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STATE-OF-THE-ART REVIEW

The Cardiac Circadian Clock

Implications for Cardiovascular Disease and its Treatment

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HIGHLIGHTS

- **The cardiomyocyte circadian clock has emerged as a central regulator of cardiac function, through temporal orchestration of fundamental cellular processes over the course of the day.**
- **Perturbations (eg, attenuation, misalignment) in governance of biological processes by the cardiomyocyte circadian clock invariably results in cardiac dysfunction.**
- **Secondary to circadian control, both the onset and severity of adverse pathologic events (eg, myocardial infarction) vary over the course of the day.**
- **Acknowledgment of, alignment with, and implementation of chronobiology is essential not only for the prevention of cardiac disease, but also for treatment of cardiovascular disease.**

SUMMARY

Virtually all aspects of physiology fluctuate with respect to the time of day. This is beautifully exemplified by cardiovascular physiology, for which blood pressure and electrophysiology exhibit robust diurnal oscillations. At molecular/biochemical levels (eg, transcription, translation, signaling, metabolism), cardiovascular-relevant tissues (such as the heart) are profoundly different during the day vs the night. Unfortunately, this in turn contributes toward 24-hour rhythms in both risk of adverse event onset (eg, arrhythmias, myocardial infarction) and pathogenesis severity (eg, extent of ischemic damage). Accumulating evidence indicates that cell-autonomous timekeeping mechanisms, termed circadian clocks, temporally govern biological processes known to play critical roles in cardiovascular function/dysfunction. In this paper, a comprehensive review of our current understanding of the cardiomyocyte circadian clock during both health and disease is detailed. Unprecedented basic, translational, and epidemiologic studies support a need to implement chronobiological considerations in strategies designed for both prevention and treatment of cardiovascular disease. (J Am Coll Cardiol Basic Trans Science 2023;8:1613-1628) © 2023 The Author. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Cardiovascular function exhibits robust fluctuations over the course of the day, at numerous levels. Continuous monitoring of blood pressure and electrophysiology in free-living heathy subjects reveals approximate 8% to 20% fluctuations in these cardiovascular parameters during a 24-hour period.[1,2] This is exemplified by lowest blood pressure during the sleep period, followed by a steep rise in the early hours of the morning.[1] Similarly, echocardiographic studies in volunteers

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ABBREVIATIONS AND ACRONYMS

CBK = cardiomyocyte-specific BMAL1 knockout

CCM = cardiomyocyte-specific CLOCK mutant

CMD = cardiometabolic disease

E4BP4 = E4 promoter binding protein 4

PAI = plasminogen activator inhibitor

PER = period

indicate day-night differences in diastolic function measures (such as left ventricular relaxation time), in the absence of fluctuations in systolic parameter (eg, left ventricular fractional shortening).[3] Daily rhythms in these physiologic functions have typically been associated with 24-hour oscillations in numerous cardiovascular-relevant neurohumoral factors (eg, autonomic/sympathetic/parasympathetic tone, renin-aldosterone-angiotensin system components, cortisol).[4-7] Many of these human-based observations have been recapitulated in animal models, affording an opportunity to gain greater insight into the mechanisms driving day-night differences in cardiovascular function.[2,8] Interrogation of cardiovascular-relevant tissues has unveiled robust fluctuations in contractile properties (eg, vasodilation/vasoconstriction of vessels, cardiac output), electrophysiologic parameters (eg, sinus node beat rate), metabolic capacities (eg, cardiac glucose oxidation, triglyceride synthesis), sensitivity to extrinsic stimuli (eg, hormones, nutrients), and the molecular underpinnings of cellular function (ie, cardiac transcriptome/proteome).[9-18] Such studies highlight the extensive and diverse nature with which the cardiovascular system is influenced by the time of day, and leads to questions regarding how temporal organization is orchestrated to ensure normal function.

As with cardiovascular physiology, cardiovascular pathology exhibits time of day dependency. Numerous reports indicate that both adverse event incidence, and as well pathologic severity, fluctuate with respect to time of day. Ischemic events serve as an example. Both myocardial infarctions and stroke have an increased incidence in the early portion of the day (6 AM to 2 PM).[4,19] Markers of cardiac damage (eg, creatine kinase) appear to be augmented in patients experiencing an myocardial infarction in the early morning hours.[20] In contrast, clinically relevant outcomes following stroke (eg, neurological deterioration) tend to be worse when stroke onset occurs in the evening.[21,22] Much like the onset of adverse ischemic events, life-threatening arrhythmias (eg, ventricular tachycardia) and sudden cardiac death exhibit increased incidence in the early hours of the morning.[4] Other forms of arrhythmias have higher prevalence at distinct times of the day, such as those associated with Brugada syndrome (which occur during the sleep period).[2] Triggers for adverse cardiovascular events, such as clotting factors (eg, platelet aggregability, plasminogen activator inhibitor [PAI]-1) and inflammatory markers (eg, monocytes, cytokines), in addition to shear stress and autonomic/sympathetic/parasympathetic tone, fluctuate over the course of the day.[4,8,23] Observations such as these, in addition to daily fluctuations in the intrinsic properties of cardiovascular tissues, have led to formulation of hypothetical models for 24-hour rhythms in adverse cardiovascular events, wherein an inappropriate temporal augmentation, alignment, or misalignment of risk factors triggers an event.[8,24] The purpose of this review is to highlight the role that intrinsic circadian clocks play in cardiovascular function/dysfunction (with a particular focus on the cardiomyocyte circadian clock) and discuss the impact that circadian biology has on cardiovascular disease treatment.

CELL-AUTONOMOUS CIRCADIAN CLOCKS

Not surprisingly, initial studies investigating 24-hour rhythms in cardiovascular physiology (and pathology) theorized that these daily fluctuations were largely secondary to sleep/wake cycle-induced alterations in neurohumoral factors. For example, changes in posture and physical/emotional arousal upon awakening were considered primary mediators of increased cortisol and adrenergic stimulation, leading to rises in blood pressure and heart rate.[4-7] Similarly, augmented shear stress (secondary to the blood pressure rise), in combination with greater platelet aggregability in the morning, would increase likelihood of adverse ischemic events in at-risk patients (ie, those with vulnerable plaques).[4] Only relatively recently has an appreciation for involvement of more intrinsic mechanisms occurred. This has been possible through use of forced dyssynchrony (FD) protocols, wherein behaviors (and the environment) are dissociated from endogenously driven (ie, intrinsic) 24-hour rhythms. More specifically, healthy volunteers remain within a controlled environment for multiple days, during which sleep/wake, feeding/fasting, and light/dark cycles are fixed to a periodicity of either 20 or 28 hours.[24] During such FD protocols, persistence of 24-hour rhythms in various cardiovascular relevant parameters (including heart rate, epinephrine/norepinephrine, platelet aggregability, and PAI-1) have been reported.[25-27] Although a 24-hour rhythm in blood pressure was also observed during FD, the peak occurred at a time corresponding to the evening (when a secondary peak is also observed in free-living subjects), suggesting that the morning rise in blood pressure likely occurs in response to waking.[28] These studies highlight that an endogenous timekeeping mechanism exists in

humans, which drives 24-hour oscillations in specific cardiovascular parameters. One such candidate mechanism is the circadian clock.

Circadian clocks are cell-autonomous molecular timekeeping mechanisms that temporally govern biological processes (such like an orchestral conductor).[29,30] Clocks are composed of a series of transcriptional-translational feedback loops, with a periodicity of approximately 24 hours.[29,30] At the core of the mechanism are 2 transcription factors, CLOCK and BMAL1, which form a functional heterodimer.[31,32] Binding of CLOCK/BMAL1 to E-boxes within target gene promoters (including multiple period [PER], cryptochrome, and REV-ERB isoforms) invariably results in induction.[33-35] Resultant protein products from distinct core clock component genes generate negative feedback loops; PER/cryptochrome inhibits transcriptional activity of CLOCK/BMAL1, whereas REV-ERBα/β represses transcription of the Bmal1 gene.[33-35] In addition to modulating expression of one another, clock components influence expression of numerous clock-controlled genes, which do not directly feedback on the clock mechanism. In doing so, it has been estimated that circadian clocks temporally govern approximately 3% to 16% of the transcriptome (in a cell/organ-specific manner).[36] Clock-controlled genes encode for proteins that impact a wide array of cellular processes, including transcription and translation, signal transduction, structural integrity, electrophysiology, and metabolism.[36] It is noteworthy that circadian clocks additionally impact biological processes through various posttranscriptional mechanisms.[37] For example, circadian clock components impact protein synthesis through direct binding to the translation machinery (eg, binding of BMAL1 to ribosomes) and/or to critical signaling components (eg, binding of PER2 to mTORC1), in addition to regulating microRNA species (which, in turn, influence mRNA stability and/or translation).[38-40] As such, circadian clocks coordinate temporal control of biological processes through both transcriptional and post-transcriptional mechanisms.

Circadian clocks are ubiquitous in nature and have been characterized within various cardiovascular-relevant cell types, including cardiomyocytes, endothelial cells, vascular smooth muscle cells, renal cells, fibroblasts, neurons, and immune cells.[23,41-46] In each case, circadian orchestration has emerged as essential for maintenance of normal cardiovascular function. Examples include vascular reactivity (endothelial and vascular smooth muscle cells), natriuresis (renal cells), wound healing (fibroblasts), autonomic/sympathetic tone (neural cells), and inflammation (immune cells). A detailed review of the function of all these cell type–specific clocks is beyond the scope of this paper; the reader is instead referred to relatively recent published literature.[23,44,47-49] Here, we provide detail regarding how cell- and animal-based studies, in combination with state-of-the-art hypothesis-testing and hypothesis-generating strategies, have yielded considerable insight into the physiologic role of the cardiomyocyte circadian clock.

THE CARDIOMYOCYTE CIRCADIAN CLOCK

One of the first studies investigating time of day-dependent rhythms in cardiac expression of a circadian clock gene was by Oishi et al,[50] who reported diurnal rhythms for *Bmal1* and *Per2* mRNA in mouse hearts. Soon thereafter, 24-hour expression patterns of major core circadian clock components were characterized in rat hearts; consistent with established workings of the clock mechanism, positive component (eg, *Bmal1*, *Clock*, *Npas2*) levels peaked close to the beginning of the sleep phase, and were essentially antiphase to negative components (eg, *Per1/2/3*, *Cry1/2*, *Rev-erbα/β*).[51] Given that the heart is composed of numerous cell types, it was likely that clock gene oscillations reported were not solely from cardiomyocytes. Similarly, it was also conceivable that diurnal patterns in gene expression were simply a myocardial response to fluctuations in the neurohumoral milieu (secondary to sleep/wake and/or fasting/feeding cycles). To expose the autonomous nature of the intrinsic cardiomyocyte circadian clock, Durgan et al[41] confirmed that mRNAs encoding for core circadian clock components oscillated in cultured adult cardiomyocytes, with a periodicity of 24 hours. Similar observations have subsequently been made for both neonatal and stem cell derived cardiomyocytes in culture, consistent with the relatively ubiquitous nature of this timekeeping mechanism.[52,53] It is noteworthy that consistent with behavioral cycle differences (eg, sleep/wake cycles) observed between diurnal humans and nocturnal rodents, 24-hour oscillations in circadian clock components are antiphase in hearts of humans vs rats/mice. For example, *Bmal1* mRNA levels peak in rat hearts at the beginning of the light phase, whereas they peak at the beginning of the night in humans[51,54,55]; as such, *Bmal1* mRNA levels are consistently peak in mammalian hearts at the awake-to-sleep-phase transition. Thus, rodents are considered appropriate preclinical models for studying the cardiac circadian clock.

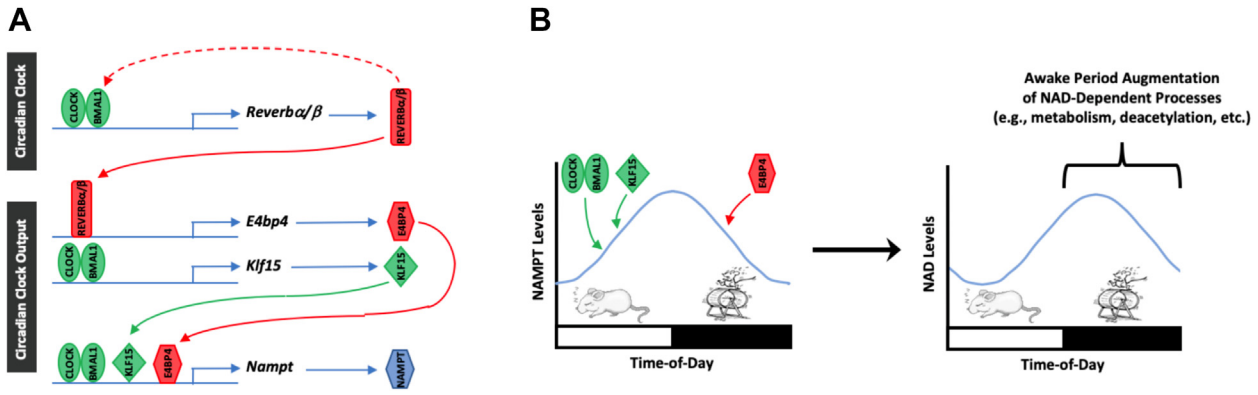
As with many areas of molecular cardiology, genetic approaches have proven extremely valuable toward identification of both the function, and

mechanisms of action, of the cardiomyocyte circadian clock. Multiple circadian clock components have been manipulated specifically within cardiomyocytes, through a variety of experimental strategies (constitutive/inducible, gain/loss of function, transgenic/knockout/adeno-associated virus). It is noteworthy that due to inherent redundancy in the mechanism, manipulation of some circadian clock components does not result in abolition of clock function (eg, deletion of a single PER isoform can largely be compensated by the remaining 2 isoforms). The most striking effects on circadian clock function have been observed when BMAL1, CLOCK, and REV-ERBα/β are targeted. More specifically, genetic deletion of BMAL1 alone, combined deletion of both REV-ERBα and REV-ERBβ, or expression of a dominant negative CLOCK mutant protein (lacking the transactivation domain), results in abolition of the cardiomyocyte circadian clock.[10,56,57] Genetic models such as these have proved valuable for enhancing our knowledge regarding orchestration of cardiac processes by this timekeeping mechanism.

The first heart clock disruption model reported was the cardiomyocyte-specific CLOCK mutant (CCM) mouse (which express a dominant negative CLOCK mutant protein that lacks a transactivation domain selectively in cardiomyocytes).[10] CCM mice exhibit attenuated diurnal fluctuations in cardiac function, signaling, and metabolism at multiple levels, in the absence of altered behaviors (such as sleep/wake and fasting/feeding cycles).[10,13] With regard to cardiac function, 24-hour rhythms in heart rate (in vivo) are attenuated, while day-night differences in both contractility (ex vivo) and myosin adenosine triphosphatase activity (in vitro) are abolished in CCM mice/hearts (relatively to littermate control animals).[10,17] Similarly, day-night variations in the intrinsic beating rate of the sinus node is abolished in cardiomyocyte-specific BMAL1 knockout (CBK) mice (although 24-hour rhythms in heart rate and other electrophysiologic parameters in vivo are only modestly affected, suggesting an important contribution of autonomic/sympathetic tone as a driver of diurnal rhythms in an intact organism).[12,58] In the case of cardiac metabolism, rates of glucose oxidation (but not fatty acid oxidation), glycogen and triglyceride synthesis, and cellular constituent turnover (eg, proteins, phospholipids, and organelles) all exhibit day-night fluctuations, which are attenuated/abolished in CCM and/or CBK mice.[10,13,14,59] More specifically, oxidative metabolism peaks during the first half of the active period (likely providing adenosine triphosphate to meet the energetic demand of increased contractility at this time), followed by fuel store replenishment during the second half of the active period (likely providing nutrients for the upcoming sleep period), and finally growth and repair during the first half of the sleep period (likely to ensure normal cellular/organ function upon awakening).[60] Consistent with the concept that circadian clocks confer the selective advantage of anticipation, facilitating temporally appropriate responses of cells to stimuli/stresses, the cardiomyocyte circadian clock appears to govern multiple signaling cascades. This includes β-adrenergic, growth hormone, and insulin signaling; day-night differences in cardiac sensitivity to these stimuli are attenuated/abolished in CCM and/or CBK mice.[10,14,61,62]

Although it is clear that the cardiomyocyte circadian clock temporally regulates critical cardiac processes, the identification of the precise mechanisms by which this occurs remains largely in its infancy. Both candidate and unbiased omics-based approaches (microarrays, RNA sequencing, chromatin immunoprecipitation sequencing, proteomics, etc.) interrogating hearts of mice with cardiomyocyte-specific circadian clock disruption have identified putative mediators linking the circadian clock with cardiac metabolism, signaling, and electrophysiology.[10,17,56,57] Early bioinformatic studies utilizing microarray data for hearts collected at 3-hour intervals over a 24-hour period from CCM, CBK, and littermate control mice applied a number of stringent filters to identify the 9 direct CLOCK/BMAL1 heterodimer target genes in the heart (4 of which were established circadian clock components).[56] Of the remaining 5 direct target genes, 4 have established functions in metabolism and signaling: 1) DGAT2 (diglyceride acyltransferase 2), involved in triglyceride synthesis; 2) PIK3R1(phosphoinositide-3-kinase regulatory subunit 1), involved in insulin/insulin-like growth factor-1 signaling; 3) USP2 (ubiquitin-specific protease 2), involved in protein turnover; and 4) nicotinamide phosphoribosyltransferase (NAMPT), involved in nicotinamide adenine dinucleotide (NAD) salvage.[56] Given the central role of NAD in a plethora of cellular processes, circadian regulation of NAMPT has been studied extensively. Molecular-based studies in the liver confirm BMAL1/CLOCK binding to E-boxes in the promoter of the NAMPT gene.[63] Moreover, Li et al[64] provided convincing evidence that the BMAL1/CLOCK-regulated transcription factor Krüppel-like factor 15 (KLF15) directly regulates NAMPT; cardiomyocyte-specific deletion of KLF15 abolishes 24-hour rhythms in cardiac NAMPT expression. Recently, Dierickx et al[57] revealed a third mechanism by which the cardiomyocyte circadian clock regulates NAMPT,

FIGURE 1 Clock Control of Cardiac NAD Levels

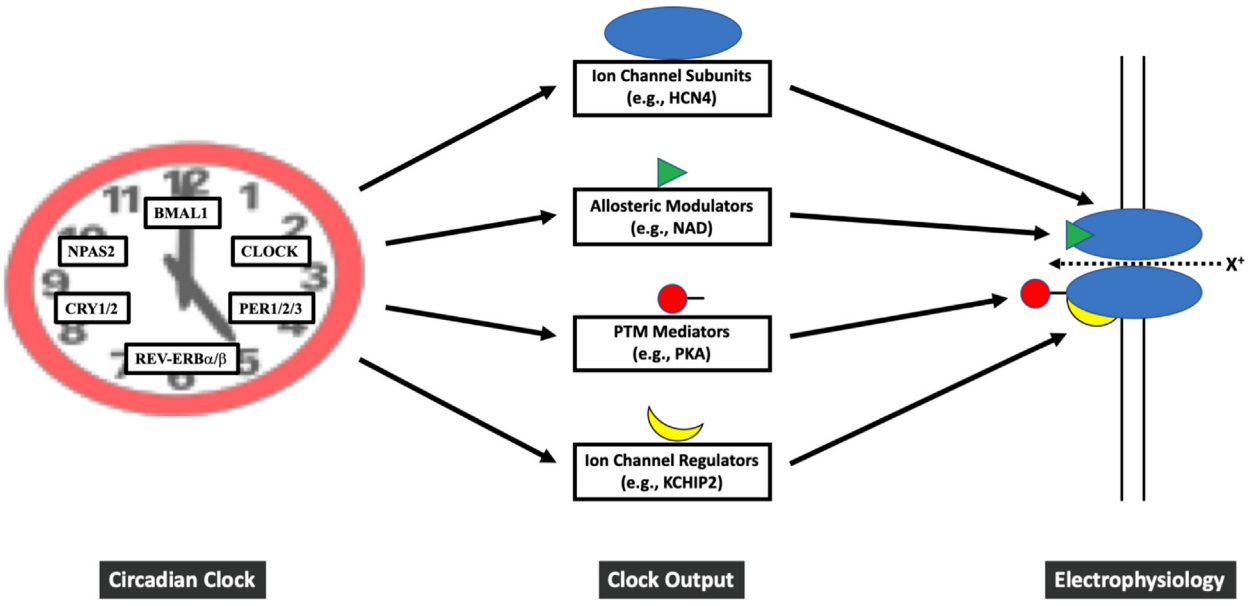


(A) Integral circadian clock components BMAL1, CLOCK, and REV-ERBα/β form a negative feedback loop of the timekeeping mechanism. The BMAL1/CLOCK heterodimer induces transcription of the clock-controlled gene Krüppel-like factor 15 (KLF15), while REV-ERBα/β represses expression of the clock-controlled gene E4 promoter binding protein 4 (E4BP4). The clock-controlled gene nicotinamide phosphoribosyltransferase (NAMPT) (a key enzyme in the nicotinamide adenine dinucleotide [NAD] salvage pathway) is induced by both the BMAL1/CLOCK heterodimer and KLF15 and is repressed by E4BP4. (B) During the sleep phase, the BMAL1/CLOCK heterodimer and KLF15 induce NAMPT (peak activation at the sleep-to-wake transition), whereas E4BP4 represses NAMPT during the awake phase (peak repression at the wake-to-sleep transition). Twenty-four-hour oscillations in NAMPT lead to augmentation of cardiac NAD levels during the awake period, thereby facilitating NAD-dependent processes at this time. Green represents activation; red represents inhibition.

involving the BMAL1/CLOCK-REV-ERBα/β axis. More specifically, REV-ERBα/β inhibits expression of the transcription factor E4 promoter binding protein 4 (E4BP4); E4BP4 directly represses NAMPT by binding to regulatory elements within the promoter.[57] As such, E4BP4 is predicted to be a significant contributor toward diurnal variations in cardiac NAMPT levels; indeed, *Nampt* mRNA oscillations are abolished in cardiomyocyte-specific E4BP4 knockout hearts.[65] Importantly, positive regulation of the NAMPT promoter by BMAL1/CLOCK and KLF15 are temporally distinct from negative regulation by E4BP4, likely operating in a coordinated manner to orchestrate 24-hour rhythms in cardiac NAMPT levels (**Figure 1**).

Similar to the aforementioned metabolism-related links, progress has been made for identifying mechanistic mediators between the cardiomyocyte circadian clock and processes such as cellular signaling and electrophysiology. With regard to electrophysiology, a large number of ion channels exhibit 24-hour oscillations in the heart, at mRNA, and/or protein levels. These include K^+ (eg, KCNA5, KCND2, KCNH2, KCNK3, HCN4), Na^+ (SCN5a) and Ca^{2+} (CACNA1D) channel subunits.[2,12,66-71] Evidence exists suggesting that several of these ion channel subunits are directly regulated by the circadian clock, such as KCNH2, HCN4, and SCN5a.[2,12,69,70] This includes loss of mRNA oscillations for these ion channel subunits following genetic disruption of the cardiomyocyte circadian clock, and in some cases direct binding of the CLOCK/BMAL1 heterodimer to their reciprocal gene promoters. In the case of HCN4, D'Souza et al[12] reported that the circadian clock within the sinus node drove 24-hour fluctuations in this gene. In addition to directly regulating ion channel levels, the circadian clock has the potential to temporally control cardiac electrophysiology through post-transcriptional mechanisms, such as reversible covalent modifications (ie, post-translational modifications), allosteric modulation (eg, nucleotide binding), and/or regulatory subunit regulation. In the latter case, a regulator of the transient outward K+ current, namely KCHIP2, is regulated by the cardiomyocyte circadian clock via both KLF15 and E4BP4 (in a manner that is similar to NAMPT regulation).[65,72] Moreover, the cardiomyocyte circadian clock regulates a plethora of signaling cascades/components (eg, AKT, AMP-activated protein kinase, extracellular signal-related kinase, mammalian target of rapamycin [mTOR], protein kinase A), several of which are known to target ion channels.[10,13,14,56,66] Similarly, various metabolism-related signals impact ion channel activity (including NAD levels),[73] leading to the possibility that the cardiomyocyte circadian clock likely affects cardiac electrophysiology through various direct and indirect mechanisms (**Figure 2**).

FIGURE 2 Direct and Indirect Regulation of Cardiac Electrophysiology by the Cardiomyocyte Circadian Clock



Evidence-based hypothetical model depicting clock control of electrophysiology through alterations in ion channel subunits, allosteric modulators, mediators of post-translational modifications (PTMs), and regulatory proteins. CRY = cryptochrome; NAD = nicotinamide adenine dinucleotide; NAMPT = nicotinamide phosphoribosyltransferase; PER, period; PKA = protein kinase A.

EVIDENCE IMPLICATING CIRCADIAN CLOCK (DYS) FUNCTION IN CARDIOVASCULAR PATHOLOGY

Consistent with governance over processes that are critical for maintenance of cellular/organ function, disruption of circadian biology typically predisposes cardiometabolic and cardiovascular disease development. In humans, genetic-induced (eg, polymorphisms in circadian clock component genes) and behavior-induced (eg, shift work) alterations in chronobiology are associated with increased risk for obesity, diabetes, hypertension, and ischemic heart disease.[74-78] Many of these epidemiologic observations are recapitulated in animal models of circadian disruption. In the case of the cardiomyocyte circadian clock, genetic ablation of this mechanism precipitates age-dependent cardiomyopathy, arrhythmogenesis, and reduced lifespan in mice.[56-58,70,79] It is noteworthy that in order for circadian clocks to maintain their selective advantage, they must remain particularly sensitive to their environment, by responding to a host of cues (termed zeitgebers or timekeepers) that adjust the timing of this mechanism. In general, the central clock (ie, the suprachiasmatic nucleus, within the hypothalamus) is primarily responsive to light (via the retinohypothalamic tract), while peripheral clocks (such as those located within cardiomyocytes) are adjusted by neurohumoral factors.[80] The latter often function in cell type-specific manners, adding an extra layer of complexity. It is therefore understandable that circadian clocks can become dysfunctional easily, particularly in disease states, when they receive "mixed" signals. In this subsection, we discuss the contribution of cardiomyocyte circadian clock (dys)function toward cardiac disease susceptibility.

SEX AND AGE. Both sex and age are considered important cardiovascular disease risk modifiers. The incidence of adverse ischemic events in younger women tends to be lower compared with age-matched men.[81] However, with age (and particularly, postmenopausal), fatal coronary heart disease is higher in women.[81] Moreover, incidence of heart failure with preserved ejection fraction is higher in women (relative to men).[82] Age is one of the strongest risk factors for cardiovascular disease development, and is a predictor for adverse outcomes.[83] Interestingly, both sex and age have been reported to impact circadian biology at specific levels. For example, circadian rhythms tend to be dampened (eg, metabolic cycles, tissue-specific oscillations of core circadian clock components) and/or phase shift (eg, sleep/

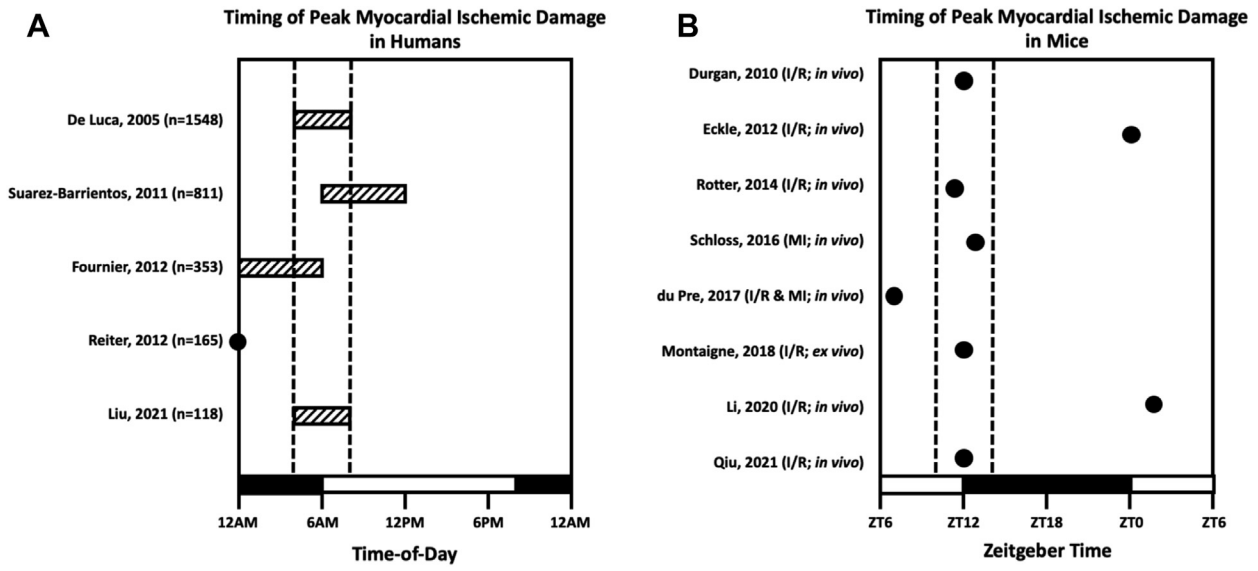
wake cycles) with age.[84] These observations lead to questions regarding whether the cardiomyocyte circadian clock is affected by sex and/or age, and if so, the extent to which altered circadian governance impacts cardiovascular disease risk. Despite an apparent lack of sex-specific differences in the cardiac circadian clock,[85] only ~50% of the cardiac transcriptome is common between males and females.[86] Similarly, ~30% of the circadian transcriptome is altered in aged hearts, yet the cardiac clock is only modestly altered (a slight phase delay).[61,87] Such observations suggest that although the cardiac circadian clock is not markedly affected by sex and/or age, circadian governance of the heart is. This likely reflects a variation of the interaction between intrinsic (ie, the circadian clock) and extrinsic (eg, the neurohumoral milieu, shear stress) circadian-related factors with respect to sex and age.

CARDIAC HYPERTROPHY. As mentioned previously, the cardiomyocyte circadian clock temporally orchestrates cellular processes, ensuring that they occur at the correct time of the day. This includes sequestration of cardiac growth and repair to the sleep period. Dissolution of this normal circadian governance and/or inappropriate challenge of the heart with a prohypertrophic stimulus during the sleep period can result in pathologic outcomes.[88] For example, genetic deletion of BMAL1 at either the whole-body or cardiomyocyte-specific levels, as well as in constitutive and inducible manners, predisposes the myocardium to hypertrophic growth.[56,61,79,89,90] Interestingly, BMAL1 deletion appears to temporally suspend the circadian clock at the beginning of the sleep phase.[56] In doing so, pro-growth signaling axes downstream of growth hormone and insulin-like growth factor 1/insulin, such as PI3K, Akt, and mTOR, become chronically active in the heart.[14,56,91] This is associated with augmented protein synthesis and cardiac growth independent of the time of day, resulting in cardiac hypertrophy.[14] Manipulation of the light/dark cycle in a manner that mimics shift work similarly leads to hypertrophic growth of the heart.[61] Interestingly, murine studies reveal that branched chain amino acids (BCAAs) (a pro-growth stimulus) only augment hypertrophic growth and pressure overload mediated adverse cardiac remodeling when consumed at the end of the active period.[91] Although it remains unknown whether a similar phenomenon occurs in humans, recent epidemiologic studies reported that consumption of protein for dinner was associated with higher cardiometabolic disease (CMD) risk factors (relative to individuals that primarily consume protein for breakfast).[92] Similar to BCAAs, isoproterenol induced cardiac hypertrophy only when administered to mice at the end of the active period.[61] This is consistent with observations in hypertensive patients; patients with nondipping hypertension (leading to the presence of a prohypertrophic stimulus during the sleep period) exhibit greater left ventricular hypertrophy (relative to patients for whom blood pressure drops during the night).[93]

Several questions regarding clock control of hypertrophic growth may include: 1) Is the circadian clock in the heart altered during hypertrophic states? 2) What are the precise mechanisms by which cardiomyocyte circadian clock disruption leads to cardiac hypertrophy? and 3) Can this mechanism be targeted pharmacologically at attenuate cardiac hypertrophy? Evidence exists for all 3 questions. In 2 rat models of compensated cardiac hypertrophy (namely transaortic constriction and the Dahl salt-sensitive rat), 24-hour oscillations in mRNAs encoding for circadian clock components and output genes are blunted (with greater attenuation observed for output genes).[51,94] As discussed previously, a number of plausible candidate mediators of hypertrophic growth in the setting of circadian disruption have been proposed, including chronic activation of mTOR; indeed, administering CBK mice with the mTOR inhibitor rapamycin is sufficient to reverse cardiac hypertrophy (as well as BCAA-induced hypertrophic growth).[14,91] However, mTOR is considered a relatively common downstream mediator of protein synthesis, for which many pathways converge.[95] What has been more elusive is identification of immediate mechanistic links emulating from the clock. Interestingly, cardiomyocyte-specific REV-ERBα/β double knockout mice develop adverse cardiac remodeling (including cardiac hypertrophy), with a similar etiology as CBK mice.[56,57] In both mouse models, REV-ERBα/β are repressed, associated with chronic E4BP4 induction.[56,57] Importantly, deletion of E4BP4 in CBK hearts completely prevents cardiac hypertrophy, adverse remodeling, and heart failure development.[65] Although the precise mechanism(s) by which E4BP4 promotes cardiac growth remain unknown, 55 direct E4BP4 target genes were identified as potential candidates. Such studies suggest that repression of cardiac E4BP4 may be cardioprotective in certain states. Consistent with this concept, the nonspecific REV-ERBα/β agonist SR9009 attenuates cardiac E4BP4 levels and concomitantly reverses cardiac remodeling in CBK mice, and does so as well during transaortic constriction-induced heart failure.[96,97]

ISCHEMIC HEART DISEASE. As highlighted previously, adverse ischemic events exhibit a robust time

FIGURE 3 Time of Day–Dependent Fluctuations in Myocardial Ischemic Tolerance



(A) Correlation of greatest myocardial damage (based on circulating damage markers) with estimated timing of ischemia onset in humans. Dashed lines represent the 4-hour window during which peak myocardial damage clusters in the majority of reported studies (4 AM to 8 AM [ie, the sleep-to-wake transition]). (B) Correlation of greatest myocardial damage (based on infarct size) with known time of ischemic onset in mice. Zeitgeber time 0 (ZT0) is the time at which lights turn on in an animal facility, while ZT12 is the time at which lights turn off. Given that rodents are nocturnal, ZT12 represents the sleep-to-wake transition. Dashed lines represent the 4-hour window during which peak myocardial damage clusters in the majority of reported studies (ZT10-14 [ie, the sleep-to-wake transition]). I/R = ischemia/reperfusion; MI = myocardial infarction.

of day dependence, with increased incidence between 6 AM and 2 PM.[4] This is in association with a rise in "triggers" for adverse ischemic events during the early waking hours, such as shear stress, clotting factors and platelet aggregability, and distinct candidate neurohumoral factors (eg, cortisol).[4] Some of these factors are directly regulated by circadian clocks (eg, PAI-1 is a direct BMAL1/CLOCK target gene), while others appear to fluctuate in response to behaviors (eg, blood pressure).[28,98] When an alignment of extrinsic (ie, behavior control of blood pressure) and intrinsic (ie, clock control of PAI-1) rhythms occurs in the early hours of the morning in at-risk individuals (ie, those with vulnerable plagues), probability of an adverse ischemic event (ie, myocardial infarction) increases. Conversely, disruption of normal circadian rhythms either acutely (eg, social jet lag, annual spring forward) or chronically (eg, shift work) increases myocardial infarction risk.[99-102] Specific single nucleotide polymorphisms in genes encoding for circadian clock components have also been associated with myocardial infarction risk.[78] Observations such as these highlight that adverse ischemic event onset is governed in a circadian fashion.

Over the past 2 decades, evidence has emerged suggesting that, in addition to the onset of ischemia, the tolerance of the myocardium to an ischemic event varies as a function of time of day. In humans, at least 5 distinct studies have sought to address this question, primarily through retrospective analysis of patient records.[20,103-106] More specifically, biomarkers of myocardial damage (eg, plasma creatine kinase, lactate dehydrogenase, and/or troponin levels) have been correlated with the estimated timing of ischemic onset. Either time of day was considered a continuous variable (for one study), or patients were subgrouped into time windows. These studies (summarized in Figure 3) suggest that tolerance of the myocardium to ischemia is lowest between 12 AM to 12 PM (relative to 12 PM to 12 AM), with a relative consensus for poor tolerance around 4 AM to 8 AM. The magnitude of these time-of-day fluctuations is not trivial in clinical terms, with up to 2-fold higher damage observed between 4 AM to 8 AM vs 4 PM to 8 PM.[20] Accordingly, it appears that in addition to greatest risk for myocardial infarction in early waking hours, greatest damage to the heart also occurs at this time.

Myocardial ischemic tolerance is influenced by a large number of biological parameters/processes,

several of which are intrinsic to cardiomyocytes (and therefore may be governed by the cardiomyocyte circadian clock).[107] Interestingly, circadian clock gene oscillations are attenuated in the rat heart within 24 hours following ischemia/reperfusion.[108] To gain greater mechanistic insight between circadian clocks and ischemic tolerance, it appears that at least 8 different rodent-based studies have examined time of day–dependent fluctuations in myocardial ischemic tolerance.[64,109-115] Consistent with differences in key experimental parameters (eg, ischemic duration, permanent ischemia vs reperfusion, in vivo vs ex vivo, age/sex of rodents), it is not surprising that some variability exists regarding outcomes. Despite this, 6 (out of 8) studies reported diurnal variations in myocardial ischemic tolerance with close temporal alignment between rodents and humans (ie, lowest tolerance concentrated around the sleep-to-wake transition) (**Figure 3**). When circadian rhythms are disrupted in rodents at a whole-organism level, through either environmental or genetic manipulation, myocardial ischemic tolerance decreases.[116,117] Although translational in nature, such interventions do not necessarily promote interrogation of the contribution of cell type-specific clocks, such as those located within cardiomyocytes. To date, only one study has selectively disrupted the cardiomyocyte circadian clock, followed by interrogation of myocardial ischemic tolerance; through genetic manipulation of the CLOCK/BMAL1 heterodimer only in cardiomyocytes, Durgan et al[109] revealed that the cardiomyocyte circadian clock was indispensable for diurnal variations in ischemia/reperfusion tolerance. Surprisingly, disruption of the cardiomyocyte circadian clock in this manner improved ischemia/reperfusion tolerance (ie, reduced infarct size).[109] Interestingly, Montaigne et al[114] investigated ischemia/reperfusion tolerance ex vivo in hearts collected from germline REV-ERBα knockout mice; in this model, cardioprotection was observed in isolated hearts subjected to ischemia/reperfusion. Given that CLOCK/BMAL1 disruption lowers REV-ERBα levels, it could be speculated inhibiting the CLOCK/BMAL1-REV-ERBα axis specifically in the heart may afford protection during ischemia/reperfusion. The reasons for opposing outcomes between germline and cardiomyocyte-specific models are unknown but likely reflect involvement of circadian clocks in other cell types that impact ischemic tolerance (eg, immune cells, fibroblasts). It is noteworthy that several laboratories have suggested that PER2 may influence myocardial ischemic tolerance through HIF1α, ANGPTL4, and/or autophagy.[110,118,119] Strangely, genetic manipulation of PER2 (germline) has been reported to either increase or decrease myocardial ischemic tolerance.[110,120] In addition, genetic deletion of PER2 alone has no impact the circadian clock, while disruption of the cardiomyocyte circadian clock does not impact PER2 diurnal variations, leading to the possibility that PER2 may exert effects independently of the clock mechanism (eg, modulation of autophagy through direct binding to mTOR).[39]

CARDIOMETABOLIC DISEASE. Circadian clocks undoubtedly regulate the responsiveness of cells/organs to factors within the extracellular milieu. During common CMD states, such as obesity and diabetes mellitus, 24-hour rhythms in various neurohumoral factors become perturbed, ranging from changes in mesor (ie, daily average) and amplitude (eg, peak-to-trough ratio), to phase (ie, the timing of the oscillation).[121,122] Concomitantly, cell type–specific circadian clocks become differentially affected, resulting in internal dyssynchrony, wherein a cell's circadian clock becomes misaligned from both the neurohumoral environment and clocks within other organs. Concepts such as these have led to the suggestion that circadian misalignment contributes toward pathologies associated with CMD states. During uncontrolled type 1 diabetes mellitus, the clock in the heart appears to exhibit a phase advance, associated with complex alterations in 24-hour patterns for various metabolically relevant genes in the heart during diabetes.[123] Studies in type 2 diabetes models (eg, *db/db* mice) suggest modest alterations in core clock components, associated with a more striking repression in established clock-controlled genes.[124] Surprisingly, 24-hour oscillations in core circadian clock components are minimally impacted in the murine heart during obesity; this is in marked contrast to circadian clocks in other metabolically active organs (eg, liver), which are impaired by obesity milieu.[121,125] Despite maintenance of core circadian clock oscillations, diurnal patterns in cardiac processes are dramatically impacted in obese mice, likely secondary to the manner with which rhythms in intrinsic (ie, clocks) and extrinsic (ie, neurohumoral) factors interact in this setting.[125] This is exemplified by cardiac metabolism; day-night differences in nonoxidative lipid metabolism are abolished in the heart during obesity.[125] Interestingly, obesity does not uniformly result in loss/attenuation of 24-hour rhythms; it also initiates/amplifies some rhythms.[125] At a transcriptomic level, interrogation of RNA sequencing data of hearts collected from control and obese mice over a 24-hour period revealed differential rhythms for genes with known functions in cellular signaling, metabolism, and cell-cell communication.[125] In the latter case, comparison of differentially oscillating

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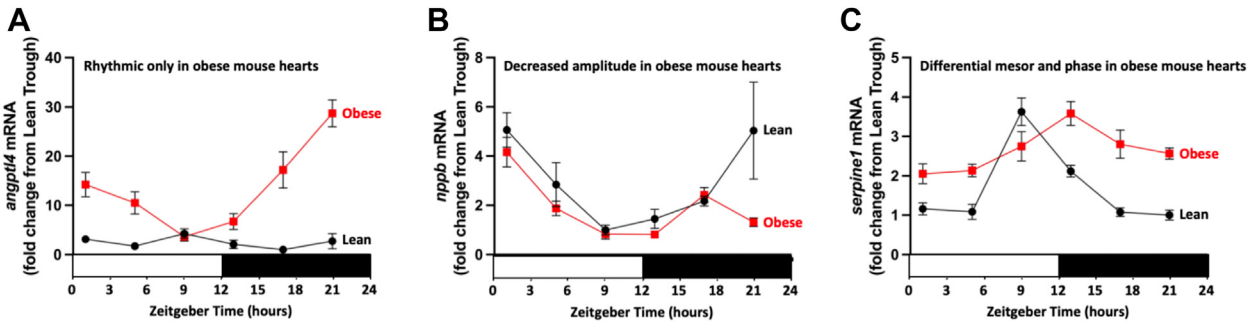
FIGURE 4 Differential 24-Hour Oscillations in Key Cardiokines During Obesity



Time of day–dependent oscillations in cardiac (A) *Angptl4*, (B) *Nppb*, and (C) *Serpine1* messenger RNA (mRNA) are altered to differing extents in obese mice compared with lean mice. Obesity was induced in mice through 20 weeks of high-fat feeding. Figures were drawn using publicly available data.

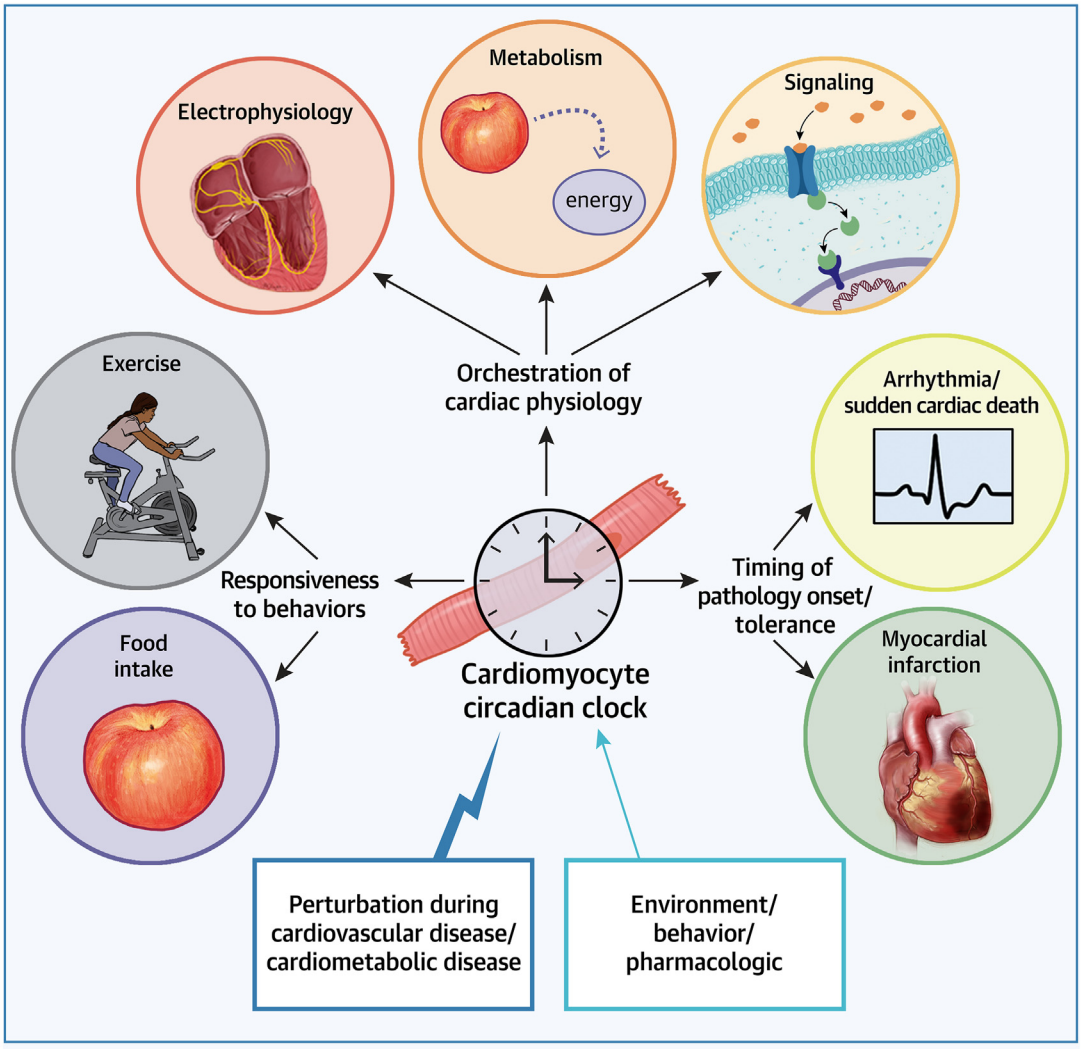
genes with known secreted proteins in human hearts revealed that 65 cardiac secreted proteins have altered 24-hour rhythms during obesity.[125,126] These include *Angptl4*, *Serpine1* (encoding for PAI1), and *Nppb* (encoding for B-type natriuretic peptide), which have established functions in insulin sensitivity, angiogenesis, clotting, natriuresis, and metabolism (Figure 4). Interestingly, Parcha et al[127] recently reported altered plasma B-type natriuretic peptide diurnal variations in obese (compared with lean) subjects. The extent to which differential rhythms in cardiokines during obesity contributes toward CMD-associated pathologies (eg, hypertension, atherosclerosis, insulin resistance) requires further investigation.

CONSIDERING CHRONOBIOLOGY FOR CARDIOVASCULAR DISEASE TREATMENT

It is undeniable that circadian orchestration of biological processes is fundamental for both health and disease. Virtually all cellular processes are temporally controlled, forming a complex network intersecting with the fourth dimension (ie, time). During pathological conditions, circadian orchestration is often perturbed, and disruption of normal rhythms in healthy organisms leads to pathology. Acknowledgment of these principles has led to the concept that perhaps circadian clocks/biology might be targeted for cardiovascular disease treatment. Many possible scenarios exist, some of which are discussed subsequently.

MANIPULATING DAILY RHYTHMS. Disruption of normal circadian rhythms, whether through behavioral/environment and/or genetic means, is detrimental in both humans and animal models, leading to increased risk of pathologies (including cardiovascular disease). Similarly, whole-body circadian rhythmicity is attenuated with age, in association with increased disease risk.[84] Taken together, such observations suggest that maintenance of normal circadian biology is critical for prevention of disease and maximizing longevity. Evidence in support of such a concept is considerable, especially in animal models.[128] What is less clear is whether amplifying biological rhythms is necessarily always an optimal disease treatment strategy (once a pathology is established). Certain beneficial scenarios can be predicted, such as amplifying cellular constituent turnover during the sleep phase to aid in the removal of protein aggregates, damaged organelles (eg, mitochondria), and stress granules that impede cellular/organ function. However, whether amplifying the morning surge of blood pressure or clotting factors would be beneficial in individuals with atherosclerosis, or accentuating the QT interval trough in Brugada patients during the sleep period, or even augmenting the evening rise in triglyceride synthesis in individuals with dyslipidemia is questionable. Cases such as these may warrant caution when considering augmentation of circadian fluctuations once pathology is established. Treatment strategies designed to achieve a state that is removed from “normal” is not a novel concept for the treatment of cardiovascular disease (consider β-blocker use in heart failure patients). Accordingly, while maintenance of normal circadian rhythmicity for disease prevention is likely advantageous, augmentation vs attenuation of rhythms for the treatment of disease may be case specific.

CENTRAL ILLUSTRATION **Roles of the Cardiomyocyte Circadian Clock in Cardiac Physiology and Pathology**



Young ME, J Am Coll Cardiol Basic Trans Science. 2023;8(12):1613–1628.

The cardiomyocyte circadian clock temporally orchestrates processes that are critical for normal cardiac function (eg, electrophysiology, metabolism, and signaling). In doing so, the cardiomyocyte circadian clock influences responsiveness of the heart to both physiologic stimuli (eg, exercise and nutrients) as well as pathologic stresses (eg, myocardial infarction). In order to maintain its physiologic advantage, the cardiomyocyte circadian clock is exquisitely sensitive to environmental cues (which are perturbed during common cardiometabolic/cardiovascular disease states).

TRANSIENTLY TARGET CIRCADIAN GOVERNANCE. In contrast to chronically targeting the circadian clock, there may be instances where it is beneficial to acutely take advantage of circadian governance of biological processes. If developed appropriately, it is theoretically possible to transiently activate cardioprotective mechanisms that outweigh potential adverse effects of long-term manipulation. One such example includes reperfusion therapy, for which a discrete therapeutic window exists. As described

previously, ischemia/reperfusion tolerance is impacted by numerous processes that are circadian regulated. This leads to the concept that pharmacologically targeting distinct circadian clock components at the time of reperfusion could potentially be employed to reduce reperfusion injury by transiently engaging circadian governance. Preclinical evidence in support of this concept includes a recent study by Reitz et al,[129] wherein a single administration of the nonselective REV-ERBα/β dual agonist SR9009 at the time of perfusion resulted in an approximate 50% decrease in infarct size, associated with attenuated heart failure development over the next 2-month time period. Whether strategies can be developed to target clocks in cell type–specific manners (eg, cardiomyocytes vs immune cells), to acutely "lock" those cells at a cardioprotective time period, is an exciting prospect.

CHRONOTHERAPEUTIC. Circadian biology has long been known to impact intervention effectiveness, particularly pharmacologic interventions. Parameters influencing optimal timing of a pharmacological intervention include diurnal variations in metabolizing enzymes, drug-specific targets, and off targets, as well as normal circadian orchestration of the targeted process.[130] Many examples exist. Consistent with reports that adverse cardiac remodeling occurs to a greater extent in patients with nocturnal hypertension, the MAPEC (ambulatory blood pressure monitoring in the reduction of cardiovascular events and effects of chronotherapy) study reported that taking at least 1 antihypertension medication at bedtime reduced risk of adverse cardiovascular events over an 8-year period.[131] Similarly, angiotensin II receptor antagonists have greater efficacy when taken in the evening (a time at which receptor levels are elevated).[132] One proposed strategy to attenuate alignment of the early morning peaks in blood pressure and platelet aggregability has been administration of aspirin at bedtime (to lower clotting risk upon awakening).[133] Given dramatic time of day-dependent oscillations in inflammatory processes, it is likely that future anti-inflammatory strategies for the treatment of cardiovascular disease will likely benefit from chrono pharmacological consideration. It is noteworthy that chronotherapeutic considerations extend beyond pharmacology and may include specific surgical interventions. For example, consistent with lower tolerance of the myocardium to ischemic events near the sleep-to-wake transition, evidence exists suggesting a modest reduction in 30-day mortality when aortic valve replacement surgery is performed in the afternoon (compared with the morning).[114,134,135]

ALIGN RHYTHMS WITH HEALTHY LIFESTYLE INTERVENTIONS. As highlighted previously, maintenance of biological rhythms is important for prevention of pathologies (including cardiovascular diseases). Currently, pharmacological interventions that selectively target circadian clocks are not clinically available (although numerous Food and Drug Administration–approved drug classes, such as β-blockers and thiazolidinediones, indirectly impact circadian clocks).[136,137] In contrast, both environment and behaviors markedly impact chronobiology through numerous mechanisms. When these entrainment signals become misaligned (as exemplified by shift work), disease risk increases. Moreover, biological clocks dictate the manner in which the body responds to common behaviors (such as food intake and physical activity) that are established cardiovascular disease risk factors. Consistent with these concepts, evidence is emerging that the timing of nutrient intake and/or physical activity dramatically impact cardiometabolic and cardiovascular parameters. For example, epidemiologic, translational, and basic science studies all suggest that shifting caloric intake toward the beginning of the day has benefit in reducing risk for obesity, diabetes mellitus, dyslipidemia, hypertension, and adverse cardiovascular events.[138] Although the body of literature regarding the timing of exercise for reduction of disease risk is significantly less (compared with diet), studies in healthy individuals and rodent models have revealed marked time-of-day differences in the responsiveness of the cardiovascular system to exercise bouts.[139-141] Collectively, such studies lead to speculation that alignment of environmental entrainment factors (eg, light) with the timing of nutrient intake and exercise (perhaps in the morning hours for healthy individuals) will likely reduce risk of future cardiovascular disease development.

POSSIBLE FUTURE DIRECTIONS

Although considerable progress has been made highlighting roles for the cardiomyocyte circadian clock during both physiologic and pathologic states, several important questions remain unanswered. These include, but are not limited to, the following:

1. What are the precise mechanisms by which the cardiomyocyte circadian clock orchestrates cardiac processes?

2. Does the cardiomyocyte circadian clock temporally regulate autocrine, paracrine (eg, cardiomyocyte-to-cardiomyocyte, cardiomyocyte-to-fibroblast), and/or endocrine (eg, cardiomyocyte-to-hepatocyte, cardiomyocyte-to-adipocyte) communication?
3. To what extent does misalignment of the cardiomyocyte circadian clock relative to the environment/milieu and/or other cells/organs contribute toward the etiology of cardiac dysfunction during disease states (eg, obesity, diabetes)?
4. Is targeting the cardiomyocyte circadian clock a feasible pharmacologic strategy for the treatment of cardiovascular disease states?
5. What roles do circadian clocks in non-cardiomyocytes play in time of day–dependent oscillations in cardiac physiology and/or pathology?

SUMMARY

Circadian clocks orchestrate cellular processes, ensuring that they are active/inactive in a temporally appropriate manner over a 24-hour period (**Central Illustration**). Disruption of this circadian governance invariably leads to cellular/organ dysfunction, ultimately precipitating pathology. This is exemplified by the cardiomyocyte circadian clock, which controls cardiac signaling, metabolism, electrophysiology, and contractility; attenuation of output from the cardiomyocyte circadian clock, or misalignment of this timekeeping mechanism with other organs and/or the environment, results in adverse cardiac remodeling and increased risk of numerous cardiovascular diseases (arrhythmias, myocardial infarction, and heart failure). Interventions designed with chronobiology in mind have the potential to improve efficacy of current cardiovascular treatment strategies and may ultimately result in novel therapeutics.

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KEY WORDS chronobiology, electrophysiology, heart failure, ischemia, metabolism

APPENDIX For an interactive version of the Central Illustration please see the online version of this paper.