduced in mice that were exposed to dLAN compared to control mice under normal 12 h light–12 h dark conditions. The circadian pattern of food intake was also altered in mice exposed to dLAN, whereas daily food intake and locomotor activity were unaffected. Finally, restricting feeding exclusively to the dark phase prevented the increase in weight due to dLAN, suggesting that these metabolic disturbances are caused by a desynchronization between food intake and activity as a result of light exposure at night [54].

### Shifting the Light–Dark Cycle

Several studies have been conducted using altered light–dark cycles and/or shift-work paradigms in order to study the effect of circadian desynchrony on physiological parameters. Disrupting the circadian rhythm by exposing C57BL/6 mice to a 10 h light–10 h dark cycle increased body weight gain and increased the levels of both insulin and leptin [55]. Inducing a chronic phase advance of the circadian rhythms in behaviour and physiology by applying a repetitive jet-lag protocol (i.e. a

6-h phase advance every 4 days for 8 weeks) had no effect on body mass or glucose levels [56]. Exposing mice to an ultradian 3 h light–3 h dark cycle gradually disrupted the animals' circadian behavioural rhythm [57]. C57BL/6J mice that were fed a high-fat diet and exposed to this ultradian light–dark cycle reduced their total daily wheel running activity, which resulted in increased weight gain, even though their energy consumption (measured in kcal/day/g body weight) was decreased. Mice that were fed a high-fat/high-sucrose diet and exposed to the ultradian light regime had no change in body weight gain or food consumption [58]. However, the ultradian light schedule did affect other metabolic parameters; specifically, the mice developed hypercholesterolaemia, hyperglycaemia and glucose intolerance, effects that were not observed in control mice housed under normal light–dark conditions. Interestingly, inducing a disruption in the circadian rhythm by phase-reversing the light–dark cycle at weekly intervals altered the intestinal microbiota of C57BL/6J mice that were fed a high-fat/high-sucrose diet [59]. Intestinal dysbiosis has been associated with obesity and metabolic syndrome and therefore contributes to the metabolic abnormalities caused by circadian disruption. In a rat model of shift work (forced activity in a slowly rotating drum for 8 h during the resting phase), both activity and food intake shifted to the forced active phase, whereas hormonal rhythms were not affected, thus resulting in circadian misalignment [60]. As a result, the rats had increased body weight, increased abdominal fat, arrhythmic blood glucose levels and reduced glucose tolerance [5]. Moreover, this rat model of shift work developed hepatic desynchronization, which was characterized by desynchronized molecular rhythms in clock genes and metabolic genes [5].

In summary, the results of the aforementioned studies indicate that environmental light conditions play a causal role in the pathophysiology of metabolic disorders (Tables 1 and 2). In addition, these results highlight the importance of maintaining a regular light–dark cycle with respect to maintaining health.

### Sleep Deprivation and the Effect on Metabolism

A disturbed circadian clock evidently affects the sleep–wake cycle. Compelling epidemiological evidence suggests a link between insufficient sleep and an increased risk of high BMI, obesity and/or type 2 diabetes [61–64]. Combining data obtained from 90 623 individuals, Cappuccio et al. concluded that individuals who report sleeping less than 5 h each night have a 46% higher risk of developing type 2 diabetes compared to individuals who sleep 7–8 h each night [64]. Partial sleep deprivation (e.g. a decrease of 5 h of sleep each night) resulted in weight gain after only 5 days, and this weight gain was likely due to increased food intake [65].

Although several rodent studies have attempted to establish a link between sleep deprivation and feeding behaviour, sleep deprivation was not found to induce an increase in body weight [66–69]. However, these animals did become hyperphagic. Disturbing the sleep patterns in mice by intermittently waking the animals in the midst of their natural sleep period was associated with increased food intake and increased adipose

tissue mass [70]. Consistent with the change in food intake, the expression of neuropeptide Y, a peptide that potently stimulates food intake and reduces energy expenditure, rapidly increased in the arcuate nucleus (ARC) in the hypothalamus; in contrast, the expression of pro-opiomelanocortin, a peptide that decreases food intake and increases energy expenditure, decreased progressively during sleep deprivation [66]. Another rodent study found that prior exposure to fragmented sleep patterns increased future complications associated with a high-fat diet, including obesity, insulin resistance and type 2 diabetes [71]. These results suggest that sleep deprivation and/or fragmented sleep increase the individual's susceptibility to develop a metabolic disorder in the future.

### Mechanisms Linking Sleep Loss to Energy Metabolism

The physiological mechanisms that link (partial) sleep loss with impaired metabolism are poorly understood. Changes in appetite regulation may account – at least in part – for the mechanism, as reduced sleep leads to decreased levels of leptin (an appetite-suppressing hormone produced by adipose tissue), increased levels of ghrelin (an appetite-stimulating hormone produced by the stomach) and increased cravings for calorie-rich foods containing high carbohydrate content [72]. Given the complex pathways that regulate feeding behaviour and energy expenditure [73], the effect of sleep loss on metabolism is likely mediated via several underlying mechanisms.

Sleep–wake behaviour is regulated by an interplay between a homeostatic process (which monitors the previous waking duration and determines the depth of sleep) and the circadian clock (which sets the body's internal sleep–wake timing) [74]. In general, sleep deprivation is believed to affect primarily the homeostatic regulatory mechanisms. However, a 6-h sleep deprivation induces a long-term suppression of electrical activity in the SCN of rats, thereby strongly reducing the amplitude of the circadian clock's electrical output [75,76]. Sleep deprivation attenuates the effect of shifting the light phase on behaviour [77–79]; moreover, we recently reported that this effect has consequences on the light response in the SCN as well [79]. Therefore, in addition to leading to subsequent deeper sleep, sleep deprivation also leads to a reduction in the amplitude of the circadian output system, which can lead to metabolic syndrome and type 2 diabetes [1,6,80].

Another possible mechanism related to chronic partial sleep loss involves the increased food intake caused by a physiological adaptation to meet the increased energy demands needed to sustain the additional wakefulness [65]. Orexinergic (also known as hypocretinergic) neurones in the lateral hypothalamus (LH) play a pivotal role in modulating both sleep–wake behaviour and metabolic physiology [81]. Orexin-expressing neurones receive information from the SCN via the dorsomedial nucleus in the hypothalamus and project to several wake-promoting structures in the brainstem and hypothalamus [82]. In addition, intracerebroventricular administration of orexin stimulates waking and increases energy expenditure. Furthermore, narcolepsy – an autoimmune disorder that

results in a loss of either orexin or its receptors – is correlated with increased BMI [83], and deleting orexin receptors increases the susceptibility to develop diet-induced obesity [84]. Interestingly, orexin levels increase during sleep loss [67,85,86], and this increase may be required in order to sustain wakefulness [86]. In our modern society in which food is readily accessible, such physiological adaptations may lead to weight gain because the food consumed contains more energy than is needed to meet the energy cost of sleep loss [65]. Because of these overlapping changes in sleep and metabolic function, the orexinergic system is an integrative neural centre that might provide promising opportunities to intervene both in sleep disorders and in related metabolic complications [1].

To conclude, it is not precisely understood how sleep deprivation is linked to an increased risk for developing obesity and obesity-related disorders, but altered feeding behaviour as a result of sleep loss likely plays a key role in this process.

### Timing of Feeding and the Effect on Metabolism

The timing of food intake is a highly modifiable behaviour that is associated with obesity. For example, skipping breakfast is associated with an increased risk of obesity, whereas dividing one's daily intake of food over more than three meals is associated with a lower risk of obesity [87]. The prevalence of night eating syndrome (an eating disorder) is high among obese individuals [88], suggesting a causal relationship between food intake at the 'wrong' time and the development of obesity. Consuming more than 33% of the daily energy intake in the evening results in a twofold increase in the likelihood of becoming obese [89]. Conversely, conventional weight-loss programmes have less effect on obese individuals with late eating habits compared to obese individuals who are early eaters [90].

Several studies using rodents support the hypothesis that eating at the 'wrong' time can contribute to obesity. Nocturnal rodents typically consume 70–80% of their food during the night (i.e. during the active phase), and mice that are restricted to feeding during the light period (i.e. during the inactive phase) have increased weight gain [91]. In addition, dLAN also shifts the animal's timing of food intake and leads to increased body weight. Interestingly, this increase in weight gain by dLAN can be prevented by restricting food intake to the active phase [54]. Likewise, obesity induced by a high-fat diet can be prevented by restricting food intake to the active phase without restricting total energy intake [92].

### Mechanisms Linking Meal Timing to Energy Metabolism

The mechanisms that link meal timing to energy metabolism are not fully understood; however, they include the readiness of metabolic organs to process carbohydrates and lipids upon food intake. For example, digestive and intestinal enzymes are expressed in a circadian-like rhythm, and the rate of gastric emptying fluctuates throughout the day. Importantly, the expression of glucose and lipid transporters and processors in the intestine also display circadian rhythmicity. White adipose

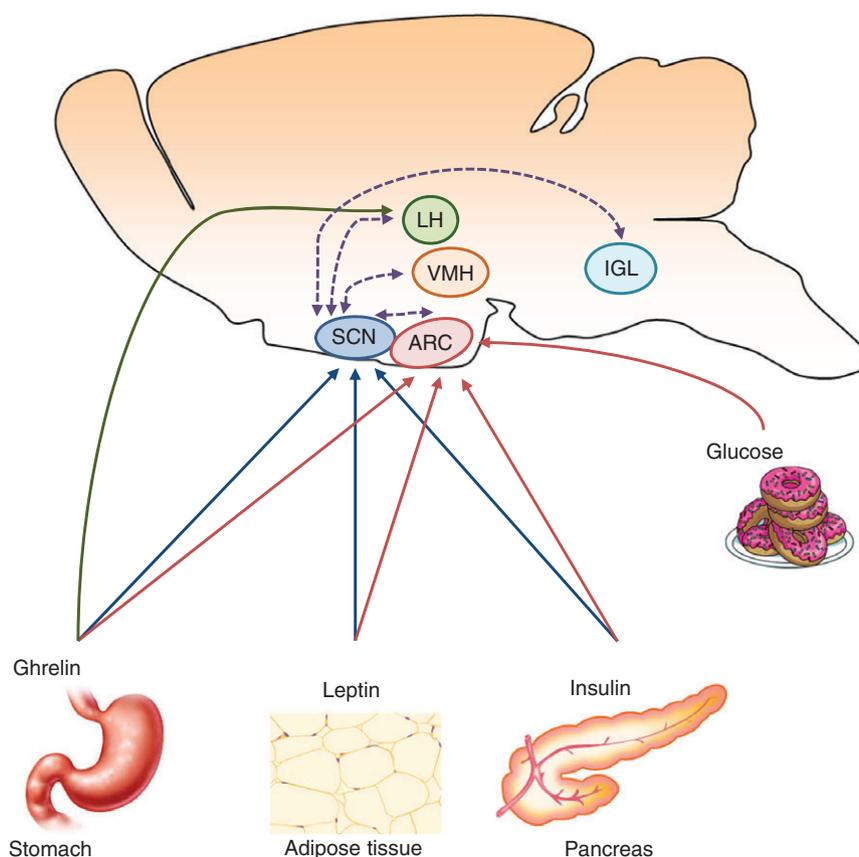
tissue prepares for the storage of excess energy by expressing proteins that mediate the uptake of nutrients from the circulation. In contrast, during periods of fasting, adipose tissue releases stored energy primarily in the form of fatty acids. Consistent with these studies, the insulin sensitivity of metabolic organs is rhythmic; thus, postprandial glucose levels are lower after an early meal than after a late meal [93]. Likewise, being awake and eating at night results in increased plasma concentrations of both insulin and glucose, as well as decreased release of the satiety hormone leptin [94]. Notably, the postprandial glucose response in some of these healthy subjects resembles a prediabetic state. Furthermore, core body temperature begins to decrease in the evening, and this may reflect a decrease in the activity of BAT [95]. Given the importance of BAT in burning excess nutrients, reduced BAT activity may underlie the increase in postprandial glucose and lipids after late eating, which may eventually lead to obesity. New weight loss intervention strategies should therefore include the timing of food intake.

### Metabolic Signals and Their Effect on the Circadian System

While the circadian clock can significantly regulate an organism's metabolism including timing of feeding, metabolic signals can influence the function of the SCN (Figure 1). Several direct and indirect pathways connect the SCN with the endocrine system (for review, see Ref. [96]). The SCN expresses receptors for the hormones leptin [97], ghrelin [98] and insulin [99], all of which can affect the central circadian clock [96]. In addition to its direct effect on SCN physiology, ghrelin can also act via orexinergic neurones in the LH and the ARC; moreover, leptin and insulin act via neurones in the ARC, which connect directly to the SCN. In addition, a neuropeptide Y-mediated feedback circuit involving the intergeniculate leaflet can signal the metabolic/feeding state of the organism to the SCN [100].

Interestingly, metabolic signals alter circadian function via molecules that directly sense fuel. For example, glucose-sensitive neurones in the ARC can communicate glucose-related information to the SCN. Another route is mediated by the AMP/ATP ratio-sensing enzyme AMP protein kinase (AMPK), which has a diurnal rhythm in the hypothalamus and can affect clock gene expression [101]. Recently, an elegant study specifically deleted sirtuin 1 expression in the ventromedial hypothalamus (VMH) and found that NAD<sup>+</sup>-sensing SIRT1 plays a role in communicating nutritional state to the central circadian pacemaker, thereby altering the behavioural phenotype [102].

When the temporal pattern in food intake is altered, metabolic signals will communicate this to the SCN and the circadian organisation. For instance it has been shown that temporal feeding restriction of mice to their resting phase, affects the oscillations in the expression of clock genes in the peripheral organs [103]. In addition, feeding rodents a high-fat diet alters their circadian behaviour and shifts their timing of food intake to the inactive phase. In humans, switching to a high-fat diet phase-shifts the expression of clock genes in monocytes [104], suggesting that a similar level of regulation is present in both rodents and humans.



**Figure 1.** Metabolic signals influence suprachiasmatic nucleus (SCN) function. Endocrine peptides such as leptin, ghrelin, insulin and glucose can directly or indirectly affect SCN function. Indirect pathways affecting the SCN involve the lateral hypothalamus (LH), the arcuate nucleus (ARC), the intergeniculate leaflet (IGL) and the ventromedial hypothalamus (VMH).

In conclusion, circadian disruption can result in changes in temporal feeding which is communicated to the SCN via metabolic signals, again stressing the importance of proper timing of food intake.

“While the central circadian pacemaker regulates metabolism, various direct and indirect pathways can communicate the metabolic state to the SCN, thereby influencing functioning of the circadian clock and altering the behavioural phenotype.”

### Implications for Society

In modern society, our circadian rhythms have become increasingly disrupted due to the widespread use of artificial lighting. Nowadays, 75% of the world's population is exposed to artificial light during the night [105], and 90% of Americans use light-emitting devices at night, which can negatively affect their sleep patterns and circadian rhythms [29]. An intensive care unit is one example of an environment with particularly high levels of nocturnal light exposure. Interestingly, decreased light exposure during daytime can also negatively affect circadian rhythmicity. For example, low lighting levels are common in nursing homes and have been associated with diminished

cognitive function [106]. Exposure to rapidly changing environments elicits a temporary state of jet lag or internal desynchrony. Frequent exposure to jet lag can repetitively induce desynchrony, and in response the circadian system must continually adapt. Given our increased social and economic demands, air travel across multiple time zones has become relatively common and approximately 20% of the work force is currently engaged in shift work. The so-called social jet lag phenomenon – in which individuals frequently shift their sleep timing due to changing social demands – has also become relatively common. Finally, certain disease states (for example, sepsis [107]) and neurodegenerative disorders such as Alzheimer's disease [108] are associated with a reduction in circadian rhythmicity.

In humans, disrupted circadian rhythms have been linked to metabolic changes (Tables 1 and 2). For example, shift workers have an increased risk of developing obesity and/or type 2 diabetes, as reported consistently by large cross-sectional [2] and prospective studies [3,4] in both men [4] and women [2,3]. This effect appears to be 'dose-dependent'; thus, the risk of developing a metabolic disorder appears to increase with the duration of shift work [3]. Obesity and insulin resistance are predictors of cardiovascular-related mortality [109]. Insulin resistance is also a risk factor for developing non-cardiovascular diseases, including cancer and kidney dysfunction [109]. More than

one-third of the population in the United States is currently obese, and the same proportion of Americans have prediabetic levels of glycosylated haemoglobin (HbA1c) [110]; thus, the identification of shift work as an additional risk factor for developing these metabolic complications is highly relevant to a large number of individuals. In addition, shift work is associated with an increased risk of breast cancer [111], osteoporosis [112] and bone fractures [113].

Evidence also suggests a link between functional variants in clock genes and health outcomes. For example, allelic variants in the circadian-related genes *Clock* [114,115] and *Rev-erba* [116] have been linked to obesity. In addition, certain polymorphisms in the clock genes *Per2* [117], *Bmal1* [118], *Cry2* [119] and *MTNR1B* [120–122] have been correlated with increased fasting blood glucose levels and an increased risk of developing type 2 diabetes. Furthermore, specific *Clock* [123,124], *Cry2* [125] and *MTNR1B* [125] gene variants determine the magnitude to which metabolic parameters improve in response to certain diets. Lastly, polymorphisms in *Per1* have been associated with the risk of developing osteoporosis in post-menopausal Korean women [126], and variants in the *Clock* gene have been associated with breast cancer [127]. Elegant experimental studies in humans show that short-term experimental disturbance in the circadian rhythm can induce metabolic disturbances [128], including decreased glucose tolerance [94]. When combined with sleep restriction, the resting metabolic rate decreases and postprandial glucose levels are elevated, placing the individual at an even higher risk for developing obesity and/or diabetes [129].

“Decreased light exposure during daytime, for instance the low lighting levels used in nursing homes, can negatively affect circadian rhythmicity, and thereby increase the risk for cognitive and metabolic complications.”

## Conclusions

Disruptions in circadian rhythm are common in modern society, and these disruptions are associated with an increased risk of developing harmful metabolic disorders. Although epidemiological studies of shift workers and subjects with polymorphisms in their clock genes indicate a relationship between disrupted circadian rhythms and metabolic disorders, they cannot determine whether this relationship is causal. On the other hand, experimental studies of circadian disruptions in humans and rodents suggest that disrupted environmental rhythmicity is a causative factor in metabolic disease. Future studies should be aimed at improving the circadian system as a promising new tool for prevention of metabolic disorders.

“The studies presented here stress the importance of recognizing circadian disruption due to shift work, jet lag, altered light exposure or sleep deprivation as a risk factor for metabolic disorders and highlight the importance of maintaining a

regular day–night cycle with respect to preserving health.”

## Conflict of interest

The authors have no conflict of interest to declare.

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