1 **Physiological links between circadian rhythms, metabolism and nutrition**

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33 **New Findings**

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- 35 • What is the topic of this review?

36 Discussion of the links between circadian, metabolic and nutritional physiology, with a particular
37 focus on mouse and human studies.

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- 39 • What advances does it highlight?

40 Links between circadian biology and metabolism exist from the molecular level through to whole
41 organism physiology. Advances highlighted include: interactions between circadian clock
42 genes/proteins and intracellular metabolic pathways; identification of the function of clocks in
43 specific peripheral tissues; effects of timed feeding; and the variable effect of obesity on rhythms.

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47 **Abstract**

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49 Circadian rhythms, metabolism and nutrition are closely inter-linked. A great deal of recent research
50 has not only investigated how aspects of metabolic physiology are driven by circadian clocks, but
51 how these circadian clocks are themselves sensitive to metabolic change. At the cellular level, novel
52 feedback loops have been identified that couple circadian 'clock genes' and their proteins to
53 expression of nuclear receptors, regulation of redox state and other major pathways. Using targeted
54 disruption of circadian clocks, mouse models are providing novel insight into the role of tissue-
55 specific clocks in glucose homeostasis and body weight regulation. The relationship between
56 circadian rhythms and obesity appears complex, with variable alteration of rhythms in obese
57 individuals. However, it is clear from animal studies that the timing and nutritional composition of
58 meals can regulate circadian rhythms, particularly in peripheral tissues. Translation of these findings
59 to human physiology now represents an important goal.

Circadian rhythms and the circadian timing system

Many biological processes, ranging from metabolic pathways to physiology and behaviour, exhibit 24-hour rhythms driven by endogenous circadian clocks. Increased morbidity occurs following prolonged desynchrony of circadian rhythms and the external environment, such as during shift work and chronic jet-lag. Of particular relevance to this symposium, shift workers exhibit greater prevalence of obesity and associated disorders (reviewed in Antunes *et al.*, 2010).

Circadian rhythms in mammals are widespread and regulate most, if not all, of the major physiological systems. These rhythms are generated by a circadian timing system, which consists of a 'master' clock within the suprachiasmatic nuclei (SCN) of the anterior hypothalamus and multiple 'peripheral' clocks located elsewhere within the brain and throughout the body (reviewed in Mohawk *et al.*, 2012). The SCN clock is synchronised to the external light-dark cycle and acts to maintain internal synchrony within the individual. In this way the circadian system is able to maintain an appropriate phase relationship to the external solar day. The mechanisms through which the SCN synchronise peripheral clocks are complex and not yet fully understood. Neuroendocrine pathways are likely to play a key role in this process, as the autonomic nervous system and hormones such as pineal melatonin and adrenal glucocorticoids are under SCN control (reviewed in Mohawk *et al.*, 2012). Through influence on sleep-wake timing the SCN also exert temporal influence over behavioural factors, such as social interaction and feeding time, that can synchronise circadian rhythmicity. Indeed, as described below, animal studies suggest timed feeding is a major regulator of the phasing of peripheral clocks.

Linking circadian rhythms, metabolism and nutrition

Much work has been conducted in this area of chronobiology over recent years. Animal and cellular studies have clearly demonstrated that rhythms and metabolism are intimately linked across multiple levels of biological organisation. Due to space limitations, this article will only focus on a subset of the available literature.

Molecular and biochemical links

The molecular mechanisms underpinning mammalian circadian rhythms have been extensively reviewed elsewhere (e.g. Buhr & Takahashi, 2013). In brief, the dominant molecular model is comprised of a set of interlocking transcriptional-translational feedback loops (TTFL). The primary loop consists of the proteins CLOCK and BMAL1 stimulating transcriptional activity of three *Period* (*Per*) and two *Cryptochrome* (*Cry*) genes. After appropriate temporal delay caused by post-translational modifications, the translated PER and CRY proteins then form protein complexes that translocate into the nucleus and inhibit their own transcription via interaction with CLOCK and BMAL1.

In addition to the primary loop, there are many interactions between the above 'clock genes', their resulting proteins and key biochemical pathways regulating intracellular metabolism. These have been reviewed in detail by others (Bass, 2012), but some examples are mentioned here. For instance circadian clock proteins act as transcription factors to drive the rhythmic expression of approximately 50% of nuclear receptors in key metabolic tissues. In turn, many of these nuclear receptors are able to regulate the expression of clock genes/proteins, thus forming additional

feedback loops linking the molecular clock to cell metabolism. Other major components of intracellular biochemistry that are reported to regulate clock function include AMPK, NAD and even cAMP (Bass, 2012).

Genetic links

Long-term changes in key aspects of metabolic physiology, such as glucose homeostasis and energy balance, are closely tied to clock gene structure. One of the predominant mouse models of disrupted circadian rhythms is the 'Clock mutant' mouse, which expresses a dominant negative version of the CLOCK protein. Studies of this mouse found wide-ranging metabolic dysregulation including: enhanced glucose response to insulin challenge and impaired gluconeogenesis (Rudic *et al.*, 2004), obesity and metabolic syndrome (Turek *et al.*, 2005). Subsequent studies of many different 'whole animal' transgenic mice with altered clock gene function have also reported abnormal metabolic phenotypes (reviewed in Bass, 2012). Similar findings have been reported in human genetic studies. Polymorphisms of human clock genes and their associated haplotypes have been associated with multiple metabolic disorders including obesity, metabolic syndrome and type 2 diabetes (e.g. Woon *et al.*, 2007; Scott *et al.*, 2008).

Although the above studies provide clear functional links between clock genes and metabolic activity, they are unable to identify the contribution of individual tissue clocks to the overall phenotype. To address this problem, later animal studies have 'knocked out' BMAL1 in a tissue-specific manner thereby creating localised clock disruption. Loss of BMAL1 in either of the liver, pancreas or skeletal muscle leads to changes in glucose metabolism. Liver-specific clock disruption causes fasting hypoglycaemia and elevated glucose clearance following acute glucose administration (Lamia *et al.*, 2008). Pancreas-specific clock disruption results in hyperglycaemia and lowered glucose tolerance, as a result of reduced insulin secretion (Marcheva *et al.*, 2010). Skeletal muscle-specific clock disruption leads to decreased insulin-dependent glucose uptake in skeletal muscles and also altered muscle glucose metabolism (Dyar *et al.*, 2014). By contrast, disruption of the clock in white adipose tissue induces obesity; this is thought to be the result of abnormal PUFA secretion from adipocytes regulating hypothalamic appetite centres and causing increased feeding during the resting phase of the day (Paschos *et al.*, 2012).

Nutritional and dietary links

The timing of food intake has many physiological consequences (reviewed in Johnston, 2014). The ability of temporally restricted feeding to influence circadian behaviour and physiology has been known for many years. Using rats, it was originally discovered that restriction of food availability to a short period each day results in an intense bout of anticipatory activity directly before the onset of the feeding period. A series of further studies revealed that the timing of this anticipatory activity possesses many of the properties of an endogenous circadian rhythm and so the existence of a food entrainable oscillator (FEO) was proposed (reviewed in Mistlberger, 2009). Intriguingly this FEO persists in SCN-lesioned animals (Mistlberger, 2009) and in mice lacking functional circadian clock genes (Storch & Weitz, 2009). However its identity is still a matter of debate in the literature.

In humans, there is an established literature describing daily changes in parameters such as glucose homeostasis. In healthy subjects, glucose tolerance lowers throughout the day, leading to the term 'afternoon diabetes'. This phenomenon persists in controlled conditions, indicating that is governed

by the internal circadian system, and is thought to involve changes in insulin signalling (reviewed in Van Cauter *et al.*, 1997). In addition, lipid metabolism exhibits circadian regulation, with elevated plasma concentration of triacylglycerol (TAG) during the biological night and an elevated post-prandial response following a night-time meal compared to the same meal consumed during the day (reviewed in Morgan *et al.*, 2003).

With the recent advances in molecular circadian biology, interest in nutritional links has again become prominent within the field. Animal studies strongly suggest that timed feeding can have beneficial physiological effects, including protection against the obesogenic and metabolic consequences of high-fat diet (Hatori *et al.*, 2012; further discussion in Johnston, 2014). Feeding time is also powerful method of synchronising rodent peripheral clocks. Indeed, when keeping animals in a light-dark cycle, restricting food availability to the time when they would usually be resting/fasting disrupts the internal synchronisation of clocks; the SCN remain synchronised to the light-dark cycle, whereas multiple peripheral clocks re-set to the timing of food availability (Damiola *et al.*, 2000). These peripheral clocks may therefore be important to the functioning of the FEO. In addition, our growing understanding of the role of clocks in individual peripheral tissues is beginning to provide physiological insight to explain temporal differences in post-prandial responses.

Rhythms in obesity

Differences in daily rhythmicity between lean and obese subjects have been observed in some studies. One of the most widely reported effects of obesity is reduced amplitude of rhythms, both physiological and molecular. However, this is not always the case and there is variation within the literature. A key point to emphasise is that the presence or nature of altered rhythms in obesity depends to a large degree on the specific molecules in question, the tissue(s) studied and other aspects of experimental design.

Daily rhythms exist in the plasma concentration of many hormones. The adipokine leptin exhibits daily rhythms that are likely to be in part driven by endogenous circadian physiology (Shea *et al.*, 2005; Otway *et al.*, 2009). Early reports indicated a decreased amplitude in leptin rhythmicity in obese humans (e.g. Sinha *et al.*, 1996), but this is not reproduced in some later work (e.g. Mantele *et al.*, 2012). Furthermore some endocrine rhythms, such as plasma melatonin, actually exhibit increased amplitude in obese insulin-sensitive men (Mantele *et al.*, 2012).

At the molecular level, reduced amplitude clock gene rhythms have been reported in mice that are obese as a result of either altered genetic background (Ando *et al.*, 2005) or imposition of a high fat diet (Kohsaka *et al.*, 2007). However, there is some inconsistency in the response to diet-induced obesity that may reflect gender of animals studied or other experimental details, such as dietary composition (reviewed in Johnston, 2014). Regarding this last point, it recently been demonstrated that altered rhythms in animals made obese by a high fat diet may reflect acute effects of the dietary intervention rather than any long-term changes in energy balance (Eckel-Mahan *et al.*, 2013).

Few studies have examined daily profiles of gene expression in obese humans. However, in one study using serial sampling of human gluteal subcutaneous fat, no differences in gene expression rhythms were found in lean vs obese individuals (Otway *et al.*, 2011). Limitations of this study include the inability to repeatedly sample visceral adipose depots, which may well have shown altered rhythms. However it does emphasise the point that not all rhythms are altered by obesity and is consistent with the principle that factors other than obesity (e.g. genetic background, acute dietary intervention) may be important regulators other than obesity per se.

Perspectives

Research linking daily rhythms and metabolism has gained a great deal of momentum over recent years. Work in model organisms has provided clear and convincing evidence to link these key physiological processes, but many important challenges lie ahead. These include translation to human physiology, functional relationship with pathophysiology, and links with nutritional physiology.

For obvious ethical and practical reasons, access to human tissues obtained in controlled experimental conditions is limited. Subcutaneous fat represents a fairly accessible tissue relevant to the study of obesity. Alternative approaches include viral transfection of cultured human cells (reviewed in Johnston, 2012) or use of blood sampling. Although not frequently considered a 'metabolic' tissue, blood is thought to provide a window into whole body physiology and its molecular rhythms are susceptible to interventions such as timed light exposure, sleep deprivation and sleep timing (Archer *et al.*, 2014). Subcutaneous fat and blood are therefore likely to play a future role in the understanding of circadian metabolism.

An ultimate goal of this work is to apply research data to optimise behaviours, thereby increasing health. This relates both to the general population and to specific groups of individuals (e.g. shift workers) experiencing circadian misalignment and associated metabolic problems. Despite the amount of translational work required, there are already encouraging signs that applying basic models of circadian biology can have a major effect on human obesity. For example, recent studies have provided associations between timing of calorie intake and success in weight loss diets (e.g. Garaulet *et al.*, 2013).

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