When the Clock stops ticking, metabolic syndrome explodes

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Circadian control depends on oscillating transcription factors, master switches synchronized by stimuli such as light and feeding. Recent studies show that altering circadian rhythmicity also results in pathophysiological changes resembling the metabolic syndrome.

Many physiological processes display day-night rhythms. Feeding behavior, lipid and carbohydrate metabolism and blood pressure control are only a few examples of processes subject to daily variation. This biological oscillation is controlled by the circadian clock, an endogenous self-sustained machinery of rhythmically acting transcriptional loops. These self-regulated feedback loops balance each other; in mammals, transcription factors like Clock and Bmal1 regulate the production of proteins like Per and Cry, which in turn regulate the production of Bmal1. Owing to this arrangement, the levels of Bmal1, Clock, Per and Cry oscillate rhythmically.

Various input signals (light and various paracrine and endocrine signals) can modulate these transcriptional pathways and ‘reset the clock.’ In addition, the circadian clock dictates the rhythmic production of output regulators, other transcription factors such as DBP, HLF and TEF. These transcription factors, in turn, regulate downstream target genes involved in different biochemical pathways, including metabolism of glucose and lipids, synthesis of cholesterol and bile acids, and others (Fig. 1).

In any given tissue, three to ten percent of transcripts show circadian rhythmicity. Many of them participate in common metabolic pathways, such as metabolism of glucose, cholesterol and fatty acids and mitochondrial oxidative phosphorylation. Dysregulation of these pathways owing to, for instance, a sedentary lifestyle and high caloric intake, results in the development of cholesterol abnormalities (dyslipidemia), insulin resistance, obesity and hypertension. These disorders often occur simultaneously and have therefore been grouped under the term ‘metabolic syndrome.’

Recent studies show that deletion of the Clock and Bmal1 genes results not only in circadian disturbances, but also in metabolic abnormalities of lipid and glucose homeostasis—a phenotype resembling the metabolic syndrome.

Turek et al. showed that mice homozygous for a loss-of-function mutation in the Clock gene have altered patterns of food intake: they eat too much, become obese and develop hyperglycemia and dyslipidemia. These Clock mutant mice develop adipocyte hypertrophy and excessive accumulation of fat in the liver (hepatic steatosis), hallmarks of the metabolic syndrome. Rudic et al. found that mutations in Bmal1 and Clock not only modified the diurnal variation in levels of plasma glucose and triglycerides, but also influenced the development of glucose intolerance and insulin resistance in response to a high-fat diet. The observed metabolic phenotypes in these mutant mice are fairly modest, indicating that the role of the Clock genes on metabolism might be modulatory and not obligatory.

The Clock genes also show patterns of rhythmic expression in peripheral organs, such as liver and adipose tissue. In obese and diabetic animals, these rhythms are disturbed, as well as the rhythmic expression of adiponectin, resistin, visfatin and leptin, adipokines that control energy homeostasis, glucose and lipid metabolism. Interestingly, Bmal1 is induced during and promotes adipocyte differentiation, possibly by activating the downstream adipogenic transcription factors Rev−erbα and SREBP1 (ref. 7). These alterations in adipocyte differentiation and adipokine expression profiles might also occur in the liver and may contribute to the phenotype of the Clock mutant mice.

An important question is whether the phenotype of the Clock mutant mice results from the alterations in circadian rhythmicity per se or from other unknown activities of the Clock gene. To answer this question, it will be of interest to characterize the phenotype of mutant mice for other genes of the circadian clock and to create tissue-specific knockout mice for the different Clock genes.

Another important question is to establish the pathways downstream of the clock genes that ultimately influence glucose and lipid metabolism. It is likely that these pathways involve well-known clock output regulators, such as DBP, Dec1 and Dec2, as well as other transcription factors known to regulate metabolic state, such as Diaphanous-related formin 2 (Drosophila), DREB2A, and DVT1/VT2. These factors are induced during circadian rhythmicity and regulate glucose and lipid metabolism in various tissues.
as SREBP1 or the nuclear receptors RORα, Rev-erbα and PPARα. For example, expression of hepatic PPARα, which is activated by fatty acids, shows circadian rhythmicity directly mediated by Clock and Bmal1 (ref. 8) and modulated by glucocorticoids (Fig. 1). Similarly, transcriptional regulation of CYP7A1, a rate-limiting enzyme in bile acid synthesis, a pathway long known to show circadian rhythmicity, is regulated by DBP and Dec2, and by nuclear receptors including PPARα10-12. Along the same line, Rev-erbα, a nuclear receptor that regulates hepatic and adipose lipid metabolism through peripheral actions, is also expressed in the suprachiasmatic nucleus, a brain region crucial for circadian rhythmicity, where it has an important role in the control of the Clock genes12. Moreover, PPARs and PPARγ regulate hepatic and adipose Rev-erbα expression, respectively14,15. Finally, recent data indicate that RORα and Rev-erbα regulate lipid metabolism and participate in the control of Clock and Bmal1 expression13,16-18. So, it appears that the Clock and nuclear receptor pathways may converge to integrate metabolic and circadian signals from the environment. As nuclear receptors are putative drug targets for combating the metabolic syndrome, studies on these interacting pathways may provide clues for optimal therapeutic management.

Plasma cholesterol (high- and low-density lipoprotein), triglycerides and coagulation factors (fibrinogen and platelets) show circadian variations19. Moreover, the incidence of cardiovascular diseases such as myocardial infarction, sudden cardiac death, stroke, unstable angina and high blood pressure appears to occur at higher frequency at specific moments of the day19. Acute jet lag, shift work and night eating, syndromes related to changes in circadian rhythmicity, occur together with alterations in one or more of the pathological components of the metabolic syndrome20,21. The finding that Clock genes impact metabolism provides a potential molecular basis for these pathophysiological conditions and may therefore suggest possibilities for new therapeutic approaches that take circadian rhythms into account. This is the basis of chronotherapeutics, which, despite being in its infancy, has already proven to be promising for improving the therapeutic index of several drugs. An example relates to the circadian variations in cholesterol synthesis through its rate-limiting enzyme HMG-CoA reductase, the expression of which shows diurnal rhythmicity, being highest during the night. For this reason, statins, widely prescribed cholesterol-lowering drugs that inhibit this enzyme, are most efficient when taken before bedtime. Thus, the observation that Clock genes control energy homeostasis may lead to the development of more finely tuned, and hopefully more optimal, treatments of obesity and its related complications.

**Author’s Comments**

Timing and serendipity had a major role in our studies on clocks and metabolism. The work began shortly after I arrived at Northwestern University as junior faculty, when Joe Takahashi and Fred Turek told me about possible body weight abnormalities in the Clock mutant mouse, a model primarily studied because of its dysregulated locomotor activity and sleep phenotypes. A powerful aspect of the Clock model was the molecular framework it had already built in studies of behavior, and I was excited to think that we might exploit the model to deepen our insight into interconnections between behavior and energy balance. Timing was important, as microarray studies had just indicated that metabolic transcriptional networks were a major output of the clock, and there were data that redox flux could directly affect activity of NPAS2, a CLOCK homolog. Our key experiment was to place mutant and isogenic littermate mice on a high-fat diet and to track changes in metabolism, in conjunction with detailed round-the-clock studies of metabolic markers (including glucose, insulin, leptin and glucocorticoids). The many all-night experiments made for close bench-partners of members of both circadian and metabolic labs and paved a new direction for our own research. At least two major questions have emerged from our original study. First, how are cell-autonomous clocks synchronized? Second, how does the clock network contribute to energy homeostasis? Understanding the pathways that link circadian activity, sleep, feeding and peripheral metabolic homeostasis, and delineating the impact of each parameter on the whole organism, will hopefully bring us one step closer to a unified ‘systems’ map of metabolic disease.

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