



Horizons in Nutritional Science

The role of the circadian clock system in nutrition and metabolism

Felino R. Cagampang* and Kimberley D. Bruce

Institute of Developmental Sciences, Faculty of Medicine, University of Southampton, MP887 Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK

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Abstract

Mammals have an endogenous timing system in the suprachiasmatic nuclei (SCN) of the hypothalamic region of the brain. This internal clock system is composed of an intracellular feedback loop that drives the expression of molecular components and their constitutive protein products to oscillate over a period of about 24 h (hence the term ‘circadian’). These circadian oscillations bring about rhythmic changes in downstream molecular pathways and physiological processes such as those involved in nutrition and metabolism. It is now emerging that the molecular components of the clock system are also found within the cells of peripheral tissues, including the gastrointestinal tract, liver and pancreas. The present review examines their role in regulating nutritional and metabolic processes. In turn, metabolic status and feeding cycles are able to feed back onto the circadian clock in the SCN and in peripheral tissues. This feedback mechanism maintains the integrity and temporal coordination between various components of the circadian clock system. Thus, alterations in environmental cues could disrupt normal clock function, which may have profound effects on the health and well-being of an individual.

Key words: Circadian clocks: Rhythms: Nutrition: Metabolism

Most mammals have evolved so that they are able to predict the 24 h day–night cycle governing their daily activities. Key to this is the development of an internal body clock that is entrained to external time cues, thus ensuring that physiological processes are carried out at the optimum time of the day or night^(1,2). This endogenous clock system runs on a near-24 h period and is termed ‘circadian’ from the Latin word *circa* and *diem*, which translates as ‘about a day’. Disruption in the integrity and temporal coordination of this clock system can lead to hormonal imbalances, sleep disorders, susceptibility to cancer and other disease states, as well as to a reduction in lifespan^(3–7).

Although malnutrition is still a major public health concern in developing countries, there has also been an escalation in the global epidemic of obesity coined as ‘globesity’ by the WHO, particularly in many industrialised societies. Serious health problems, including the development of diabetes mellitus, CVD, hypertension, stroke and certain types of cancers, can arise as a consequence of being overweight or obese, and this could overwhelm the healthcare infrastructure of societies. Key to how we prevent obesity is to maintain

energy homeostasis, i.e. the balance in energy intake and energy expenditure. Major components of energy homeostasis, including the sleep–wake cycle, feeding behaviour, thermoregulation and metabolism, exhibit circadian rhythms which are controlled and coordinated by the circadian clock system^(8–10). Conversely, external stimuli such as light, temperature, and the timing and type of nutrient intake can also influence clock function. Thus, further investigation into the relationship between the circadian clock system and nutrition will reveal mechanisms involved in energy homeostasis and the pathogenesis of obesity. The present review summarises recent findings on the importance of the endogenous circadian clock system in the regulation of nutritional and metabolic processes.

Molecular aspect of the circadian clock system

Daily oscillations in the gene and protein components of the endogenous molecular clock network mediate circadian rhythms in both physiological and metabolic outputs. These oscillations are generated through a series of positive and

Abbreviations: BMAL1, brain and muscle ARNT-like protein 1; CK1 ϵ , casein kinase 1 epsilon; CLOCK, circadian locomotor output cycles kaput; CRY, cryptochrome; GI, gastrointestinal; PER, Period; REV-ERB α , reverse erythroblastosis virus α ; ROR α , retinoic acid receptor-related orphan receptor α .

* **Corresponding author:** Dr F. R. Cagampang, email f.cagampang@soton.ac.uk

negative feedback loops and involve a number of genes collectively known as 'clock' genes and their constitutive proteins (see Fig. 1). These genes include the brain and muscle ARNT-like protein 1 (*Bmal1*; also known as *Mop3* or *Arntl*), circadian locomotor output cycles *kaput* (*Clock*), Period 1 (*Per1*), Period 2 (*Per2*), Period 3 (*Per3*), cryptochrome 1 (*Cry1*) and cryptochrome 2 (*Cry2*)^(11–13). The positive drivers to this system are the two basic helix–loop–helix Period–Arnt–Single-minded domain-containing transcription factors CLOCK and BMAL1, which form a heterodimer complex. CLOCK is a histone acetyltransferase, and its activity is stimulated following heterodimerisation with BMAL1⁽¹⁴⁾. The CLOCK–BMAL1 dimer binds to the E-box elements in the *Per1*, *Per2*, *Per3*, *Cry1* and *Cry2* genes and activates their transcription, facilitated by histone acetylation.

Following translation, PER and CRY proteins form complexes and translocate back to the nucleus where they then exert a negative feedback effect on the transcriptional activity of the CLOCK–BMAL1 heterodimer, thus inhibiting their expression and completing the feedback loop. In this feedback loop, the protein kinase casein kinase 1 epsilon (CK1ε) has been shown to phosphorylate the PER proteins that have accumulated in the cytoplasm. As the phosphorylated forms of PER become unstable, they are then degraded by ubiquitinylation. On the other hand, the accumulation of CRY proteins in the cytoplasm promotes the formation of stable CK1ε–PER–CRY complexes, and subsequently enters the nucleus⁽¹⁵⁾. This series of events allows the clock genes to exhibit an oscillatory pattern of expression over a period of about 24 h. Typically, CLOCK and BMAL1 dimerise in the

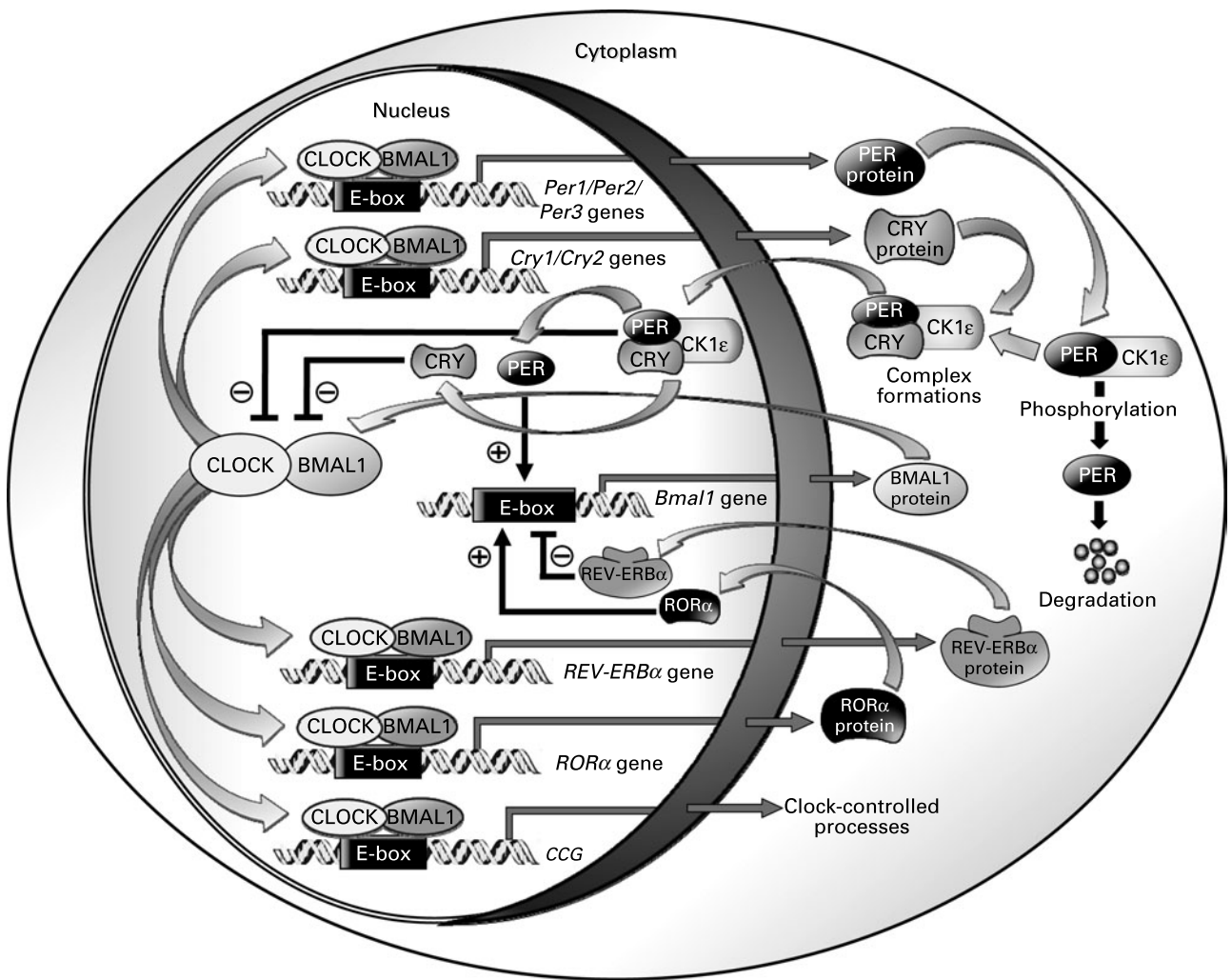


Fig. 1. The core mechanism of the circadian clock in the suprachiasmatic nuclei and peripheral tissues. The cellular oscillator is composed of a positive limb (circadian locomotor output cycles *kaput* (CLOCK) and brain and muscle ARNT-like protein 1 (BMAL1)) and a negative limb (cryptochrome (CRY) and period (PER)). CLOCK and BMAL1 dimerise in the cytoplasm and translocate to the nucleus. The CLOCK–BMAL1 heterodimer then binds to enhancer (E-box) sequences located in the promoter region of the *Per* and *Cry* genes, as well as other clock-controlled genes (*CCG*) activating their transcription. After translation, PER and CRY undergo nuclear translocation and inhibit CLOCK–BMAL1, resulting in decreased transcription of their own genes. Casein kinase 1 epsilon (CK1ε) periodically binds to and phosphorylates the PER proteins, which form heterodimers with each other and interact with CRY. The phosphorylation of the PER proteins prevents nuclear entry and also increases their ubiquitinylation, which leads to degradation. However, this can be overcome when the PER–CK1ε protein complex is bound to CRY. The autoregulatory transcription–translation loop comprising CLOCK–BMAL1 and PER–CRY constitutes the core clock and generates 24 h rhythms of gene expression. Retinoic acid receptor-related orphan receptor α (*RORα*) stimulates and reverse erythroblastosis virus α (*REV-ERBα*) inhibits *Bmal1* transcription.

early morning. Subsequently, the transcription of *Per* and *Cry* peak at noon, to inhibit the activity of CLOCK–BMAL1 complexes leading to decreased expression of *Per* and *Cry*. Eventually, levels of PER and CRY will become too low to maintain this negative feedback, and CLOCK and BMAL1 will begin to complex again, reinitiating the cycle⁽¹⁶⁾. *Bmal1* expression is also negatively regulated by the transcription factor reverse erythroblastosis virus α (REV-ERB α)⁽¹⁷⁾ and positively regulated by retinoic acid receptor-related orphan receptor α (ROR α)⁽¹⁸⁾ via the ROR α response element in the *Bmal1* promoter⁽¹⁹⁾. These interlocking positive and negative transcriptional–translational feedback loops regulate numerous downstream clock-controlled genes with key roles in metabolic processes, which are central to how the clock systems generate circadian rhythms in nutrition and metabolism.

The mammalian circadian clock system

The circadian clock system in mammals consists of a master pacemaker clock and clock gene networks in peripheral tissues (see Fig. 2). The master pacemaker clock, also known as the central clock, is found in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus of the brain, adjacent to the optic chiasm. The SCN clock is composed of multiple, single-cell circadian oscillators numbering between 10 000–20 000 neurons, which, when synchronised, generate coordinated circadian outputs that orchestrate overt rhythms^(20–23). The critical role of the SCN was first recognised when circadian rhythms of activity, drinking and feeding were abolished by electrolytic lesions of this area in the rat brain⁽²⁴⁾. SCN grafts on SCN-ablated animals have been shown to restore circadian locomotor rhythms^(25,26). Circadian rhythms generated by the SCN clock are reset daily by daylight, ensuring that the central clock is kept entrained to the external day–night cycle. In the absence of such daylight cues, as when an individual is kept in total darkness for an extended period of time, rhythms will eventually ‘free-run’ and drift across the entire day⁽²⁷⁾. As an example, mammals that are exposed to a continuous lighting environment, such as reindeer living above the Arctic Circle, lack circadian oscillations of clock components⁽²⁸⁾. Thus, daylight is a potent ‘zeitgeber’ (German for ‘time-givers’), which is able to synchronise or ‘entrain’ the clock system in the SCN to the day–night cycle. The position of the SCN, which is adjacent to the optic chiasm, makes it ideal to receive visual input for light entrainment via the retinohypothalamic tract⁽²⁹⁾. Light is detected by a particular type of retinal ganglion cell containing the photopigment melanopsin⁽³⁰⁾. These photic inputs are then transduced to the SCN neurons through a number of neurotransmitters, including glutamate and pituitary adenylate cyclase-activating peptide, which are thought to transcriptionally induce *Per1* expression through ion channel activation and the intracellular kinase–cAMP response element-binding (CREB) cascade⁽³¹⁾.

Similar clock systems are found within the cells in non-SCN neurons and in peripheral tissues, including the heart^(32,33), liver^(32,34), gastrointestinal (GI) tract^(35,36) and pancreas^(37,38), in both humans and animals. However, the rhythmicity of clock gene expression in peripheral tissues can be up to 4 h

out of phase with the rhythms found in the SCN^(32,39,40). This is due to the hierarchical organisation of the central clock, which sends signals to peripheral oscillators in order to maintain circadian rhythms in these tissues. There could be a time delay in the receipt of these SCN signals, or the signals may be sent at different times of the day for different tissues depending on their function. For example, certain metabolic pathways have to be activated in muscles at a particular time of the day while simultaneously reducing the activity of the GI tract⁽⁴¹⁾. Thus, the opposing autonomic tone redirects blood away from the abdomen towards tissues involved in movement. The mechanisms by which the SCN accomplishes this task are not well understood but may involve humoral mediators such as prokineticin-2^(42,43), arginine vasopressin^(44,45), cardiolipin-like cytokine⁽⁴⁶⁾, vasoactive intestinal polypeptide⁽⁴⁷⁾, orexin⁽⁴⁸⁾, pituitary adenylate cyclase-activating peptide⁽⁴⁹⁾ and transforming growth factor- α ⁽⁵⁰⁾, or the release of the hormone melatonin by the pineal gland during darkness^(51,52). The SCN can also communicate time-of-day signals to peripheral tissues via neural outputs, such as the rhythmic change in the parasympathetic/sympathetic balance^(53,54). The sympathetic projection, in particular, is critical in maintaining the physiological rhythms in peripheral tissues, such as glucose homeostasis by the liver^(55,56). Moreover, the selectivity in communication between the SCN and peripheral tissues is such that parasympathetic and sympathetic branches of the autonomous nervous system are able to innervate different compartments within the same tissue. For example, the subcutaneous and intra-abdominal fat pads are innervated by separate parasympathetic and sympathetic motor neurons⁽⁵⁷⁾. The sympathetic input to adipocytes has been reported to be essential for the regulation of daily rhythms in leptin release from the fat depot⁽⁵⁸⁾. Thus, altered autonomic outflow from the SCN can result in an imbalanced rhythm between different fat compartments, and this may bring about increased fat accumulation and obesity⁽⁵⁹⁾.

In addition to daylight, other zeitgebers are able to entrain the central and/or peripheral clock. These include body temperature, mealtimes, restricted feeding and scheduled physical exercise^(60–62). These zeitgebers are particularly important for the circadian clock system in peripheral tissues which cannot perceive daylight cues. In contrast, the central clock in the SCN is largely light responsive and can be entrained by the light–dark cycle^(63,64). Moreover, the intercellular connections of the SCN clocks enable them to maintain coherence in generating rhythms indefinitely *in vivo* and for several weeks in brain explants⁽⁶⁵⁾. In contrast, cells in the periphery are less communicative and rapidly desynchronise in animals with SCN lesions⁽⁶⁶⁾ or in tissue culture^(40,67). Therefore, the rhythms generated by oscillators in peripheral tissues must be entrained by the central SCN clock so that they can be in synchrony with each other. For peripheral tissues involved in nutritional and metabolic processes, such as the stomach, intestine, liver and pancreas, feeding schedules and restricted feeding become powerful zeitgebers^(60,68). Importantly, alterations in these peripheral zeitgebers could uncouple the phases of the peripheral and central clocks. Under normal conditions, the SCN clock synchronises the oscillators in the periphery in

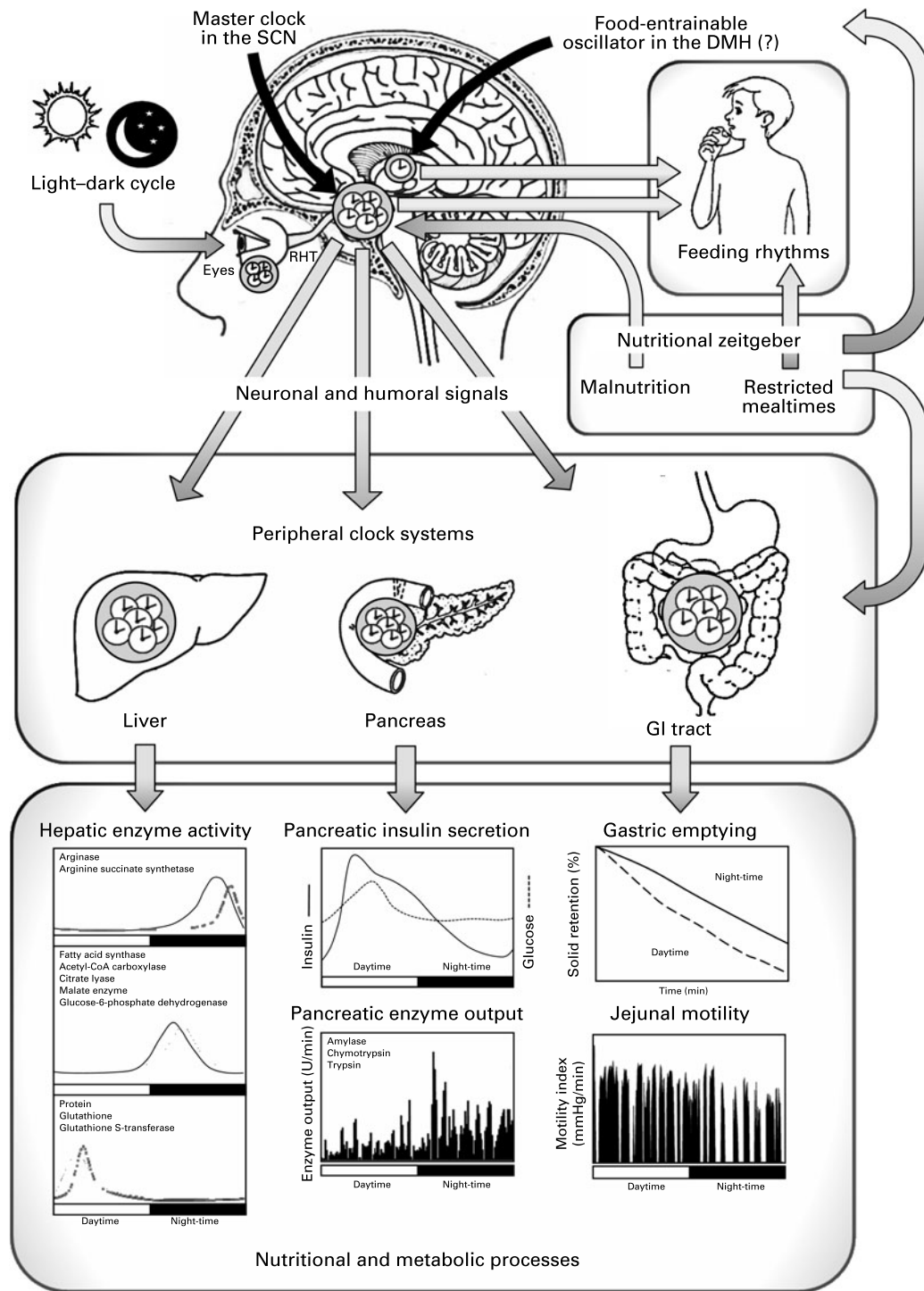


Fig. 2. The mammalian circadian clock system and its link to nutrition and metabolism. Photic signals received by retinal cells in the eyes are conveyed to the master clock in the suprachiasmatic nucleus (SCN) via the retinohypothalamic tract (RHT). The clock system in the SCN then conveys this zeitgeber to peripheral clock systems in the liver, pancreas and gastrointestinal (GI) tract via humoral and neuronal signals, which in turn regulate rhythms in nutritional and metabolic processes. Feeding rhythms, including food anticipatory activities, are also regulated by the master SCN clock. Nutritional zeitgebers such as malnutrition (over- or undernutrition) and restricted mealtimes can also entrain the clock systems and rhythmic processes in peripheral tissues, as well as feeding rhythms via a food-entrainable oscillator in non-SCN regions of the brain. DMH, dorsal medial nucleus of hypothalamus.

an indirect fashion. Hence, when nocturnal feeding animals such as rodents are restricted to meals during the day, the rhythms in food anticipatory activity, energy metabolism and gene expression in peripheral tissues will entrain to the

day^(34,69,70), while gene expression rhythms in the SCN remain entrained to the day–night cycle^(11,63). This may be viewed as an adaptive mechanism to changes in food availability while maintaining synchrony with the day–night

cycle. The synchronisation of the peripheral clocks by feeding makes sense because circadian metabolism is a major clock output in many peripheral cells. However, long-term exposure to compounding effects of the different zeitgeber signals can result in poor metabolic health. Long-term shift work, frequent crossing of multiple time zones or irregular lifestyle with poor sleep, where changes in activity–sleep pattern are coupled with altered mealtimes, may also be considered risk factors in the development of various cardiometabolic and GI pathologies^(71–74).

Circadian clock system in extra-suprachiasmatic nuclei brain regions: how they regulate appetite and food intake

The canonical hypothalamic regions of the mammalian brain involved in food intake regulation include the lateral hypothalamus and the ventromedial nucleus, the former being associated with hunger and the latter linked to satiety^(75,76). In addition, neurons in other regions within the hypothalamus, including the dorsomedial, paraventricular and arcuate nuclei, secrete peptides that are involved in hunger and satiety. These neuropeptides can stimulate appetite, which include neuropeptide Y and Agouti-related protein, while others, including cocaine- and amphetamine-regulated transcript and pro-opiomelanocortin and its derivatives (e.g. α -melanocyte-stimulating hormone, melanocortin), diminish appetite^(76,77). Gene and protein expression patterns of these neuropeptides exhibit a circadian rhythm in the rodent brain^(78–80) and may therefore be required for the effect of circadian cues to affect appetite. In one study, it has been shown that increased food intake in mice at the onset of darkness is reduced in animals lacking neuropeptide Y⁽⁸¹⁾. It further went on to show that mechanisms implicated with increased food intake brought about by food deprivation are distinct from those involved in response to feeding at the onset of the dark period of the light–dark cycle. Circadian rhythms in clock gene and protein expression have been shown in these regions of the rat brain by immunohistochemistry^(82,83), by *in situ* hybridisation⁽⁸⁴⁾ or by monitoring reporter constructs such as luciferase that is driven by clock gene promoters^(85–87). Currently, it is difficult to distinguish autonomous circadian rhythmicity in these extra-SCN brain regions from those imposed by inputs from the SCN. One could therefore assume that circadian rhythms of these peptides are dependent on the SCN. However, differential coupling of the extra-SCN clocks to their SCN counterparts due to multiple zeitgeber effects may result in changes in the phase, amplitude and phase-resetting kinetics in their oscillations, thus allowing plasticity of the circadian clock systems to integrate a wide range of temporal information.

Circadian rhythms of neuropeptides involved in appetite regulation may explain the persistent basic pattern of eating three meals per day in humans. This eating pattern has even been observed in individuals isolated from external time cues such as the day–night transition and day length⁽⁸⁸⁾. A regular mealtime helps maintain a stable internal temporal order of the circadian clock system. Thus, abandonment of regular eating patterns due to the increasing demand imposed

by contemporary 24 h societies may be disrupting nutritional and metabolic processes and can have profound effects on long-term health and well-being^(89–91).

The metabolic status of an individual is also transmitted in a circadian-dependent manner via humoral signals from peripheral tissues to the brain regions that control appetite⁽⁹²⁾. The hormone leptin, which suppresses appetite and is produced primarily in adipocytes, is secreted in a circadian manner^(93,94) and is therefore suggested to be under the control of the SCN clock via its sympathetic input to the adipocytes⁽⁵⁸⁾. In humans, night-time plasma leptin levels are high when appetite decreases, favouring fasting and nocturnal rest, and low during the day, when hunger increases. In obese individuals, however, daytime and night-time leptin levels are much higher compared with lean healthy subjects, indicating a state of leptin resistance⁽⁹⁵⁾. Nevertheless, the circulating diurnal leptin rhythm is maintained. Leptin is also expressed in non-adipose tissues such as the stomach^(96,97). Gastric leptin levels oscillate in a circadian manner where leptin levels are high at night but low during the day⁽⁹⁸⁾. This would suggest that gastric leptin is involved in regulating appetite by inducing satiety. One other hormone that reciprocates the action of leptin on appetite is ghrelin. Ghrelin is produced in the stomach and in other tissues including the pancreas and hypothalamus^(99,100). It is involved in stimulating appetite via its action on neuropeptide Y in the lateral hypothalamus^(101,102) and can also alter clock function in the SCN *in vitro*^(103,104). Ghrelin oscillates with feeding⁽¹⁰⁵⁾, making this peptide a putative candidate for food-related entraining signals. In addition, elevated levels of ghrelin were found during the early part of the night in sleeping subjects, decreasing in the morning before awakening⁽¹⁰⁵⁾. Sleep deprivation can increase circulating ghrelin levels and this is accompanied by heightened hunger sensation⁽¹⁰⁶⁾. Thus, ghrelin may be a signal involved in the cross-talk between the peripheral and central circadian clock system. However, circulating ghrelin levels are lower in obese individuals⁽¹⁰⁷⁾, whereas in anorectic patients, fasting ghrelin levels are significantly higher than in control subjects⁽¹⁰⁸⁾. The temporal relationship between ghrelin and leptin indicates that, apart from the increase in serum ghrelin levels during the early part of the night, the diurnal rhythm of ghrelin is actually in-phase with that of circulating leptin levels. Hence, the night-time increase in circulating ghrelin levels may offset the appetite-suppressive effect produced by increased leptin. Interestingly, this temporal relationship is evident in both humans and rodents whose eating patterns are completely different^(105,109).

In parallel to the circadian changes in neuropeptide levels and humoral signals from peripheral tissues, there also exists a circadian rhythm in macronutrient selection. In most mammals, the time of the day can influence the choice and quantity of macronutrient that is consumed. It has been shown in rats that at the beginning of their active phase at night when their glycogen reserves are low, their preference for carbohydrate increases with parallel increases in neuropeptide Y levels in the paraventricular nucleus of the hypothalamus^(110,111). By the end of their activity phase early in the morning, preference shifts to fat over protein and carbohydrates, which release

energy more slowly over the resting phase⁽¹¹²⁾. Similarly in humans, a carbohydrate-rich diet is favoured during breakfast and high-fat diets are preferred during evening meals⁽¹¹³⁾. Carbohydrates are metabolised better during breakfast because the body is metabolically poised to respond to a glucose stimulus⁽¹¹⁴⁾. It is therefore sensible to ingest a sufficient quantity of energy that will enable the individual to become more alert and thus break the lethargy upon waking. On the other hand, circulating glucose levels are lower during sleep, when GI transit slows down, so it would be logical to think that an evening meal should not contain too much carbohydrate. Nevertheless, it remains to be elucidated whether the distribution of the macronutrients during the day is associated with obesity.

Circadian clock system in the gastrointestinal tract and its effect on the digestive cycle

Studies in rodents have shown that the GI tract contains functional clock genes^(36,115,116). The presence of these clock genes in the myenteric plexus, which acts as the local nervous system within the digestive system, and in the epithelial cells suggests that clock genes are involved in the generation of daily rhythms of GI function and activities, such as gastric emptying, colonic motility, gastric secretion and enzymatic activities, maintenance and repair of protective mucosal barriers, nutrient transport in the small intestine, and epithelial cell proliferation^(117,118). Rhythmic expression of these clocks can also vary between sections of the GI tract. In one such study, it has been reported that the rhythms of clock genes in the duodenum were phase-advanced to rhythms in the colon⁽¹¹⁹⁾, and parallel the direction of the passage of food through the gut.

The production and secretion of various key metabolites in the GI tract^(118,120) and gastric secretions^(120–122) also display circadian rhythmicity. In diurnal species including humans, gastric secretions in the fasted state are at their maximum during the night and low in the morning^(120,122). This is coupled to slower gastric emptying and intestinal absorption rates after an evening meal compared with rates after morning meals⁽¹²³⁾. Nocturnal species such as the rat also exhibit high gastric secretion during the dark phase when gastric pepsin is low⁽¹²⁴⁾.

The circadian clock system in the intestine could also play an important role in nutrient absorption. Fats, carbohydrates and proteins are hydrolysed in the small intestine and the products of this hydrolysis are absorbed via intrinsic membrane transporter proteins. Interestingly, gene expression levels of these transporters exhibit circadian rhythmicity. In rodents, the Na-glucose transporter SGLT1, the GLUT GLUT2 and GLUT5^(125–127), and the proton-coupled oligopeptide transporter 1^(128,129) show peak expression at night. The circadian rhythms of these nutrient transporters are lost in *Clock*-mutant mice⁽¹³⁰⁾ but is maintained in food-deprived animals⁽¹³¹⁾, suggesting that the circadian clock system in the intestinal lumen is more important in the regulation of these transporters than the presence of food. The significance of the circadian clock system in the small intestine may therefore lie in its ability to anticipate luminal food exposure which

would allow the intestinal epithelium and its transporter system to be optimally prepared for the absorption of nutrients.

Indigestible food, on the other hand, that is unable to pass through the pylorus of the stomach to the duodenum (i.e. beginning of the small intestine) is emptied by a powerful muscular contraction propagated by the migrating myoelectric complex. In healthy individuals, the speed of migrating myoelectric complex propagation during the day is more than double compared with night-time values⁽¹³²⁾. Likewise, colonic motility is lower in the evening but increases during the day, particularly following awakening or following a meal⁽¹³³⁾. Thus, in humans, healthy individuals have bowel movements more often during the waking hours in the morning or subsequent to a meal but rarely during the night.

Since clock genes of the GI tract are expressed in a circadian manner, they are likely to be important regulators of GI tract activity. Therefore, disruption in circadian rhythms such as in shift work or travel across multiple time zones can upset the natural processing of food by the GI tract and could lead to abdominal bloating, poor nutrient absorption, diarrhoea or constipation⁽¹³⁴⁾. Understanding the mechanisms underlying circadian variations in GI tract activity might therefore be useful in the diagnosis and prevention of these GI disorders.

Circadian clock system in the liver and pancreas: how they affect metabolism

The liver plays an important role in adjusting metabolic processes to the daily feeding cycles. This role is manifested by the vast number and variety of genes and proteins in the liver shown to be expressed in a circadian manner, which strongly suggests that the circadian clock system is vital in liver physiology^(67,135,136). In addition to the clock genes themselves exhibiting circadian rhythms^(32,40,137), rhythms were also observed in those genes involved in vital liver-specific processes, including rate-limiting steps in urea, sugar, alcohol and bile metabolism⁽¹³⁶⁾. Urea formation is central to the function of the liver, and the proteins that control several steps in the urea cycle vary across the circadian cycle. In rodents, the majority of these proteins peak during the dark phase of the light–dark cycle when they are actively feeding, and digestion would present amino acids to the hepatocytes⁽³⁴⁾. Key enzymes involved in cholesterol metabolism show robust peak levels during the dark period of the light–dark cycle⁽³⁴⁾. Enzymes involved in fructose metabolism as well as those involved in glycolysis and steps in the citric acid cycle also exhibited rhythmic oscillations, with expression levels increasing during the dark phase of the cycle^(136,138). Moreover, the transcription of genes encoding these metabolic enzymes is elevated during the early part of the night in anticipation of the start of night-time feeding in rodents^(34,139). Thus, there is a synchronous activation of a plethora of genes and their constitutive proteins critical to metabolism. Such temporal regulation optimises hepatic processing of night-time meals and metabolic efficiency, and implicates food-entrained circadian regulation for most of the genes in



the rodent liver. The hepatic circadian clock system is therefore likely to be important for many aspects of liver physiology, such as clearance of drugs and toxins, which is impaired in mice lacking clock-regulated transcription factors⁽¹⁴⁰⁾.

In humans, it is difficult to directly assess the circadian clock system in the liver so proxy parameters are used, such as plasma glucose levels and insulin production. Humans show high glucose levels and insulin secretion rates shortly before awakening in anticipation of glucose demand during the active period^(141,142). This would suggest that under normal feeding conditions, these rhythms are regulated by the circadian clock system in the SCN and not by the rhythms in food intake^(143,144). Daily rhythms in glucose tolerance have also been reported, with a lower plasma glucose response to bolus glucose administration in the morning compared with responses in the evening^(145,146). Interestingly, even though humans are active during the light phase of the light–dark cycle and rodents are active during the dark phase, both shows similar variations in glucose and insulin concentrations in the dark phase of the cycle⁽¹⁴⁷⁾.

In the pancreas, the role of the circadian clock system has only been recently elucidated. The pancreas regulates sugar and fat metabolism via controlled production of digestive enzymes and hormones in response to food availability and physiological demands. Circadian rhythms in pancreatic enzyme secretion are well documented in rodents, showing a night-time increase in amylase and the rate-limiting enzyme ornithine decarboxylase in polyamine biosynthesis^(148,149). Rodent studies have also shown that clock genes are expressed in a circadian manner in the pancreas, particularly in the insulin-producing β -cells of the islets of Langerhans^(38,150). Normally after meals, the β -cells produce insulin to stimulate glucose uptake and storage by the muscle and fat cells, and also to stop glucose production and secretion by the liver. The importance of the circadian clock system is therefore reflected by a robust circadian pattern of insulin release in isolated pancreatic islet⁽¹⁵¹⁾. Hence, loss of clock function in the pancreas can lead to metabolic disease pathologies. In studies using mice lacking one of the essential clock genes, *Bmal1*, in the pancreas, severe glucose intolerance and defective insulin production were observed, triggering the onset of a diabetes mellitus-like phenotype^(37,38). Moreover, the pancreatic islets in these mutant mice were found to be smaller and less efficient in producing insulin. In human subjects, circadian variation in the secretion of the pancreatic enzymes amylase and trypsin has been reported^(121,152). There is also a well-defined circadian rhythm of insulin secretion rates, where plasma insulin levels increase in the early morning, peaking by the afternoon and declining during the night⁽¹⁵³⁾.

The effect of the food-entrainable zeitgeber on the circadian clock function

The preceding sections have highlighted the circumstances by which changes in the feeding schedule in nocturnal rodents can alter the rhythmic expression of circadian clock genes in

the GI tract, liver and pancreas, without necessarily altering the expression pattern of the central clock in the SCN^(36,154). Hence, food is a very potent zeitgeber for peripheral clock systems. If rodents have access to food only during the light period when they are normally asleep, they will adjust to this feeding schedule within a few days and will display food anticipatory activity, including increased locomotor activity, body temperature, digestive enzyme activity and GI motility, a few hours before food becomes available^(154–156). Moreover, clock gene expression rhythms in these organs shift to realign with the new feeding schedule^(60,63). These circadian activities are normally entrained by the central clock in the SCN. Thus, it would appear that clock gene expression in these organs, which are intimately involved in feeding, have become entrained to changes in the timing of feeding. Nonetheless, when the animals regain access to food during the dark period, the clock system in the SCN, whose rhythms remained unaffected by changes in the feeding schedule, regains the control of and re-entrains the peripheral clocks.

It remains unclear what signals associated with feeding cause the shifting of clock gene expression in peripheral tissues. Total parenteral nutrition, which bypasses the GI tract, during the light period in rats shifted the peak expression of hepatic clock genes⁽¹⁵⁷⁾. This would suggest that factors directly associated with feeding, such as the taste of food, stomach distension, or direct physical contact of food with the GI lining, are not involved in entraining clock gene expression. In fact, nutrient availability at a cellular level has the greatest influence on molecular changes in the peripheral clock system. In addition, others have suggested that palatability as well as the nutritional value of the diet plays some role in entraining food anticipatory activity^(158,159).

It remains a point of contention whether food anticipatory activity requires a food-entrainable oscillator since it is unlikely that the SCN clock is responsible for the changes in peripheral clock gene expression in response to timed feeding⁽¹⁶⁰⁾. In SCN-lesioned rats, food anticipatory activity is still evident⁽¹⁶¹⁾. Therefore, the question remains, whether a second neuronal circadian system exists that can be entrained by the feeding schedule and has an influence over circadian rhythms in peripheral tissues. The exact location of this putative oscillator remains uncertain. Initially thought to be located in the GI tract, recent studies have suggested that at least important components of this food-entrainable oscillator are located in the dorsomedial hypothalamic nucleus^(162,163). Ablation of this nucleus resulted in the abolition of food anticipatory activity and the pre-meal rise in body temperature⁽¹⁶³⁾. Nevertheless, one can argue that the central clock can still synchronise the clocks in peripheral tissues indirectly through its influence on the rest–activity cycles, which in turn drives humoral outputs resulting in feeding rhythms.

From an adaptive point of view, food anticipatory activity allows the organism to activate its arousal, appetite, digestive secretions and metabolism just before receiving food, allowing it to cope advantageously with predictable feeding availability. Hence when food is abundant, the light-entrainable oscillator in the SCN becomes responsible for driving circadian rhythms,

but when food is scarce or is only available at certain times, the food-entrainable oscillator takes charge of a subset of rhythms, thus improving food access, but without encroaching on other rhythmic processes which continue to be governed by the light-entrainable SCN^(154,164).

Poor nutrition can deregulate the clock system and increase the risk of metabolic disease

It is clear that the circadian clock system is profoundly influenced by nutrient intake. Therefore, it is unsurprising that excessive or imbalanced diets can have negative effects on the orchestration of core clock genes and their downstream transcriptional targets. A recent study in mice has shown that rhythmic expression of the clock gene *Rev-erba*, which links circadian rhythms and metabolism in peripheral tissues, is disrupted in pancreatic β -cells in response to high-fat diet exposure⁽¹⁶⁵⁾. In addition, the rhythmic pattern of insulin secretion was impaired, which suggests that *Rev-erba* plays an important role in β -cell adaptation to nutritional stimuli. Thus, the plasticity of the clock system appears to become a maladaptive process when the organism is exposed to an obesogenic diet. In another study in mice, exposure to high-fat nutrition resulted in long-term abnormal clock and clock-controlled gene expression patterns in peripheral tissues such as the liver⁽¹⁶⁶⁾. Similar observations have also been made in adipose tissue and the hypothalamus, whereby high-fat nutrition does not only induce molecular alterations in the clock system, but also changes behaviour rhythms⁽¹⁶⁷⁾. Collectively, these data imply that poor diets can cause an imbalance in the molecular components of both the peripheral and central clock systems. Since many of the clock-controlled genes have direct metabolic outputs, diet-induced perturbations in core clock genes can directly lead to altered metabolism, leading to an increased risk of metabolic disease. This notion is supported by studies in mice whereby inactivation of the key clock components *Bmal1* and *Clock* resulted in metabolic pathologies such as obesity, fatty liver, hyperinsulinaemia, hyperglycaemia, hyperlipidaemia and hyperleptinaemia^(37,168,169); co-existing pathologies which bear a striking resemblance to the human metabolic syndrome. The dramatic rise in the prevalence of the metabolic syndrome in recent times necessitates the need to understand its pathogenesis. Therefore, the circadian clock system is now an emerging research target and a putative candidate mechanism linking dietary influences to metabolic disease susceptibility.

Concluding statements

The circadian clock system is fundamental to a range of physiological processes as demonstrated by the temporal and pronounced activity of a plethora of systems involved in nutrition and metabolism. Disrupted circadian rhythms can lead to attenuated circadian feeding rhythms, hyperphagia, GI pathologies, metabolic disease and reduced life expectancy. As food components and feeding time have the ability to reset biological rhythms, it is of paramount importance to understand the relationship between food, feeding and the circadian

clock system. In so doing, we may be able to use food or feeding times as a therapeutic intervention to reset or re-entrain the circadian clock system for better functionality of physiological systems, preventing obesity, promoting well-being and extending lifespan.

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