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Temporal Partitioning of Cardiac Metabolism by the Cardiomyocyte Circadian Clock

Martin E. Young

Division of Cardiovascular Disease, Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama, USA

Abstract

Cell autonomous circadian clocks have emerged as critical mediators of twenty four hour rhythms in cellular processes. In doing so, these molecular timekeepers confer the selective advantage of anticipation, allowing cells/organs to prepare for stimuli/stresses before their onset. The heart is subjected to dramatic fluctuations in energetic demand and nutrient supply in association with sleep/wake and fasting/feeding cycles. Recent studies suggest that the cardiomyocyte circadian clock orchestrates daily rhythms in both oxidative and non-oxidative glucose and fatty acid metabolism, as well as protein turnover. Here, we review this evidence, and discuss whether disruption of these rhythms can contribute towards cardiovascular disease.

Time-of-day-dependent rhythms are observed in multiple aspects of life, as well as death. In terms of cardiovascular parameters, blood pressure, heart rate, and cardiac output all increase sharply at the beginning of the awake period, which is associated with increased risk of adverse ischemic events, arrhythmias, and sudden cardiac death at this time (reviewed in (Durgan & Young, 2010)). Classically, daily rhythms in cardiovascular physiology and pathophysiology have been attributed to fluctuations in neurohumoral factors and sheer stress associated with sleep/wake and fasting/feeding cycles (Young, 2006; Durgan & Young, 2010). Elegant studies by Shea and Scheer have recently revealed that 24-hr oscillations in multiple cardiovascular functions are intrinsically driven in healthy human subjects, thus highlighting a need to reevaluate our understanding of the mechanisms modulating cardiovascular processes (Scheer *et al.*, 1999; Scheer *et al.*, 2010). One intrinsic mechanism capable of driving 24-hr rhythms in cellular processes is the circadian clock.

Circadian clocks are cell autonomous mechanisms composed of transcriptional positive and negative feedback loops, with a free running period of approximately 24-hr (reviewed in (Takahashi *et al.*, 2008)). Clock components are expressed within individual cells, in a ubiquitous manner, and can operate in the absence of extrinsic cues. At the core of the mammalian mechanism are two transcription factors, CLOCK and BMAL1, which heterodimerize and bind to E-boxes in the promoters of target genes (Gekakis *et al.*, 1998;

Address for correspondence: Martin E. Young, D.Phil., Division of Cardiovascular Diseases, Department of Medicine, University of Alabama at Birmingham, 703 19th St. S., ZRB 308, Birmingham, Alabama, 35294, USA, Tel # 205-934-2328, Fax # 205-975-5104, meyoung@uab.edu.

Competing Interests

None of the authors have any conflicts of interests.

Hogenesch *et al.*, 1998). Target genes include repressors of CLOCK/BMAL1 (e.g., PER1/2/3, CRY1/2, REV-ERB α), thus resulting in 24-hr oscillations in the activity of this heterodimer (Takahashi *et al.*, 2008). The CLOCK/BMAL1 heterodimer also modulates expression of non-core clock components, so called clock output genes (CCGs). Translation of CCG mRNAs enables circadian clocks to influence cellular processes over the course of the day (Figure 1). In general, circadian clocks confer a selective advantage by temporally partitioning cellular processes in anticipation of predicted daily fluctuations in the environment. Two major cycles that the heart must contend with on a daily basis include sleep/wake and fasting/feeding cycles.

Following initial characterization of the cell autonomous cardiomyocyte circadian clock (Durgan *et al.*, 2005), we developed two distinct mouse models wherein this mechanism was genetically disrupted, namely cardiomyocyte-specific CLOCK mutant (CCM) and cardiomyocyte-specific BMAL1 knockout (CBK) mice (Durgan *et al.*, 2006; Bray *et al.*, 2008; Durgan *et al.*, 2011b; Young *et al.*, 2014). Given the transcriptional nature of this mechanism, unbiased gene expression microarrays were performed initially, revealing that approximately 10% of the cardiac transcriptome is regulated by the cardiomyocyte circadian clock (Bray *et al.*, 2008; Young *et al.*, 2014). Gene ontology analysis clustered CCGs into various categories, including transcription, signal transduction, transport, and metabolism (Bray *et al.*, 2008; Young *et al.*, 2014). The latter was reinforced by an unbiased proteomics approach, indicating that approximately 4% of the cardiac proteome is modulated by the cardiomyocyte circadian clock, and that many of the identified proteins influence metabolic processes (Podobed *et al.*, 2014). Collectively, these observations led to the hypothesis that the cardiomyocyte circadian clock allows the heart to predict/prepare for fluctuations in energetic demand and/or nutrient availability associated with sleep/wake and fasting/feeding cycles, through metabolic modulation.

During periods of physical activity (i.e., increased workload), the heart matches increased energetic demand primarily through stimulation of glucose utilization (Allard *et al.*, 1994; Goodwin *et al.*, 1998). Consistent with this knowledge, glucose oxidation is more than 2-fold higher in rodent hearts during the middle of the active (dark) phase (relative to the sleep (light) phase; Figure 2) (Young *et al.*, 2001; Durgan *et al.*, 2007; Durgan *et al.*, 2011a). Evidence suggesting mediation by the cardiomyocyte circadian clock includes: 1) glucose oxidation rhythms are observed in *ex vivo* perfused hearts (in the absence of neurohumoral influence), even when cardiac work was constant (Durgan *et al.*, 2011a); 2) rhythms are absent in CCM hearts, which exhibit chronically low glucose oxidation rates (Durgan *et al.*, 2011a); and 3) peak glucose oxidation rates are markedly attenuated in CBK hearts (Young *et al.*, 2014). In contrast, cardiac fatty acid oxidation rates do not exhibit a robust time-of-day-dependent oscillation, consistent with baseline requirements of this parameter independent of workload (although fatty acid oxidation is slightly higher at the sleep-to-wake transition; Figure 2) (Bray *et al.*, 2008).

Unlike sleep/wake cycles, feeding/fasting cycles are less predictable for the animal in the wild, being dependent on successful forage for food during the awake period. For the *ad libitum* fed laboratory rodent (mimicking a constant abundance of food, independent of time-of-day or season, much like humans in Westernized countries), one might predict

increased nutrient storage during the awake/feeding period, in anticipation of an upcoming sleep/fasting period. Indeed, both glycogen and triglyceride synthesis oscillate in the *ex vivo* perfused mouse heart, peaking in the middle and end of the active (dark) phase (respectively; Figure 2) (Tsai *et al.*, 2010; Durgan *et al.*, 2011a). Importantly, these 24-hr oscillations are absent in CCM hearts, indicative of mediation by the cardiomyocyte circadian clock (Tsai *et al.*, 2010; Durgan *et al.*, 2011a). Less is known regarding whether the cardiomyocyte circadian clock also anticipates unsuccessful forage for food (i.e., prolongation of the sleep phase fast); during this situation, energetic demand of the heart will remain elevated during the continued search for food (and predation avoidance). Whether 24-hr rhythms in glycogen and triglyceride turnover persist in the heart during prolonged fasting has not been addressed (although glycogen rhythms persist in the liver during fasting) (Ishikawa & Shimazu, 1976).

Less is known regarding circadian influences on cardiac protein metabolism. Unpublished studies suggest that protein synthesis exhibits a 24-hr oscillation in the murine heart, peaking at the beginning of the sleep (light) phase, with a prominent trough in the middle of the awake (dark) phase (Figure 2). Circumstantial evidence suggests possible mediation by an intrinsic mechanism (e.g., the clock), as: 1) these rhythms persist *ex vivo*; 2) pro-growth signals such as sheer stress/workload, insulin, and amino acids, all peak in the middle of the active (dark) phase, when cardiac protein synthesis troughs; and 3) protein synthesis is altered (elevated) in CBK hearts (He *et al.*, 2016b). These observations have led us to hypothesize that the cardiomyocyte circadian clock decreases protein synthesis during the active period to spare ATP for contraction, yet increases protein synthesis at the beginning of the sleep phase in an attempt to replace damaged proteins (in anticipation of the next awake period). If true, one would predict that the cardiomyocyte circadian clock would also promote protein degradation at the beginning of the sleep phase, thus facilitating replacement of damaged proteins. Consistent with this hypothesis, recent studies reveal that both autophagy (He *et al.*, 2016a) and proteasome activity (unpublished observations) are increased in the heart at the beginning of the sleep phase (Figure 2).

Figure 2 summarizes evidence-based temporal partitioning of cardiac metabolic processes by the cardiomyocyte circadian clock. Major questions that now can be asked include: 1) How do intrinsic (i.e., cardiomyocyte circadian clock) and extrinsic (e.g., neurohumoral factors, such as insulin, b-adrenergic stimulation, and various nutrients) influences coordinate cardiac metabolism in the *in vivo* setting?; 2) What are the molecular links between the cardiomyocyte circadian clock and metabolic processes?; and 3) What, if any, are the pathological consequences of cardiomyocyte circadian clock control of cardiac metabolism? With regards to the latter, evidence has emerged suggesting that the heart exhibits profound time-of-day-dependent oscillations in its responsiveness to pathologic stresses, including ischemia/reperfusion and pro-hypertrophic stimuli (Durgan *et al.*, 2010; Durgan *et al.*, 2011b). Consistent with greater protein synthesis at the beginning of the sleep phase, challenging mice with isoproterenol at this time leads to greater hypertrophic growth of the heart (compared to isoproterenol challenge during the awake phase) (Durgan *et al.*, 2011b). Such observations may provide insight regarding the pathogenesis of hypertrophic cardiomyopathy associated with non-dipping hypertension and sleep apnea, when the heart is challenged at an inappropriate time of day. Interestingly, ischemia/reperfusion tolerance is highest in the heart at the beginning of the sleep phase (Durgan *et al.*, 2010), a time when

autophagy (a cardioprotective mechanism) is activated. We speculate that disruption of the cardiomyocyte circadian clock by common behaviors such as sleep/wake disruption, shift work, frequent travel across time zones, and nighttime eating significantly contribute towards cardiovascular disease risk through impairment of normal metabolic rhythms.

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Recent Findings

1. Cardiac glucose utilization peaks during the middle of the active period, likely to meet increased energetic demands at this time.
2. Synthesis of glycogen and triglyceride peak in the heart in the middle and end of the active period (respectively), likely in anticipation of the upcoming sleep/fasting period.
3. Protein turnover increases in the heart at the beginning of the sleep phase, likely to promote growth and repair at this time.
4. Temporal partitioning of metabolic processes in the heart is mediated in large part by the cardiomyocyte circadian clock.

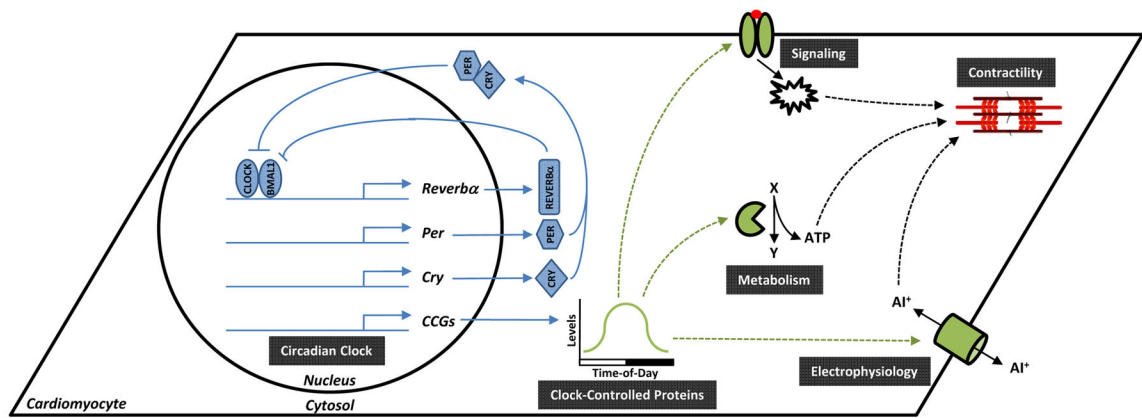


Figure 1. The cardiomyocyte circadian clock mechanism. Figure illustrates the positive and negative feedback loops of the cardiomyocyte circadian clock (blue), hypothetical clock controlled proteins (green), and the possible ways in which this mechanism influences cardiac contractility over the course of the day (black). Abbreviations include: AI⁺, anion; CCGs, clock-controlled genes; Cry, cryptochrome isoforms 1 and 2; Per, period isoforms 1, 2, and 3; X and Y, hypothetical metabolites.

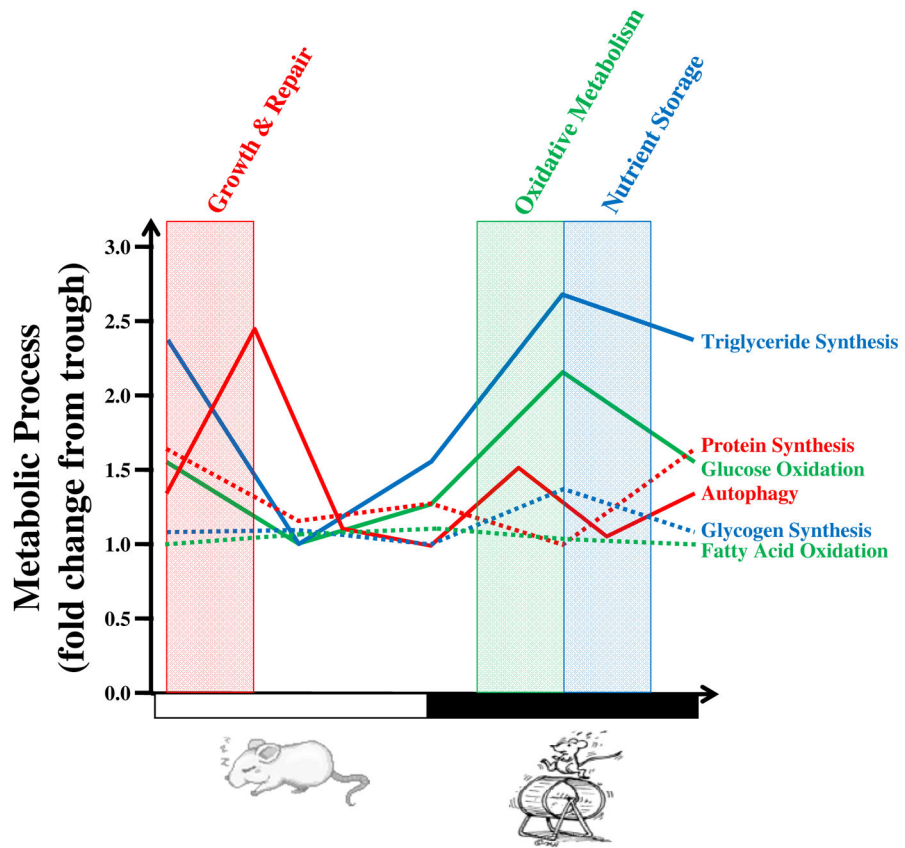


Figure 2. Temporal partitioning of metabolic processes in the heart. Figure illustrates fold changes in distinct cardiac metabolic processes with respect to time-of-day, which is based on both published and unpublished findings in the *ad libitum* fed mouse. Assessments include metabolic flux (glucose and fatty acid oxidation, glycogen and triglyceride synthesis, as well as protein synthesis) and protein levels (LC3II/I ratio for autophagy). Shaded areas indicate the 4-hr periods of greatest oxidative metabolism, nutrient storage, and growth/repair.