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THE MOLECULAR CLOCK AS A METABOLIC RHEOSTAT

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Abstract

Circadian clocks are biologic oscillators present in all photosensitive species that produce 24-hour cycles in the transcription of rate-limiting metabolic enzymes in anticipation of the light-dark cycle. In mammals, the clock drives energetic cycles to maintain physiologic constancy during the daily switch in behavioral (sleep/wake) and nutritional (fasting/feeding) states. A molecular connection between circadian clocks and tissue metabolism was first established with the discovery that 24-hour transcriptional rhythms are cell-autonomous and self-sustained in most tissues and comprise a robust temporal network throughout the body. A major window in understanding how the clock is coupled to metabolism was opened with discovery of metabolic syndrome pathologies in multi-tissue circadian mutant mice including susceptibility to diet-induced obesity and diabetes. Using conditional transgenesis and dynamic metabolic testing we have pinpointed tissue-specific roles of the clock in energy and glucose homeostasis, with our most detailed understanding of this process in endocrine pancreas. Here we review evidence for dynamic regulation of insulin secretion and oxidative metabolic functions by the clock transcription pathway to regulate homeostatic responses to feeding and fasting. These studies indicate that clock transcription is a determinant of tissue function and provide a reference for understanding molecular pathologies linking circadian desynchrony to metabolic disease.

Keywords

Circadian clock; Pancreatic islet; Metabolism; Glucose homeostasis; Transcription regulation

INTRODUCTION

Circadian clocks are self-sustained biologic oscillators found in all photosensitive species and drive transcriptional and metabolic processes in anticipation of the rising and setting of the sun each day. In mammals, the clock system maintains homeostasis of neurobehavioral and energetic states during alternating phases in the sleep/wake and fasting/feeding cycle. A transformation in our understanding of the molecular basis of circadian control of metabolism was made possible by the identification of core clock genes and the subsequent

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CONFLICT OF INTEREST

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recognition that cellular oscillation of gene expression occurs not only within master pacemaker neurons of the hypothalamic suprachiasmatic nucleus (SCN), but also within nearly all cells throughout the body, including organs critical for metabolism and weight regulation. Subsequent genetic studies have begun to provide mechanistic insight into the integrated physiologic function of the clock, revealing temporal integration amongst transcriptional, post-transcriptional, and bioenergetic processes, and establishing that metabolic and circadian systems exhibit bi-directional regulation.

An entry point to understanding how the molecular clock is coupled to metabolism was opened with the discovery of metabolic syndrome pathologies in multi-tissue circadian mutant mice. More recent insight into specific effects of the clock within distinct metabolic organs has emerged with the application of Cre-LoxP-based conditional mutagenesis and chemically-inducible recombination. The combination of genetics, dynamic endocrine testing, and cell-based physiologic analyses has provided a detailed portrait of the circadian organization of metabolism. Classical studies first emphasized the hierarchical anatomic organization of the circadian system, establishing that light entrains brain pacemaker neurons within the SCN, which in turn synchronize cellular clocks within extra-SCN neurons and peripheral metabolic tissues [1-3]. More recently, a revision in this model has occurred with the discovery that circadian oscillation of gene expression occurs across a wide range of cell types and can even be observed in fibroblasts maintained *ex vivo* [4]. In addition to its role as coordinator of behavior and physiology with the external light-dark environment, the clock system is also integral to the communication between brain and peripheral tissues, and this dynamic communication is dependent upon both time-of-day and nutrient state. For instance, during sleep, clock function in oxidative tissues is especially important in regulating mitochondrial respiration and thereby in providing sufficient glucose to feed the brain. Indeed, deficiency of clock function in liver and skeletal muscle causes mitochondrial pathologies corresponding to those found in human metabolic myopathy, a syndrome characterized by fasting-induced hypoketotic hypoglycemia [5,6]. In contrast, during the postprandial state, clock function within endocrine pancreas is required for nutrient storage, a point emphasized by the observation that abrogation of clock function isolated to pancreas results in impaired glucose-stimulated insulin secretion and β-cell failure [7,8]. In addition to clock control of metabolism, the clock also functions as a metabolic rheostat to adjust metabolism in response to metabolic flux across the 24-hr light-dark cycle, though the underlying mechanisms are not well understood. The yin-yang of clocks in both fasting and fed conditions represents an added layer of complexity in understanding how temporal homeostasis is maintained across the day-night cycle. Our emphasis in this review is to highlight cell-type specific mechanisms through which rhythmic clock transcription factors anticipate and coordinate nutrient responsiveness and to indicate evidence for feedback effects of nutrient on core properties of the clock.

GENETIC MECHANISMS IN CIRCADIAN INTEGRATION OF ENERGETICS AND METABOLISM

Circadian clocks are encoded by a transcription-translation feedback loop that maintains internal temporal organization in synchrony with the solar cycle. In plants, circadian clocks

coordinate oxygenic photosynthesis and DNA repair processes to maximize these events with exposure to sunlight each day [9]. Indeed in photosynthetic eubacteria, misalignment of the external light cycle with the genetically-determined period length of the organism in both short and long period mutants results in reduced fitness, reproduction, and survival [10]. Likewise, evidence in eubacteria using mixed cultures harboring clocks of varying intrinsic period length demonstrates a selective advantage in the alignment between internal period length and the external light-dark cycle [11]. Such reductionist experiments in unicellular organisms provide conceptual insight when considering the impact of the clock system in mammals. Indeed, among the most striking pathologies observed in mice harboring mutations in core clock genes are abnormalities in energetics--weight regulation and glucose homeostasis. In extending the idea that clocks benefit energetic processes in prokaryotes to animals, one must consider the added complexity of the central nervous system and the multiple cell types and organ systems involved in metabolism. Specifically, the brain clock within the hypothalamic SCN controls sleep-wake and fasting-feeding cycles, in addition to rhythmicity of autonomic and neuroendocrine pathways [3]. A more complete view of circadian homeostasis must also take into consideration the effect of clocks within peripheral tissues. Local organ clocks impact metabolic function by anticipating the varying requirement for anabolic and catabolic processes across the daily fasting-feeding/sleep-wake cycle. Unraveling the role of the clock system is of direct relevance to human health since mounting evidence from both longitudinal population studies and clinical investigation have demonstrated a pronounced effect of shiftwork, jetlag, and sleep disorders on metabolic function. Genome-wide association studies also indicate linkage between polymorphisms in the clock gene *CRY2* and the melatonin receptor (*MTNR1B*) with glucose homeostasis in man [12-14]. Indeed, the prevalence of circadian environmental disruption may well be increasing with the spread in exposure to blue light through electronic media devices [15]. Collectively, these observations raise interest in understanding underlying mechanisms accounting for the interrelationship between clock function and metabolic systems in health and in conditions of environmental disruption of the system.

Our studies of clock function were serendipitous in that the first mammalian circadian mutant animal to be discovered by positional cloning, the $Clock^{\Delta 19}$ mutant mouse, was identified at Northwestern University, and there were early hints that these animals displayed abnormalities in body weight. Importantly, this animal expressed a dominant negative mutation rather than a loss-of-function allele, resulting in formation of a competent partner for the heterologous factor BMAL1 that was incapable of transcriptional activation [16]. Using longitudinal high fat feeding, we found that $Clock^{\Delta 19}$ mutant animals display increased susceptibility to diet-induced obesity, increased hepatic steatosis and visceral adiposity, as well as both hyperlipidemia and hyperglycemia, a cluster of pathologies associated with the metabolic syndrome in man [17]. However, a paradox in the multi-tissue mutants was that the animals gained weight but did not develop classical hyperinsulinemia —indicating a primary deficiency in β-cell function in circadian mutant animals, as discussed further below. An ensuing series of studies confirmed the concept that clock disruption across multiple metabolic tissues and systems exerts strong effects on metabolic

homeostasis, and as discussed below, clock functions in distinct metabolic tissues in unique ways that are both dependent upon time of day and nutrient state of the animal [18-20].

Since the original studies in circadian mutant animals were performed in multi-tissue mutants which developed hyperglycemia, the subsequent observation that multi-tissue *Bmal1* knockout animals develop fasting-induced hypoglycemia was initially perplexing, although later studies using liver-specific conditional knockouts demonstrated a role of *Bmal1* within liver in mitochondrial oxidative function, supporting a role for clock transcription factors in biosynthetic pathways during fasting. These studies emphasize the concept that the analysis of clock gene mutant animals is dependent both on nutrient condition (fasting/feeding) and on the specific tissue in which the clock is expressed [5,6]. This idea that distinct effects of the clock in different tissues act in opposition is especially elucidated by the application of Cre-LoxP conditional mutagenesis in pancreas. Indeed, selective ablation of the clock within endocrine pancreas in *Bmal1*$^{fx/fx}$*; Pdx-Cre* mutant mice results in even more severe early-life hypoinsulinemic diabetes than in the global mutants, confirming the idea that in the setting of the multi-tissue mutant, glucose homeostasis reflects the net (and opposing) actions of clock function in both endocrine pancreas and oxidative tissue (e.g. liver) [7]. Moreover, studies in identically-sized isolated islets from circadian mutant mice confirm the observation that loss of core clock activator expression results in impaired glucose stimulated insulin secretion. Further, life-long clock deregulation in pancreas also leads to impaired islet growth and reduced proliferation *in vivo*. Subsequent research in three other groups has corroborated these observations [8,21,22], including work from the Dibner laboratory which has monitored live-cell clock oscillations within isolated α- and β-cells of human islet cells, indicating that the expression of autonomous rhythmic gene expression may be a general feature of endocrine pancreas [22]. Together, studies of clock function in the islet provide a paradigm for understanding the integrated role of peripheral tissue circadian rhythmicity in organismal metabolic homeostasis.

The initial investigation of circadian regulation of pancreatic islet function was based upon reverse genetic approaches and physiologic analyses, revealing that clock ablation results in defective glucose-stimulated insulin secretion. However, at the time of the original studies of clock function in islets, the most in-depth approach to analyzing transcriptional targets relied upon microarray analyses which were limited by low resolution and difficulty in detecting alterations in transcripts expressed in low abundance, and/or those whose steady state levels were long and thus unchanged within the context of steady state comparisons. Subsequently, rapid advances derived from the advent of next-generation sequencing have opened new vistas on our ability to dissect the basis of genome-wide regulation of rhythmic transcription and the specific role of clock activator and repressors in this process. A first phase in the application of next-generation sequencing has been the identification of the cis-regulatory map of core clock transcription factor (TF) chromatin binding *in vivo* in liver; this work has established new insight into the genomic basis of clock regulation within peripheral tissues [23,24]. More recent studies using global run on-sequencing (GRO-Seq) in liver have revealed that enhancer RNA (eRNA) transcription corresponds with both the phase of transcription of gene bodies and the phase-specific binding of core clock TFs within these loci [25]. These observations are consistent with a model in which circadian

TFs regulate physiology through the control of tissue-specific enhancers. Nonetheless, a gap remains in our understanding of genomic mechanisms of clock regulation across tissues, specifically in pancreas.

Analyses of circadian regulation of genome-wide transcription within pancreas raises intriguing questions in the context of understanding the etiology of diabetes mellitus since both genetic and environmental studies implicate transcriptional deregulation within the pancreatic β-cells and liver in the pathophysiology of human diabetes. Although developmental studies have provided detailed understanding of lineage- determining TFs in the β-cell, less is known concerning the dynamic role of TFs in the differentiated islet. In this regard, a surprise has been the discovery that monogenic disorders causing β-cell failure in humans arise from heritable mutations in TFs originally identified in studies of hepatic organogenesis, including HNF1α/β and HNF4α, in addition to PDX1, NEUROD1, PAX4, and KLF11 [26]. Of note, the majority of DNA sequence variants associated with diabetes risk in humans map to non-coding DNA regions, which are thought to alter the transcription of nearby genes by inhibiting TF binding within distal enhancers, indicating a role for transcriptional control outside of gene promoters in β cell function and disease [27-29]. Altered expression of circadian TFs in islets from type 2 diabetic humans and the identification of a sequence variant at the *CRY2* locus in human glucose metabolism mentioned above collectively suggest that clock-mediated transcriptional regulation at islet enhancers may likewise contribute to organismal glucose homeostasis. However, how rhythmic transcription on a tissue-level coordinates physiologic homeostasis in the whole organism remains a question for future study. Overall, studies of the β-cell molecular clock will elucidate how glucose homeostasis is coupled to the light/dark cycle and the transcriptional determinants of circadian physiology.

CLOCK-NAD$^+$- SIRTUIN PATHWAY IN METABOLIC EPIGENETICS AND AGING

In addition to the application of genomic methodologies towards understanding clock function in pancreas and diabetes, parallel research has shown integral integration of metabolic and circadian systems through feedback signaling involving the NAD$^+$-biosynthetic pathway and its control of the NAD$^+$-dependent sirtuin (SIRT) family of class III histone deacetylases. Indeed, the discovery that SIRT1, the mammalian ortholog of the founding member of the yeast sirtuin family (Sir2), is bound in a complex with CLOCK/BMAL1, and that CLOCK/BMAL1 regulate the rate-limiting enzyme in the NAD$^+$ biosynthetic salvage pathway (NAMPT), has established a central feedback loop involving CLOCK/BMAL1-SIRT1-NAMPT/NAD$^+$. An intriguing aspect of the CLOCK/BMAL1-NAD$^+$/SIRTUIN connection is that the sirtuin family generally has been well established as a key factor in the coupling of nutrient with lifespan regulation from yeast to animals. Longevity in animals involves interactions between environmental factors, such as nutrition and physical activity, and genetically-encoded factors, such as the cellular circadian clock and regulators of cell cycle, yet the interplay between genes and environment in lifespan determination remains incompletely understood. The circadian system is unique in its role in maintaining daily rhythms of sleep, feeding, and metabolism with the environmental light

cycle, while also participating in lifespan regulation. Indeed, a hallmark of aging in humans and experimental animals has been the finding that both sleep and circadian homeostasis decline chronologically, indicating that diseases of aging involve impaired coordination of sleep, circadian behavior, and cell metabolism [30]. Indeed, microarray and next-generation sequencing studies have revealed oscillation of more than 10% of the transcriptome, including many rate-limiting enzymes in intermediary metabolism [23,31-34]. Surprisingly, one of the most robust outputs of the clock across multiple tissues is the regulation of biosynthesis of NAD^+, a central molecule in electron transport and cell redox. NAD^+ is also an obligate cofactor for the class III sirtuin deacetylases, which couple nutrient sensing to oxidative metabolism and lifespan determination.

As noted above, at the biochemical level, a clue to the molecular mechanisms coupling circadian rhythms, sleep, and metabolism during aging originated with our discovery that a major transcriptional output of the clock is NAMPT (nicotinamide mononucleotide phosphoribosyl transferase), the rate-limiting enzyme in NAD^+ biosynthesis [35]. NAMPT maintains essential levels of cellular NAD^+ for both electron transport reactions and for its activity as a cofactor for the NAD^+-dependent sirtuin deacetylases and PARPs, which are activated by low glucose and genotoxic stress. A major advance came when several laboratories demonstrated that SIRT1, a well characterized deacetylase that promotes mobilization of oxidative fuel, is present in tight physical complexes with, and modulates the activity of, the transcriptional activators in the forward limb of the clock (CLOCK/BMAL1). Since SIRT1 activity modulates CLOCK/BMAL1, and in turn, since SIRT1 is dependent upon NAD^+ bioavailability for its activity, the SIRT1-CLOCK/BMAL1 pathway represents a novel “feedback loop” [35-38]. Importantly, SIRT1 belongs to a family of 7 class III NAD^+-dependent histone deacetylases that are nutrient-responsive epigenetic regulators. Indeed, we find that circadian disruption in *Bmal1* mutant mice leads to profound NAD^+ deficiency in combination with mitochondrial failure—similar to the phenotype of the human metabolic myopathy syndrome in children who present with fatty liver, hypoglycemia, and skeletal and cardiac myopathies, an observation discussed in the following sections.

While NAD^+ biosynthesis occurs predominately in liver, skeletal muscle, and adipose tissue of the adult animal, current evidence suggests that NAMPT and its biosynthetic product NMN also circulate in blood and are delivered through non-autonomous pathways to non-biosynthetic cells in brain and other tissues [39]. Indeed, both genetic and pharmacologic studies have shown that allosteric activation of NAMPT and/or administration of NAD^+ precursors (e.g. NMN or NR) exert potent neuroprotective effects [40,41]; likewise, overexpression of SIRT1 is also neuroprotective [42], supporting the hypothesis that physiologic and pharmacologic activation of NAD^+-SIRT1 plays an important role in CNS healthspan. A major feature of aging in mice involves the age-related decline in NAMPT and intracellular NAD^+ levels in peripheral liver, fat, and skeletal muscle, in addition to circulating NMN [43,44], whereas NAD^+ supplementation with either NMN or NR reverses aging pathologies in myopathic disease [45] and age-related insulin resistance in wild-type C57BL6 mice [44] through mechanisms involving both enhanced oxidative metabolism and improved insulin sensitivity. In addition to evidence that NAD^+ deficiency contributes to

metabolic disorders of aging, recent studies indicate that loss of SIRT1 in brain disrupts circadian behavior [46], although it is still not known whether pacemaker neurons per se are influenced by NAD^+ biosynthesis, nor do we know the extent to which NAD^+ bioavailability might impact sleep. An age-associated decline in NAD^+ also restricts the availability of $NADP^+$, an essential electron acceptor and the major reducing equivalent in cells, exposing both brain and peripheral biosynthetic tissues to excessive oxidative stress. Despite strides in understanding the role of the NAD^+-SIRT1 pathway in circadian systems, a major gap exists in understanding the impact of age-related decline in NAD/$NADP^+$ in peripheral tissues on the coordination of sleep-wake/fasting-feeding and metabolic cycles. Moreover, we do not yet know the impact of deficiency in NAD^+ on SIRT1-mediated gene transcription rhythms in brain cells important in circadian function and sleep.

Clock-NAD^+ cycle links circadian systems to metabolic programming

We pursued the idea that disruption of clock and its effect on NAD^+ biosynthesis in turn impairs mitochondrial function. We reasoned that alterations in the phase (i.e., alignment of intrinsic NAD^+ oscillation with the fasting/feeding cycle) or absolute levels of NAD^+ biosynthesis and the activity of NAD^+-dependent deacetylases, might represent an important node through which the circadian process influences fuel switching from glycolytic to oxidative substrate. Although there is indeed intensive study of the sirtuin deacetylases as agents involved in stress, aging, and nutrient adaptation as we review below—the concept that intrinsic (rather than nutrient-driven) control of NAD^+ biosynthesis might in turn regulate mitochondrial function is novel. We began with a focus on sirtuin3 (SIRT3) since this is a mitochondrial—localized deacetylase that participates in fatty acid oxidation [47]. Surprisingly, there is very little understanding of the factors *in vivo* that regulate SIRT3 function, and it is has been an uncharted area to determine the consequences of the circadian cycle on NAD^+-responsive enzymes. In exciting new studies, we have shown that clock control of NAD^+-biosynthesis plays a critical role in mitochondrial function. With respect to bioenergetics and oxidative metabolism, a barrier in the past has been a lack of available approaches in which the circadian system can be manipulated and the consequences of this disruption on fuel flux analyzed at the cell and organellar levels.

A major step in understanding how the clock-NAD^+ cycle impacts physiology came from our observation that circadian mutant mice die when subject to a prolonged fast and also exhibit muscle and heart failure—all hallmarks of mitochondrial disease. We began our investigation into the effect of NAD^+ deficiency on mitochondria function in circadian mutant animals using a multi-faceted approach including unbiased proteomics, which led to the identification of abnormal acetylation of enzymes involved in lipid oxidation, amino acid catabolism, tricarboxylic acid (TCA) cycle, electron transport chain (ETC), and superoxide dismutase pathways. Importantly, loss-of-function mutations in several of these oxidative enzymes have also been identified in the human metabolic myopathy syndrome and in both glioblastoma and renal cell carcinoma, indicating a broader effect of the clock-NAD^+ pathway on mitochondrial metabolism in both normal and transformed cells. Using tissue- and cell-based bioenergetics assays, we discovered that abrogation of the clock impairs electron transfer from lipid to the TCA cycle, in addition to increased mitochondrial-production of superoxide free radical, increasing sensitivity to genotoxic stress. Our work

also showed that cells exhibit an autonomous rhythm of oxygen consumption, glucose oxidation, and mitochondrial lipid catabolism. Importantly, the oxygen consumption cycle in muscle is directly linked to metabolism of NAD^+ and activity of the mitochondrial NAD^+-dependent deacetylase SIRT3 [6].

Although the aforementioned work has pinpointed specific defects in clock control of mitochondrial function, several unanswered questions remain in dissecting the effect of clock-NAD^+ rhythms on physiology and cell biology. First, it is not yet known how NAD^+ deficiency locally within skeletal muscle contributes to respiration or exercise tolerance in circadian mutant mice or in animals subjected to environmental circadian disruption. Though skeletal muscle ablation of the clock has been achieved in our group and others, the biochemical pathways through which clock abrogation impairs oxidative capacity remain largely unknown [48].

Second, we still do not know whether clock abrogation and NAD^+ deficiency in liver or skeletal muscle impacts overall energy balance and alters the capacity to utilize carbohydrate and lipid as a fuel source. New pharmacologic [41] and genetic means to raise NAD^+ both globally in the whole animal and selectively within either liver or skeletal muscle are now available and will be powerful tools in evaluating the potential to boost NAD^+ as a therapeutic strategy in myopathy and liver defects of circadian mutant animals. Third, in addition to its function as a cofactor for the class III histone deacetylases, NAD^+ is also a cofactor for the poly-ADP-ribosylases, critical factors in DNA repair and stress response, though the possible interaction between rhythmic regulation of NAD^+ and PARP activity is not known. Lastly, NAD^+ functions as an electron transport molecule, and as such, it is a direct marker of cellular redox state and the balance between glycolytic and oxidative metabolism. Whether NAD^+ might participate in the bidirectional communication between metabolism and the clock system remains an area of intensive investigation. In summary, discovery of the clock as an upstream regulator of NAD^+ provides a wealth of opportunity to dissect the interrelationship between circadian rhythms, physiology, and epigenetics.

FEEDBACK TO THE CLOCK BY NUTRIENT AND METABOLIC STATE

In animals, clocks are organized hierarchically, with brain pacemaker cells synchronizing peripheral tissue clocks, leading to the prevailing model of the neural clock as an anterograde director of metabolism. However, there is plasticity in the interrelationship between brain and peripheral tissue clocks since these oscillators can become uncoupled from each other and, in turn, from the environmental light cycle, a concept that was first demonstrated by experimentally restricting food access to the daytime when mice are normally resting [49,50]. Surprisingly, nutritional environment affects both behavioral and molecular oscillators since high fat chow induces period lengthening and altered amplitude and phase of physiologic and metabolic rhythms, consistent with a bidirectional relationship between the clock and metabolism [51]. Further, isocaloric high-fat feeding provided during the incorrect circadian time (subjective daytime), when mice are normally sleeping and fasting, causes more exaggerated obesity than providing the same calories during the correct time (subjective nighttime) [52]. Consistent with time-dependent effects of diet on metabolic

pathologies, the converse observation has also been made, that is, restricting high-fat feeding to the subjective night reduces adverse metabolic pathologies such as steatosis and insulin resistance [53]. Studies in human subjects also indicate that the time-of-feeding contributes to metabolic disease, a concept originally formulated with description of the “night eating syndrome”, a mood disorder associated with increased food intake in the evening [54]. Human analyses, including genome-wide association studies, population based case-control investigations, and clinical research, have cumulatively indicated a strong interrelationship amongst circadian disruption, obesity, diabetes mellitus, and metabolic syndrome [55]. Moreover, certain inflammatory and cardiovascular events, including thrombosis and nocturnal asthma, exhibit pronounced circadian variation. Together, these observations indicate feedback pathways through which both diet and metabolic conditions alter circadian function. At the molecular level, coupling of circadian and metabolic pathways has been revealed through demonstration that the cis-acting networks (cistromes) of core clock transcription factors exhibit extensive interconnection with metabolic gene networks [23,56-58]. However, how changes in metabolic and nutrient conditions can reset transcriptional or behavioral cycles remains incompletely understood, and as such, this topic represents an area of intensive investigation.

SUMMARY AND FUTURE DIRECTIONS

A major window in understanding how the clock is coupled to metabolism was opened with the discovery of metabolic syndrome pathologies in multi-tissue circadian mutant mice including susceptibility to diet-induced obesity, mis-timed feeding rhythms, hypoinsulinemia, and energetic collapse upon fasting. Using Cre-LoxP conditional transgenesis and dynamic endocrine testing, we have pinpointed the tissue-specific role of the clock in energy and glucose homeostasis, with our most detailed understanding of this process in liver, muscle, and endocrine pancreas. In the post-prandial condition, the β-cell clock is essential for nutrient and adenyl cyclase-induced insulin exocytosis. In contrast, the hepatocyte and myocyte clocks are required for oxidative metabolism. Circadian mutant mice die upon prolonged fasting due to mitochondrial failure, a defect that we have tied to the bioavailability of NAD^+, a cofactor of class III histone deacetylases and poly-ADP ribosylase enzymes involved in adjusting metabolic and gene regulation in response to environmental change, including glucose deprivation, oxidative damage, and cell stress. Indeed, we have found that liver and myoblasts exhibit an autonomous rhythm of oxygen consumption, glucose oxidation, and mitochondrial lipid catabolism that is directly linked to an autonomous rhythm of NAD^+ metabolism and, consequently, to cyclic activity of the mitochondrial NAD^+-dependent deacetylase SIRT3. NAD^+ supplementation improves respiration in live animals, indicating that circadian control of NAD^+ metabolism plays a key role in cellular and organismal respiration [45]. A future challenge will be to determine the cell and molecular basis for the interplay between nutritional and circadian processes important in metabolic health and disease states.

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References

1. Lowrey PL, Takahashi JS. Genetics of the mammalian circadian system: photic entrainment, circadian pacemaker mechanisms, and posttranslational regulation. Annu Rev Genet. 2000; 34:533–562. [PubMed: 11092838]
2. Bass J, Takahashi JS. Circadian integration of metabolism and energetics. Science. 2010; 330:1349–1354. [PubMed: 21127246]
3. Dibner C, Schibler U, Albrecht U. The mammalian circadian timing system: organization and coordination of central and peripheral clocks. Annu Rev Physiol. 2010; 72:517–549. [PubMed: 20148687]
4. Balsalobre A, Damiola F, Schibler U. A serum shock induces circadian gene expression in mammalian tissue culture cells. Cell. 1998; 93:929–937. [PubMed: 9635423]
5. Lamia KA, Storch KF, Weitz CJ. Physiological significance of a peripheral tissue circadian clock. Proc Natl Acad Sci U S A. 2008; 105:15172–15177. [PubMed: 18779586]
6. Peek CB, Affinati AH, Ramsey KM, et al. Circadian clock NAD+ cycle drives mitochondrial oxidative metabolism in mice. Science. 2013; 342:1243417. [PubMed: 24051248]
7. Marcheva B, Ramsey KM, Buhr ED, et al. Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. Nature. 2010; 466:571–572. [PubMed: 20671699]
8. Sadacca LA, Lamia KA, deLemos AS, Blum B, Weitz CJ. An intrinsic circadian clock of the pancreas is required for normal insulin release and glucose homeostasis in mice. Diabetologia. 2011; 54:120–124. [PubMed: 20890745]
9. Doherty CJ, Kay SA. Circadian control of global gene expression patterns. Annu Rev Genet. 2010; 44:419–444. [PubMed: 20809800]
10. Johnson CH, Golden SS, Kondo T. Adaptive significance of circadian programs in cyanobacteria. Trends Microbiol. 1998; 6:407–410. [PubMed: 9807785]
11. Ouyang Y, Andersson CR, Kondo T, Golden SS, Johnson CH. Resonating circadian clocks enhance fitness in cyanobacteria. Proc Natl Acad Sci U S A. 1998; 95:8660–8664. [PubMed: 9671734]
12. Bouatia-Naji N, Bonnefond A, Cavalcanti-Proenca C, et al. A variant near MTNR1B is associated with increased fasting plasma glucose levels and type 2 diabetes risk. Nat Genet. 2009; 41:89–94. [PubMed: 19060909]
13. Dupuis J, Langenberg C, Prokopenko I, et al. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet. 2010; 42:105–116. [PubMed: 20081858]
14. Mulder H, Nagorny CL, Lyssenko V, Groop L. Melatonin receptors in pancreatic islets: good morning to a novel type 2 diabetes gene. Diabetologia. 2009; 52:1240–1249. [PubMed: 19377888]
15. Chang AM, Aeschbach D, Duffy JF, Czeisler CA. Evening use of light-emitting eReaders negatively affects sleep, circadian timing, and next-morning alertness. Proc Natl Acad Sci U S A. 2015; 112:1232–1237. [PubMed: 25535358]
16. Gekakis N, Staknis D, Nguyen HB, et al. Role of the CLOCK protein in the mammalian circadian mechanism. Science. 1998; 280:1564–1569. [PubMed: 9616112]
17. Turek FW, Joshu C, Kohsaka A, et al. Obesity and metabolic syndrome in circadian Clock mutant mice. Science. 2005; 308:1043–1045. [PubMed: 15845877]
18. Maury E, Hong HK, Bass J. Circadian disruption in the pathogenesis of metabolic syndrome. Diabetes Metab. 2014; 40:338–346. [PubMed: 24433933]
19. Everett LJ, Lazar MA. Nuclear receptor Rev-erbalpha: up, down, and all around. Trends Endocrinol Metab. 2014; 25:586–592. [PubMed: 25066191]

20. Asher G, Schibler U. Crosstalk between components of circadian and metabolic cycles in mammals. Cell Metab. 2011; 13:125–137. [PubMed: 21284980]
21. Lee J, Moulik M, Fang Z, et al. Bmal1 and beta-cell clock are required for adaptation to circadian disruption, and their loss of function leads to oxidative stress-induced beta-cell failure in mice. Mol Cell Biol. 2013; 33:2327–2338. [PubMed: 23547261]
22. Pulimeno P, Mannic T, Sage D, et al. Autonomous and self-sustained circadian oscillators displayed in human islet cells. Diabetologia. 2013; 56:497–507. [PubMed: 23242133]
23. Koike N, Yoo SH, Huang HC, et al. Transcriptional architecture and chromatin landscape of the core circadian clock in mammals. Science. 2012; 338:349–354. [PubMed: 22936566]
24. Menet JS, Rodriguez J, Abruzzi KC, Rosbash M. Nascent-Seq reveals novel features of mouse circadian transcriptional regulation. eLife. 2012; 1:e00011. [PubMed: 23150795]
25. Fang B, Everett LJ, Jager J, et al. Circadian enhancers coordinate multiple phases of rhythmic gene transcription in vivo. Cell. 2014; 159:1140–1152. [PubMed: 25416951]
26. Bell GI, Polonsky KS. Diabetes mellitus and genetically programmed defects in beta-cell function. Nature. 2001; 414:788–791. [PubMed: 11742410]
27. Gaulton KJ, Nammo T, Pasquali L, et al. A map of open chromatin in human pancreatic islets. Nat Genet. 2010; 42:255–259. [PubMed: 20118932]
28. Kameswaran V, Bramswig NC, McKenna LB, et al. Epigenetic regulation of the DLK1-MEG3 microRNA cluster in human type 2 diabetic islets. Cell Metab. 2014; 19:135–145. [PubMed: 24374217]
29. Pasquali L, Gaulton KJ, Rodriguez-Segui SA, et al. Pancreatic islet enhancer clusters enriched in type 2 diabetes risk-associated variants. Nat Genet. 2014; 46:136–143. [PubMed: 24413736]
30. Froy O. Circadian rhythms, aging, and life span in mammals. Physiology (Bethesda). 2011; 26:225–235. [PubMed: 21841071]
31. Panda S, Antoch MP, Miller BH, et al. Coordinated transcription of key pathways in the mouse by the circadian clock. Cell. 2002; 109:307–320. [PubMed: 12015981]
32. Akhtar RA, Reddy AB, Maywood ES, et al. Circadian cycling of the mouse liver transcriptome, as revealed by cDNA microarray, is driven by the suprachiasmatic nucleus. Curr Biol. 2002; 12:540–550. [PubMed: 11937022]
33. Storch KF, Lipan O, Leykin I, et al. Extensive and divergent circadian gene expression in liver and heart. Nature. 2002; 417:78–83. [PubMed: 11967526]
34. Zhang R, Lahens NF, Ballance HI, Hughes ME, Hogenesch JB. A circadian gene expression atlas in mammals: implications for biology and medicine. Proc Natl Acad Sci U S A. 2014; 111:16219–16224. [PubMed: 25349387]
35. Ramsey KM, Yoshino J, Brace CS, et al. Circadian clock feedback cycle through NAMPT-mediated NAD+ biosynthesis. Science. 2009; 324:651–654. [PubMed: 19299583]
36. Nakahata Y, Sahar S, Astarita G, Kaluzova M, Sassone-Corsi P. Circadian control of the NAD+ salvage pathway by CLOCK-SIRT1. Science. 2009; 324:654–657. [PubMed: 19286518]
37. Asher G, Gatfield D, Stratmann M, et al. SIRT1 regulates circadian clock gene expression through PER2 deacetylation. Cell. 2008; 134:317–328. [PubMed: 18662546]
38. Nakahata Y, Kaluzova M, Grimaldi B, et al. The NAD+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. Cell. 2008; 134:329–340. [PubMed: 18662547]
39. Revollo JR, Korner A, Mills KF, et al. Nampt/PBEF/Visfatin regulates insulin secretion in beta cells as a systemic NAD biosynthetic enzyme. Cell Metab. 2007; 6:363–375. [PubMed: 17983582]
40. Sasaki Y, Vohra BP, Lund FE, Milbrandt J. Nicotinamide mononucleotide adenylyl transferase-mediated axonal protection requires enzymatic activity but not increased levels of neuronal nicotinamide adenine dinucleotide. J Neurosci. 2009; 29:5525–5535. [PubMed: 19403820]
41. Wang G, Han T, Nijhawan D, et al. P7C3 neuroprotective chemicals function by activating the rate-limiting enzyme in NAD salvage. Cell. 2014; 158:1324–1334. [PubMed: 25215490]
42. Araki T, Sasaki Y, Milbrandt J. Increased nuclear NAD biosynthesis and SIRT1 activation prevent axonal degeneration. Science. 2004; 305:1010–1013. [PubMed: 15310905]

43. Ramsey KM, Mills KF, Satoh A, Imai S. Age-associated loss of Sirt1-mediated enhancement of glucose-stimulated insulin secretion in beta cell-specific Sirt1-overexpressing (BESTO) mice. Aging Cell. 2008; 7:78–88. [PubMed: 18005249]
44. Yoshino J, Mills KF, Yoon MJ, Imai S. Nicotinamide mononucleotide, a key NAD(+) intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. Cell Metab. 2011; 14:528–536. [PubMed: 21982712]
45. Canto C, Houtkooper RH, Pirinen E, et al. The NAD(+) precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity. Cell Metab. 2012; 15:838–847. [PubMed: 22682224]
46. Chang HC, Guarente L. SIRT1 mediates central circadian control in the SCN by a mechanism that decays with aging. Cell. 2013; 153:1448–1460. [PubMed: 23791176]
47. Hirschey MD, Shimazu T, Goetzman E, et al. SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. Nature;. 464:121–125.
48. Dyar KA, Ciciliot S, Wright LE, et al. Muscle insulin sensitivity and glucose metabolism are controlled by the intrinsic muscle clock. Mol Metab. 2014; 3:29–41. [PubMed: 24567902]
49. Damiola F, Le Minh N, Preitner N, et al. Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. Genes Dev. 2000; 14:2950–2961. [PubMed: 11114885]
50. Stokkan KA, Yamazaki S, Tei H, Sakaki Y, Menaker M. Entrainment of the circadian clock in the liver by feeding. Science. 2001; 291:490–493. [PubMed: 11161204]
51. Kohsaka A, Laposky AD, Ramsey KM, et al. High-fat diet disrupts behavioral and molecular circadian rhythms in mice. Cell Metab. 2007; 6:414–421. [PubMed: 17983587]
52. Arble DM, Bass J, Laposky AD, Vitaterna MH, Turek FW. Circadian timing of food intake contributes to weight gain. Obesity (Silver Spring). 2009; 17:2100–2102. [PubMed: 19730426]
53. Hatori M, Vollmers C, Zarrinpar A, et al. Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. Cell Metab. 2012; 15:848–860. [PubMed: 22608008]
54. Stunkard A, Lu XY. Rapid changes in night eating: considering mechanisms. Eat Weight Disord. 2010; 15:e2–e8. [PubMed: 20571317]
55. Maury E, Ramsey KM, Bass J. Circadian rhythms and metabolic syndrome: from experimental genetics to human disease. Circ Res. 2010; 106:447–462. [PubMed: 20167942]
56. Cho H, Zhao X, Hatori M, et al. Regulation of circadian behaviour and metabolism by REV-ERB-alpha and REV-ERB-beta. Nature. 2012; 485:123–127. [PubMed: 22460952]
57. Feng D, Liu T, Sun Z, et al. A circadian rhythm orchestrated by histone deacetylase 3 controls hepatic lipid metabolism. Science;. 331:1315–1319.
58. Rey G, Cesbron F, Rougemont J, et al. Genome-wide and phase-specific DNA-binding rhythms of BMAL1 control circadian output functions in mouse liver. PLoS Biol. 2011; 9:e1000595. [PubMed: 21364973]

CALLOUTS

- Local organ clocks impact metabolic function by anticipating the varying requirement for anabolic and catabolic processes across the daily fasting-feeding/sleep-wake cycle.
- Clock disruption in distinct tissues exerts strong effects on metabolic homeostasis by deregulating unique organ-specific functions that are both dependent upon time-of-day and nutrient state of the animal.
- Selective ablation of the clock within endocrine pancreas results in severe early-life hyperglycemia, whereas ablation in liver causes hypoglycemia, revealing that glucose homeostasis reflects the net (and opposing) actions of clock function in both endocrine pancreas and oxidative tissue.