Chronobiological Influence over Cardiovascular Function: The Good, The Bad, and The Ugly

Samir Rana, **Sumanth D. Prabhu**, **Martin E. Young**

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

Abstract

Essentially all biological processes fluctuate over the course of the day, observed at cellular (e.g., transcription, translation, signaling), organ (e.g., contractility, metabolism), and whole body (e.g., physical activity, appetite) levels. It is therefore not surprising that both cardiovascular physiology (e.g., heart rate, blood pressure) and pathophysiology (e.g., onset of adverse cardiovascular events) oscillate during the 24-hr day. Chronobiological influence over biological processes involves a complex interaction of factors that are extrinsic (e.g., neurohumoral factors) and intrinsic (e.g., circadian clocks) to cells. Here we focus on circadian governance of six fundamentally important processes: metabolism, signaling, electrophysiology, extracellular matrix, clotting, and inflammation. In each case, we discuss: 1) the physiologic significance for circadian regulation of these processes (i.e., 'The Good'); 2) the pathologic consequence of circadian governance impairment (i.e., 'The Bad'); and 3) whether persistence/augmentation of circadian influences contribute to pathogenesis during distinct disease states (i.e., 'The Ugly'). Finally, the translational impact of chronobiology on cardiovascular disease is highlighted.

PRELUDE

The purpose of this review article is to highlight recent advances made with regards to our understanding of how temporal organization of biological processes across the 24-hr hour day impact cardiovascular physiology and pathology. The majority of these studies fall into two major categories: 1) observational and interventional studies in humans; and 2) experimental studies in animal models (most commonly rats and mice). A great deal of heterogeneity exists between these studies, including the resolution of the time intervals reported (ranging from minutes to hours), the environmental conditions (e.g., light/dark cycles versus constant lighting), the natural sleep-wake patterns of the organism (e.g., diurnal humans versus nocturnal rodents), and whether sleep/wake status or neurohumoral markers of 'circadian phase' were assessed. In an attempt to reach a wider audience, this review article often discusses findings in terms of sleep and awake periods, making the assumption that non-shift work humans typically wake up in the morning (around 6am), while rodents wake at the beginning of the dark phase. 'Time-of-day fluctuation' is utilized to describe whether a parameter changes as a function of the time of the day, while 'circadian rhythm' is

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.

reserved to describe 24-hr oscillations in parameters that occur independently of environmental influences (i.e., intrinsically driven). Along those lines, intrinsic and extrinsic will be utilize to describe effects that originate from within or outside (respectively) a cell, organ, or organism (to be specified when the term is utilized).

Keywords

Chronobiology; electrophysiology; extracellular matrix; inflammation; metabolism

Introduction

Organisms have evolved numerous strategies allowing them to accommodate daily fluctuations in stimuli/stresses on Earth. In the case of mammals, dramatic changes in both environmental factors (e.g., light intensity, temperature, humidity, predator/prey presence, etc.) and behaviors (e.g., sleep/wake and fasting/feeding cycles) must be dealt with on a daily basis. For example, upon awakening, physical activity increases as the organism forages for food, avoids predation, engages in reproductive and husbandry activities, etc.; these activities must occur, regardless of whether the forage for food is successful. Biological processes influencing cognitive function (including alertness), neuroendocrine activity, metabolic homeostasis, repair mechanisms, and cardiovascular function must occur in a cohesive and coordinated manner, thus ensuring the organism maintains a selective advantage.[1, 2]

It has become increasingly apparent that temporal control of many of these processes does not operate solely at the level of stimulus-response coupling, but also involves an element of anticipation (i.e., daily rhythms in many biological processes occur prior to, and therefore independent of, alterations in behavioral/environmental factors). Anticipatory mechanisms allow cells/organs/systems to prepare for stimuli/stresses prior to their onset, thus ensuring optimal physiologic responses in a temporally appropriate manner. The purpose of this article is to provide an overview of chronobiological influence over cardiovascular function, the mechanisms responsible for daily temporal partitioning of biological processes, and why this form of regulation may be advantageous ('The Good'). Moreover, evidence will be discussed suggesting not only that circadian disruption precipitates cardiovascular dysfunction ('The Bad'), but will also provide specific examples in which persistence/augmentation of circadian governance may play a causal role in cardiovascular disease ('The Ugly').

Circadian Rhythms in Cardiovascular Physiology and Pathophysiology

In healthy subjects, blood pressure, heart rate, and cardiac output decrease while sleeping.[3, 4] Upon awakening, these parameters increase rapidly in association with neurohumoral fluctuations (e.g., sympathetic and autonomic tone, cortisol, epinephrine, etc.).[5–7] Such observations led to early suggestions that time-of-day fluctuations in cardiovascular function occurred in response to changes in mental and physical stresses (secondary to sleep/wake, and possibly fasting/feeding, cycles). However, early studies by Kleitman (in the 1930's) and Aschoff (in the 1960's) revealed that approximate 24-hr fluctuations persist for distinct

physiologic parameters (e.g., body temperature) when environmental conditions (e.g., lighting) are kept constant.[8, 9] Regarding cardiovascular parameters, Reinberg and colleagues similarly reported persistence of approximate 24-hr fluctuations in heart rate and blood pressure when healthy volunteers were housed under constant environmental conditions.[10] Recently, studies by Scheer and Shea have utilized a protocol designed to dissociate both environmental and behavioral influences on 24-hr fluctuations in cardiovascular parameters.[11–13] More specifically, when either 20-hr or 28-hr environmental/behavioral cycles (combined light/dark, sleep/wake and fasting/feeding cycles; termed dyssynchrony protocol) were enforced in healthy volunteers, 24-hr fluctuations in both heart rate and blood pressure persisted. However, although the endogenous 24-hr rhythm in heart rate was similar to that observed under native conditions (rising in the morning), the endogenous 24-hr rhythm in blood pressure peaked in the early evening (this less prominent evening peak is also observed in free living healthy individuals).[11, 12, 14] These studies suggest that time-of-day fluctuations in cardiovascular parameters are driven by a combination of mediators that are both extrinsic (e.g., environment- and/or behavior- related) and intrinsic to the body. One intrinsic timekeeping mechanism is the circadian clock.

Circadian clocks are cell autonomous molecular mechanisms comprised of a series of transcriptional/translational feedback loops with a cycle duration of approximately 24-hrs. [1, 2] Although initially considered to be restricted to specialized neurons within the hypothalamus (more specifically the suprachiasmatic nucleus [SCN]; also known as the central clock), it is now clear that circadian clocks are found in all mammalian cells.[15, 16] At the core of the mammalian clock are two transcription factors, CLOCK and BMAL1, which upon heterodimerization, bind to E-boxes within the promoter of various target genes (typically leading to induction).[17, 18] These target genes include negative feedback loop components, such as Period (PER), Cryptochrome (CRY), and REV-ERB isoforms, which repress the CLOCK/BMAL1 heterodimer (in transcriptional and post-translational manners). [19–21] Notably, circadian clock components (both those detailed here, as well as others; see review by Takahashi et al for a more comprehensive description of the circadian clock mechanism[2]) not only influence expression of one another, but also modulate expression of genes that do not directly feedback onto the CLOCK/BMAL1 heterodimer (so called clock controlled genes; CCGs). CCGs play an important role in modulating cellular function over the course of the day. It has been estimated that 3% to 10% of an organ's transcriptome is circadian regulated, either directly (i.e., a CCG) or indirectly.[22] Both unbiased (e.g., transcriptomic) and candidate (e.g., RT-PCR) approaches implicate clock control of critical cellular processes, including transcription, translation, posttranslational modifications, protein turnover, signaling, electrophysiology, and metabolism (as discussed in greater detail below).

The use of genetically modified mouse models has provided important insights regarding circadian governance of cardiovascular function. A striking phenotype is observed following germline deletion of BMAL1; 24-hr fluctuations in both heart rate and blood pressure are abolished.[23] However, these mice exhibit disruption of normal sleep/wake and fasting/feeding cycles, thus hindering dissociation of contributions from cell autonomous clocks versus behaviors.[23, 24] Cell type specific modulation of circadian clock components has

proven to be very insightful. For example, cardiomyocyte-specific CLOCK mutant (CCM) mice exhibit attenuated time-of-day fluctuations in both heart rate and contractility.[25] With regard to blood pressure, both endothelial and smooth muscle cell specific BMAL1 knockout mice have revealed that approximately one third of the daily oscillation in blood pressure is dependent on the smooth muscle cell clock.[26, 27] Collectively, studies in both humans (e.g., dyssynchrony protocol) and rodents (e.g., genetically modified mice) suggest that extrinsic (e.g., neurohumoral changes associated with behaviors) and intrinsic (e.g., circadian clocks) influences act in synchrony to mediate 24-hr fluctuations in multiple aspects of cardiovascular physiology (i.e., 'The Good').

Circadian clocks are exquisitely sensitivity to a plethora of stimuli/stresses, allowing them to adjust to environmental perturbations (associated with, for example, seasonal fluctuations, geographical location, food sources, etc.).[16] However, as a consequence, circadian clocks can become dysregulated during disease states (e.g., obesity, diabetes mellitus, sleep apnea, etc.) and/or secondary to 'abnormal' behaviors (e.g., eating during the usual sleep period, etc.).[28–31] When considering a hypothetical example (such as mRNA levels of a circadian clock component, whose 24-hr fluctuation fits a simple cosine curve; Figure 1A), circadian dysregulation can manifest at the level of altered mesor (i.e., daily average level), amplitude (i.e., mesor-to-peak difference; Figure 1B), and/or phase (i.e., timing of the trough/peak). Interestingly, shift workers (estimated at over 25% of the workforce) have increased risk of obesity, diabetes mellitus, gastrointestinal disorders, cognitive decline, cancer, and cardiovascular disease (although it should be noted that circadian dysregulation is likely just one of several contributing factors to increased disease risk in this population).[32, 33] Similarly, genetic polymorphisms in circadian clock components have also been described in humans, which are associated with increased obesity, diabetes mellitus, and hypertension incidence.[34, 35] Genetic manipulation of circadian clock components in rodents also leads to numerous cardiometabolic disease states.[36, 37] Moreover, germline deletion of BMAL1 results in age-onset cardiomyopathy, a phenotype that is recapitulated in cardiomyocyte-specific BMAL1 knockout (CBK) mice, revealing a critical role of the cardiomyocyte circadian clock in maintenance of normal cardiac structure and function.[38, 39] Collectively, the aforementioned observations suggest that circadian disruption is frequently associated with pathology (i.e., 'The Bad').

Not all circadian clocks are regulated in an identical fashion. A striking example of differential regulation involves consideration of the central (i.e., SCN) clock versus peripheral clocks. The SCN is entrained on a daily basis by light (via the retinal-hypothalamic tract), while peripheral clocks are entrained primarily via neurohumoral signals (some of which originate from the SCN).[16] Central and peripheral clocks can therefore become misaligned when physical activity or food intake occurs at an inappropriate time-of-day. For example, forcing rodents to consume food only during the sleep period phase shifts peripheral clocks, without effecting the central clock.[31] An additional layer of complexity exists: not all peripheral clocks respond equally to a distinct entrainment signal. In the case of altering food availability, the liver clock responds quickly, whereas the heart clock responds slowly.[40] As such, internal dyssynchrony can occur, observed at the levels of both central-to-peripheral and peripheral-to-peripheral clock misalignment. This form of circadian misalignment might occur readily in humans, and has

the potential not only to attenuate a physiologic process, but also to augment pathology. With respect to the latter, the onset of adverse CV events (e.g., myocardial infarction, stroke, arrhythmia, and abdominal aorta rupture) exhibit time-of-day fluctuations in humans, rising steeply during early morning hours in predisposed individuals (e.g., presence of vulnerable plaque, ischemic heart disease, vessel wall thinning).[41–44] This leads to speculation that circadian control of cardiovascular processes may contribute towards disease onset in the presence of risk factors (i.e., 'The Ugly'). Subsequent sections in this review will elaborate further on this concept, providing specific examples.

Circadian Control of Biological Processes Influencing Cardiovascular (Dys)function

As highlighted above, it is becoming increasingly clear that biological processes known to be essential for cardiovascular function exhibit time-of-day fluctuations. Clearly, it is not feasible to provide a detailed review of all circadian regulated processes. Accordingly, here we have elected to highlight circadian regulation of six biological processes known to play critical roles in cardiovascular function and dysfunction: metabolism, cellular signaling, electrophysiology, extracellular matrix, clotting, and inflammation. In each case, we will discuss: 1) the physiologic significance for circadian regulation of these processes (i.e., 'The Good'); 2) the pathologic consequence of circadian governance impairment (i.e., 'The Bad'); and 3) whether persistence/augmentation of circadian influences contributes to pathogenesis during distinct disease states (i.e., 'The Ugly').

Metabolism

The Good.

Consistent with fluctuations in energetic demand and nutrient availability during normal sleep/wake and fasting/feeding cycles, metabolic fluxes change dramatically over the course of the day, at both whole body and tissue-specific levels.[45, 46] This form of metabolic plasticity serves multiple purposes. In addition to homeostatic roles (e.g., maintenance of blood nutrients within a physiologic range), temporal partitioning of metabolism ensures that a cell's needs are met at all times of the day. Examples include providing ATP for augmented myosin cross-bridge cycling and ion channel activity during periods of increased physical activity, providing building blocks for replacement of cellular constituents during reparative periods, as well as providing metabolism-derived signaling molecules (e.g., AMP) and posttranslational modification precursors (e.g., UDP-glucosamine for protein O-GlcNAcylation) at an appropriate time-of-day.[47]

With regard to the cardiovascular system, circadian governance of cardiac metabolism has been investigated to the greatest extent (as reviewed previously[47–49]). Rodent studies reveal that upon awakening, myocardial oxidative metabolism increases (particularly glucose oxidation, secondary to increased glucose uptake and mitochondrial oxidative capacity), enabling the heart to meet increased energetic demand at this time.[25, 50–52] During the latter half of the awake period, anabolic processes become more active, including replenishment of myocardial glycogen and triglyceride (which are subsequently utilized during the sleep

period).[52, 53] The beginning of the sleep period is a time of increased cellular constituent turnover, when processes such as protein synthesis and degradation (e.g., autophagy) are activated simultaneously (theoretically enhancing cellular function integrity prior to awakening).[54, 55] Daily fluctuations in cardiac metabolism appear to be mediated in large part by the cardiomyocyte circadian clock (as these rhythms are severely attenuated following genetic clock ablation in murine models).[25, 52, 54] Moreover, many time-of-day fluctuations in cardiac metabolism have been described in ex vivo perfused rodent hearts, during which acute neurohumoral influences are excluded.

Multiple mechanistic links between the cardiomyocyte circadian clock and cardiac metabolism have been proposed, including direct clock control of metabolic enzymes (e.g., diacylglycerol acyltransferase 2 [DGAT2; involved in triglyceride synthesis], nicotinamide phosphoribosyltransferase [NAMPT; involved in NAD metabolism]) and signaling components (e.g., regulatory subunit of phosphoinositide-3-kinase [p85α; involved in insulin signaling]).[39] In vivo, factors intrinsic (i.e., cardiomyocyte circadian clock) and extrinsic (i.e., neurohumoral factors) to the heart likely act cooperatively, thus facilitating synchrony of metabolic rhythms. For example, increased contractility and β-adrenergic stimulation upon awakening will both augment glucose uptake and glycolytic flux, while post-prandial secretion of insulin during the awake period will accentuate nutrient uptake and storage.[56, 57] Although little is known regarding circadian control of cardiac metabolism in humans, numerous studies have detailed daily fluctuations in whole body metabolism; dyssynchrony and misalignment protocols (see above) suggest that multiple aspects of metabolic homeostasis (e.g., glucose tolerance) are regulated in part by endogenous circadian clocks.[13, 58]

The Bad.

As temporal partitioning of cardiac metabolism plays important roles in ATP provision, cellular constituent integrity, and signaling, it is not surprising that disruption of circadian governance over cardiac metabolism yields detrimental effects. Genetic manipulation of either CLOCK or BMAL1 in mice results in a model of temporal suspension, such that the clock is essentially 'paused' at the beginning of the sleep phase (a time-of-day at which glucose utilization is low, while protein turnover is elevated).[25, 39] As such, CCM and CBK mouse hearts exhibit attenuated rates of glucose utilization and augmented rates of protein synthesis independent of the time-of-day.[25, 39, 52, 54] These metabolic perturbations would be predicted to limit ATP generation during periods of high energetic demand, and promote cardiac remodeling; this phenotype is observed in both CCM and CBK mice.

Workload challenge studies serve as excellent examples. Exercise simulation ex vivo (i.e., increased afterload plus epinephrine) unveils greatest contractile reserve and glucose utilization in wild-type hearts during the active period.[25] In marked contrast, CCM hearts do not show time-of-day fluctuations in either contractile reserve or glucose utilization during this acute workload challenge; these parameters are chronically blunted, at levels similar to those observed in wild-type hearts at the beginning of the sleep phase.[25] Similarly, when CCM mice are provided running wheels, these mice exhibit an approximate 2-fold lower voluntary running capacity, compared to wild-type littermates.[59] Moreover, when challenged

with increased workload chronically (daily isoproterenol injection, for one week), wild-type hearts exhibit greatest hypertrophic (remodeling) responses at the beginning of the sleep phase (consistent with increased protein synthesis at this time); this time-of-day specific remodeling was absent in CCM hearts.[60] Interestingly, both CCM and CBK hearts exhibit a pro-hypertrophic phenotype even in the absence of an interventional stimulus/stress.[39, 60, 61] In the case of CBK hearts, hypertrophic growth worsens with age, ultimately precipitating dilated cardiomyopathy (a phenotype also observed in germline BMAL1 null mice).[39] This pro-hypertrophic phenotype is in part secondary to chronic mTOR-mediated protein synthesis, as the mTOR inhibitor rapamycin normalized protein synthesis and cardiac mass in CBK mice.[54]

In contrast to rodent studies, little is known regarding the impact of circadian disruption on cardiac metabolism in humans. However, both behavioral (e.g., shift work, sleep disorders, etc) and genetic (e.g., polymorphisms in clock genes) influences on circadian rhythms negatively impact whole body metabolism, predisposing individuals to common cardiometabolic diseases (e.g., obesity, diabetes mellitus).[32–35, 62]

The Ugly.

While disruption of circadian control over cardiac metabolism is detrimental, we further postulate that persistence of daily oscillations in metabolic processes may also accelerate cardiac disease (particularly in the presence of common cardiovascular risk factors). One example might be the increased risk of left ventricular (LV) hypertrophy reported in non-dipping hypertensives.[63] In healthy individuals, blood pressure decreases more than 10% during the sleep period.[4] We have hypothesized that circadian governance of metabolism ensures that pro-hypertrophic stimuli (e.g., shear stress associated with increased blood pressure) is not normally present when myocardial cellular constituent turnover is active. During non-dipping hypertension, normal lowering of blood pressure during sleeping is attenuated, such that blood pressure remains elevated at a time when processes involved in cardiac remodeling are augmented (which could hypothetically promote LV hypertrophy).[64] Evidence in support of this concept was highlighted in the previous sub-section (i.e., isoproterenol promotes adverse remodeling only during the sleep phase).[60]

A second hypothetical example is lipotoxicity during dyslipidemic conditions, such as obesity (Figure 2). A mismatch between fatty acid (FA) availability and utilization (oxidation plus storage as triglyceride) can lead to accumulation of lipid-based signaling molecules (e.g., ceramide, phospholipids, acyl-carnitines) within cardiomyocytes, resulting in lipotoxic cardiac dysfunction.[65–67] Prior studies report circadian regulation of triglyceride turnover in the heart, with high rates of triglyceride synthesis during the awake period, and high rates of lipolysis during the sleep period.[53] Persistence of this circadian governance during dyslipidemic states (e.g., obesity) augments expansion of the myocardial triglyceride pool specifically during the awake period (i.e., a time at which both triglyceride synthesis capacity and substrate are elevated).[53] This expanded triglyceride pool can subsequently undergo lipolysis during the sleep period (a time at which oxidative metabolism is low), thus facilitating lipotoxic species generation. Given that multiple lipid-based species are known to influence cellular signaling, inflammation, and electrophysiology, we postulate that

augmentation of triglyceride turnover rhythms contributes toward the increased incidence of myocardial infarction and sudden cardiac death risk in the early hours of the morning, particularly in obese individuals.

Cellular Signaling

The Good.

Neural activity (e.g., sympathetic tone), autocrine/paracrine/endocrine signals (e.g., cortisol), and shear stresses (e.g., blood pressure) all fluctuate as a function of time-of-day, and elicit cellular responses through numerous signaling pathways.[4, 6, 68] Equally important to the level of the stimulus is the sensitivity of the target tissue, which is also subject to daily oscillations; fluctuations in the stimulus and target tissue sensitivity collectively impact activation state of a signaling pathway at different times of the day. Changes in absolute levels of signaling cascade components (via protein turnover), as well as posttranslational modifications (e.g., phosphorylation, acetylation, O-GlcNAcylation, etc.) and pathway endpoints (e.g., transcriptional, metabolic, and functional outcomes) have been described over a 24-hr period in various tissues, including many that are critical to cardiovascular function.[22, 52, 68–72]

In the heart, phosphorylation and/or activation status of signaling components such as AMPK (AMP-activated protein kinase; energetic/nutritional sensor), AKT (protein kinase B; influences cell survival, growth, and metabolism), GSK3β (glycogen synthase kinase β; cell survival and metabolism modulator), mTOR (mammalian target of rapamycin; nutrient and growth factor sensor), PKA (protein kinase A; mediator of β-adrenergic signaling), ERK (extracellular signal-regulated kinase; involved in stress and growth factor response), and calcineurin (calcium-regulated protein phosphatase) exhibit time-of-day fluctuations.[53–55, 59, 73] Increased PKA activity at the beginning of the active period has been proposed to promote contractility (through, for example, increased phospholamban phosphorylation and subsequent SERCA2 activity), while increased calcineurin activity at the awake-to-sleep transition appears to reverse PKA-mediated effects.[73] Interestingly, key components of this system are regulated by the cardiomyocyte circadian clock; 24-hr oscillations in both calcineurin and the regulatory subunit of PKA are lost in CCM hearts.[25] Such observations, as well as reports that β-adrenergic receptor density exhibits time-of-day fluctuations in the heart[74], suggest that factors intrinsic and extrinsic to the heart collaboratively mediate 24-hr rhythms in this signaling system.

The cardiomyocyte circadian clock has also been implicated as a significant modulator of insulin signaling in the heart. Time-of-day fluctuations in multiple insulin signaling components are disrupted in both CCM and CBK hearts.[39, 54] One example includes the regulatory subunit of PI3K (p85α), a direct BMAL1/CLOCK target gene.[39] Interestingly, cardiac insulin sensitivity is higher at the beginning of sleep phase, a time at which circulating insulin levels are usually low.[54, 68] When mice are subjected to prolonged continuous fasting (thus chronically lowering insulin levels), the phosphorylation status of downstream insulin signaling components (mTOR and S6) still fluctuate, with peak levels at the awake-to-sleep transition.[55] This suggests that the cardiomyocyte circadian clock augments insulin signaling at this time, regardless of feeding status. It has been suggested

that this occurs to promote protein synthesis during the reparative sleep phase. It is noteworthy that circadian governance of cardiac sensitivity to neurohumoral influences that result in differential transcriptional responses have been reported; for example, cardiac transcriptional response to both fatty acids and thyroid hormone is augmented during the awake period.[75, 76]

The Bad.

As highlighted above, 24-hr fluctuations in signaling are the product of changes in both the extracellular milieu (e.g., neurohumoral factors and shear stress) and intracellular sensitivity, both of which can be mediated by circadian clocks. When stimulus and sensitivity rhythms are in alignment, mesor-to-peak differences (i.e., amplitude) in the activation status of a signaling cascade are augmented. Conversely, when 24-hr rhythms of these two parameters are antiphase, the amplitude of signaling cascade activation is attenuated. Hypothetically, both scenarios could present benefit for homeostasis, by maximizing performance in response to an anticipated stimulus (e.g., heart rate increase during exercise) and/or ensuring tolerance to a stress (e.g., limiting hypertrophic growth in response to a daily rise in blood pressure). Therefore, depending on the nature of circadian governance, loss of normal rhythms will result in either chronic activation or inactivation of a signaling cascade. A number of examples exist. Genetic disruption of CLOCK and/or BMAL1 specifically in cardiomyocytes leads to loss of time-of-day fluctuations in the phosphorylation status of numerous kinases; for some, phosphorylation is chronically high (e.g., AKT, mTOR, ULK1, and ERK1/2), while for others it is chronically low (e.g., AMPK, GSK3β).[53, 54, 59] Many of these signaling components have been implicated in adverse cardiac remodeling. For example, chronic activation of the PI3K/AKT/mTOR axis (as observed in CCM and CBK hearts) is typically associated with hypertrophic growth. Circadian disruption at the whole body level also leads to aberrant AKT signaling in the heart and vasculature.[77, 78] In the latter, perturbations in AKT were associated with impaired day-night differences in eNOS and endothelial function (e.g., acetylcholine-mediated vasodilation).[78]

The Ugly.

Daily fluctuations in cellular signaling undoubtedly have the potential to contribute towards known time-of-day fluctuations in both the onset of, and susceptibility to, cardiovascular pathology. Cardiac ischemia/reperfusion (I/R) tolerance is influenced by a host of secondary messengers (e.g., cAMP, cGMP, Ca^{2+}) and posttranslational modifications (e.g., phosphorylation, acetylation, O-GlcNAcylation, sumoylation, nitrosylation), as well as numerous signaling cascade components (e.g., PKA, PKG, PKC, PKB/AKT, GSK3β, calcineurin).[79, 80] Almost all of these factors have been reported to be circadian regulated. However, it is noteworthy that time-of-day fluctuations may differ between basal and stressed states. Calcineurin serves as an excellent example. In the basal state, calcineurin activity (which is known to decrease I/R tolerance[81]) peaks in the heart at the beginning of the sleep phase (which is inconsistent with time-of-day fluctuations in cardiac I/R tolerance).[73, 82] However, both pharmacological and genetic inhibition of calcineurin attenuate I/R-induced damage only at the beginning of the active period (with no effect at the beginning of the sleep phase), suggesting that I/R-induced activation of calcineurin contributes to day-night differences in I/R tolerance.[82] Such observations would be explained by greater

induction of calcineurin activators (e.g., Ca^{2+}, calpain) and/or greater repression of calcineurin inhibitors (e.g., MCIP) in response to I/R at the beginning of the active phase.[82] Daily fluctuations in the sensitivity of the cardiovascular system to stresses/stimuli may also help explain why inappropriately stressing the heart during the sleep phase (e.g., shift work, sleep apnea, non-dipping hypertension) is associated with greater risk of cardiovascular mortality. A steep rise in susceptibility to cardiac arrhythmias is also observed at the sleep-to-wake transition, a time at which PKA is more active, leading to speculation that increased PKA activity at this time may play a contributing role (e.g., through promotion of Ca^{2+} sparks).[41, 73, 83] Such a concept will be discussed further in the Electrophysiology subsection below.

Electrophysiology

The Good.

Twenty-four hour rhythms in cardiac electrophysiology are firmly established. In humans, heart rate increases sharply during the morning, associated with changes in cognitive and physical activity status.[3, 4] Consistent with circadian governance, studies by Hu et al in 2004 revealed that when healthy volunteers were forced to live a 28-hour day (i.e., forced dyssynchrony), a 24-hr rhythm in heart rate persisted.[14] The endogenous 24-hr rhythm peaked at a time corresponding to 10am, and had an amplitude of approx. 11%;[14] a normal daily rhythm (i.e., that observed in volunteers experiencing a normal 24-hr day) has an amplitude of approx. 50%.[4] Time-of-day fluctuations have also been reported in humans for heart rate variability (HRV) and QT interval, which are increased during the early morning and night, respectively;[84, 85] 24-hr rhythms in these parameters persist when environmental/ behavioral parameters are modulated, again suggesting mediation (at least in part) by an intrinsic timekeeping mechanism.[86, 87]

The use of genetically modified mouse models reveals a complex contribution of circadian clocks. For example, day-night differences in heart rate are severely attenuated in both germline BMAL1 knockout and CLOCK [19] mutant mice; in both cases, the mesor (daily average) heart rate is lower (i.e., bradycardia).[23] However, germline deletion of the CLOCK paralogue NPAS2 also leads to bradycardia, but does not influence day-night differences in heart rate.[23] Given that germline disruption of circadian clock components can impact both behaviors and systemic factors[36], cell type specific models have been employed. Cardiomyocyte-specific CLOCK mutant mice exhibit a reduced amplitude of day-night differences in heart rate (with bradycardia again being observed); the amplitude was partially attenuated (by approximately one third).[25] In contrast, cardiomyocyte-specific inducible Bmal1 knockout mice exhibit normal day-night differences in heart rate.[88] These observations suggest that circadian regulation of extra-cardiac influences (such as autonomic tone) likely play an important role in mediating time-of-day fluctuations in heart rate, with a lower contribution of the cardiomyocyte circadian clock. Interestingly, deletion of PPARγ (a putative regulator of BMAL1) in either endothelial or vascular smooth muscle cells diminishes day-night differences in heart rate, suggesting a complex interplay between autonomic control, the cardiomyocyte circadian clock, and additional local oscillators.[89]

Multiple K^{+}, Na^{+}, and Ca^{2+} ion channels are circadian regulated in the heart. These include KCHIP2, KCNA5, KCND2, KCNH2, KCNK3, SCN5a, and CACNA1D.[59, 88, 90–93] Additional channel subunits have also been identified through omics approaches, but have yet to be validated. In several instances, daily rhythms in ion channel components are associated with predicted day-night differences in cardiomyocyte electrophysiological properties. For example, steady state current (IKur) density was greater in rat myocytes during the active period (relative to the sleep period), a time at which KCNA5 protein levels are higher.[93] In many instances, rhythms in these ion channel subunits are lost following disruption of circadian clocks (both whole body and cardiomyocyte-specific) and/or autonomic blockade. CACNA1D, encoding for a subunit in L-type voltage gated ion channels, is rhythmic at both mRNA and protein expression levels in wild-type hearts, but not CCM hearts.[59] Another subunit of this channel, CACNA1C, may also be regulated by the cardiomyocyte clock, not only at the level of expression, but also post-translationally via the PI3K/AKT signaling axis (which is clock controlled; see Signaling sub-section).[94] Together, these studies illustrate that the electrophysiologic properties of the heart are markedly different depending on the time-of-day, which likely play an important role in accommodating daily fluctuations in contractile demands.

The Bad.

As highlighted in the preceding subsection, genetic disruption of the central circadian clock components (i.e., BMAL1, CLOCK, and/or NPAS2) in either germline, cardiomyocyte specific, and/or constitutive/inducible manners, results in a bradycardia phenotype.[23] In addition, Schroder et al have reported that cardiomyocyte-specific inducible Bmal1 knockout mice exhibit an increased QTc interval (particularly during the sleep period), which is known to increase arrhythmia risk in humans.[88] Consistent with this, many of these mouse models exhibit pro-arrhythmic phenotypes. For example, ECG recording in cardiomyocyte-specific inducible Bmal1 knockout mice revealed increased episodes of pauses that were not observed prior to deletion of Bmal1.[88] Moreover, an increased preload challenge ex vivo results in greater arrhythmia incidence in the cardiomyocyte-specific inducible Bmal1 knockout hearts.[88] Studies by Jeyaraj et al suggest that the transcription factor Klf15 serves as a mechanistic link between the circadian clock and KCIP2 (transient outward potassium current subunit); genetic deletion of KLF15 abolishes time-of-day fluctuations in both KCIP2 and QTc interval.[92] Importantly, QTc interval was elevated throughout the 24-hr period in KLF15 knockout mice, associated with increased susceptibility to pacing-induced arrhythmias.[92] Thus, impairment of normal circadian governance over critical ion channels in the heart can increase arrhythmia risk. Notably, cardiac arrhythmia susceptibility is also increased secondary to morphological (e.g., hypertrophy, fibrosis) and conduction (e.g., connexin) abnormalities; genetic deletion of circadian clock components adversely affects these parameters. For example, CCM and CBK mice both exhibit increased cardiomyocyte size and fibrosis, which is exaggerated with age.[39, 60, 61] Connexin-40 is circadian regulated in the atria, being chronically repressed in CCM hearts; interestingly, both connexin-40 null and CCM mice exhibit bradycardia.[25] Collectively, these observations suggest that circadian disruption may lead to increased arrhythmia susceptibility through a host of potential mechanisms.

The Ugly.

The incidence of sudden cardiac death has a striking time-of-day fluctuation, rising sharply in the early hours of the morning, and remaining elevated throughout most of the awake period.[41, 95] Such observations have led to suggestions that pro-arrhythmic triggers, such as increased workload and adrenergic stimulation (which are themselves subject to circadian governance), potentially play a significant role in the dependency of sudden cardiac death to the time-of-day.[41] This leads to questions regarding why these triggers do not precipitate arrhythmias on a daily basis in all individuals. Numerous possibilities exist. One is that in healthy individuals, protective mechanisms are present during the awake period, thus reducing arrhythmogenesis susceptibility at this time. For example, HRV (a strong anti-arrhythmic 'mechanism') normally increases in the early hours of the morning, thus affording protection (Figure 3).[84] However, during ischemic heart disease, time-of-day fluctuations in HRV are severely attenuated, and are chronically low, resulting in a loss of protection.[96] It is noteworthy that the heart exhibits increased sensitivity to distinct ion channel inhibitors (e.g., oubain[97]) at certain times of the day, leading to speculation that time-of-day fluctuations in ion channel activity may make the heart more susceptible to elevated stresses and/or arrhythmic triggers present upon awakening during disease states.

Aberrant circadian governance of ion channels in specific disease states is also possible. For example, in preceding sections, we hypothesized that lipid-based signaling species likely accumulate in the heart during the sleep phase, which have the potential to impair electrophysiology-based cardioprotective mechanisms in dyslipidemic subjects upon awakening. Moreover, if temporal regulation of cardiac ion channels become phase shifted, pro-arrhythmic triggers and susceptibility would become aligned. Interestingly, circadian clocks (and therefore clock controlled processes) are phase shifted through environmental exposures (e.g., light) and behaviors (e.g., food intake), as well as during numerous disease states (e.g., diabetes mellitus).[16, 28–31] Another possibility involves factors that increase arrhythmia susceptibility independent of the time-of-day, such as structural/morphological impairments (e.g., fibrosis).[98] In some instances, a combination of these distinct mechanisms has the potential to contribute (e.g., a diabetic patient with ischemic heart disease may exhibit chronically low HRV, a phase shift in the circadian clock, and adverse cardiac remodeling).

Extracellular Matrix

The Good.

The extracellular matrix (ECM) has a diverse array of functions, ranging from maintenance of tissue integrity, to modulation of platelet aggregability, interaction with inflammatory cells, and serving as a reservoir for numerous paracrine signals.[99] Although often perceived as relatively static in certain tissues (e.g., skin) and following injury (e.g., scars), the ECM is fairly dynamic in many tissues, during physiologic conditions. In the heart, approximately 20% of collagen (a primary ECM component) undergoes daily turnover (compared to only 5% in skin).[100] Evidence is emerging that the ECM is circadian regulated, such that processes involved in turnover are more active at one time of the day relative to another.

Recent studies investigating collagen synthesis/secretion in tendons serve as an excellent example. In tendons, the size of collagen fibrils vary as a function of time-of-day, associated with increased collagen-1 protein levels at the beginning of the sleep phase.[101] Through the use of complementary molecular and biochemical studies, Chang *et al* revealed increased collagen synthesis during the active period, which was subsequently secreted towards the active-to-sleep phase transition.[101] Circadian regulation of multiple mediators/ regulators of collagen-1 translation and secretion was identified, including Sec61a2 (Sec61 translocon alpha 2 subunit; involved in transfer of collagen into endoplasmic reticulum [ER]), Mia3 (melanoma inhibitory activity family, member 3; involved in cargo loading of collagen in ER), Pde4d (phosphodiesterase 4d; modulates phosphorylation status of transport machinery components via protein kinase A), and Vps33b (vacuolar protein sorting 33 homolog B; involved in vesicle transport).[101] Secretion of new collagen at the beginning of the sleep phase potentially aids in tendon repair and strengthening prior to awakening (and physical stressors associated with muscle activity).

Circadian governance of the ECM also includes fibroblast activation in response to injury (critical for generation of new ECM and subsequent fibrosis); here we will consider skin laceration as a physiologic injury (as this can occur as a consequence of normal daily physical activities). Hoyle *et al* reported approximate 2-fold faster wound healing following laceration during the active period (compared to the sleep period) in both mice and humans, secondary to greater mobilization and activation of fibroblasts.[102] Use of genetically modified mouse models confirmed mediation of this daily rhythm by the intrinsic fibroblast circadian clock, leading to speculation that temporal governance occurs in anticipation of greater likelihood of injury during the awake period (while animals forage for food and avoid predation).

Unbiased transcriptomic studies support the concept that the ECM is also regulated in a circadian fashion within cardiovascular tissues (such as heart and aorta).[103, 104] For example, multiple isoforms of collagen (COL; e.g., COL1A1, COL3A1, COL4A1, COL4A2), matrix metalloproteases (MMP; e.g., MMP13), tissue inhibitors of MMPs (TIMPs; e.g., TIMP3), and secretory pathway components (e.g., VSP33b), exhibit time-of-day fluctuations in wild-type hearts, which are abolished in CBK hearts, suggesting temporal governance by the cardiomyocyte circadian clock.[39] Further studies are required to characterize fully the extent to which ECM in cardiovascular tissues is circadian regulated, and whether temporal control is critical for anticipation of daily fluctuations in parameters such as shear stress and contractile demand.

The Bad.

Both germline and tissue-specific manipulation of circadian clock components result in increased fibrosis in multiple tissues. For example, mouse tendons isolated from germline CLOCK $^{\Delta 19}$ mutant mice exhibit abnormally large collagen deposits.[101] This same mouse model exhibits increased sensitivity to bleomycin-induced pulmonary fibrosis, as well as augmented age-onset fibrosis in the heart.[77, 105] The latter fibrotic phenotype may be secondary to disruption of local oscillators (as opposed to systemic effects). Indeed, similar to CLOCK $^{\Delta 19}$ mutant mice, CCM mice exhibit augmented age-onset cardiac fibrosis.[60]

Moreover, modest fibrosis is also observed in CBK mouse hearts, which worsens with age. [39, 61] Given that CCM and CBK mice are models of temporal suspension at the beginning of the sleep phase, a time at which collagen secretion is predicted to be increased (based on studies in the tendon), it is conceivable that continuous collagen secretion throughout the day leads to this pro-fibrotic state. Consistent with this concept, expression of multiple circadian-regulated collagen isoforms are elevated in CBK hearts.[61]

Questions arise regarding how dysfunction of the *cardiomyocyte* circadian clock could cause *myofibroblast* activation. Prior studies suggest that factors such as TGFβ, AngII, and miRNAs (packaged within exosomes) act in a paracrine manner, transmitting signals from cardiomyocytes to fibroblasts (and vice versa);[106, 107] circadian regulation of these factors has been proposed in numerous tissues. Interestingly, the phosphorylated form of SMAD2 is dramatically elevated in CBK hearts, consistent with augmented TGFβ signaling and subsequent fibrosis.[61] Not only does circadian disruption lead to cardiac fibrosis, but so too does circadian misalignment. The most compelling evidence comes from studies investigating tau mutant hamsters, which due to a spontaneous mutation in the CK1ε gene, have an intrinsic circadian clock with a periodicity of 22 hours.[108] When these mutant hamsters are housed in a normal 12-hr light 12-hr dark cycle (i.e., misalignment between the intrinsic 22-hr clock and the extrinsic 24-hr environment) they develop cardiac (and renal) fibrosis. However, fibrosis is prevented when tau mutant hamsters are housed in an 11-hr light 11-hr dark cycle, thus re-establishing circadian alignment.[108] The potential translational importance of these observations is underscored when considering shift workers, who routinely experience circadian-related stresses (and have increased CVD risk). The circadian environment is also disrupted when patients are admitted to the critical care unit, during which patients are exposed to irregular lighting, noise, sleep, and nutritional patterns. Recent studies by Alibhai *et al* reported that disrupting the light/dark cycle following an MI was sufficient to increase infarct expansion and fibrosis in mice (associated with a worsening of contractility).[109]

The Ugly.

Fibroblast activation and fibrosis following an adverse event (such as myocardial infarction; MI) is a double-edged sword: too little fibrosis results in a scar with poor structural integrity (increasing rupture susceptibility), whereas excessive fibrosis contributes to tissue/organ dysfunction (through, for example, myocardial stiffening and/or promotion of prolonged inflammation). This leads to the question whether circadian governance over the ECM and/or fibroblast activation impacts tolerance of the heart to adverse stress. Here, we will consider ischemic events, non-dipping hypertension, and arrhythmias. Observational studies indicate that in addition to an increased incidence of MI in the morning, MI tolerance appears to be low at this time (resulting in greater myocardial damage).[41, 110] Similar studies in rodents confirm lowest tolerance of the heart to MI or I/R at the beginning of the active period; ischemic events at this time lead to augmented fibrosis.[82, 111] As mentioned above, fibroblast mobilization and activation potential is increased during the awake period, raising the possibility that hyperactivation of myofibroblasts in the morning results in excessive fibrosis, thereby exacerbating myocardial damage.

Cardiac fibrosis is also a hallmark of hypertension, likely playing a significant role in the etiology of contractile dysfunction.[112] Prolongation of elevated blood pressure during the sleep period in non-dipping hypertensives predisposes to worse clinical outcomes, such as left ventricular hypertrophy.[63] As highlighted above, the sleep phase appears to be a reparative period, including replacement of the ECM; persistence of shear and hemodynamic stresses during sleep could potentially augment collagen secretion, thereby worsening fibrosis. A similar scenario would be hypothesized during sleep apnea, during which inappropriate sympathetic tone during sleep is associated with cardiac fibrosis.[113] Finally, arrhythmia incidence increases dramatically upon awakening (persisting throughout the active period).[41] Arrhythmia risk is increased by numerous mechanisms (discussed in the Electrophysiology subsection), one of which includes fibrosis; excess ECM interferes with transmission of electrical impulses across the myocardium, thus precipitating arrhythmogenesis.[98] This raises the possibility that excess ECM remodeling during the sleep period, in combination with 'event triggering substrates' during the active period, contribute towards greater arrhythmia risk at this time.

Clotting

The Good.

Blood clotting is a normal physiologic process involving a complex cascade of enzymes that facilitate fibrin polymerization and platelet activation.[114] Any mismatch between coagulation and fibrinolytic activity can result not only in thrombotic events (e.g., thromboembolism, myocardial infarction), but also in hemorrhagic events (e.g., intracerebral, subarachnoid). The balance between coagulation and fibrinolysis is under strict circadian governance.[115, 116] Plasminogen activator inhibitor-1 (PAI-1; inhibits fibrinolytic activity through inhibition of tissue plasminogen activator [tPA]) exhibits time-of-day fluctuation in numerous tissues.[117] In humans, circulating PAI-1 levels peak in the early morning (approximately 0630); these oscillations persist during dyssynchrony protocols (as do rhythms in platelet activation), suggesting mediation by endogenous circadian clocks.[118] Indeed, the CLOCK/BMAL1 heterodimer directly regulates human PAI-1 transcription in endothelial cells, by binding to E-boxes in the PAI-1 gene promoter. [119] Likewise, fibrinolytic activity also exhibits 24-hr rhythms, with lowest activity in the morning hours.[120] Platelets play an important role in hemostasis and thrombosis, and are also under strict circadian governance.[121–123] In healthy adults, platelet function is enhanced during the early morning hours (8am-noon), along with increased levels of fibrinogen and factor VIII activity during the wake phase.[124] Furthermore, human platelet activation is subject to robust endogenous circadian oscillations, evidenced by a corresponding morning peak (~0900hr) in platelet surface activated glycoproteins (GP) IIb-IIIa, GPIb, and P-selectin (required for platelet aggregation).[125] Collectively, these observations support greater clotting potential upon awakening, hypothetically in anticipation of increased likelihood of lacerations at this time.

The Bad.

Shift work leads to disruption of circadian governance of multiple critical biological processes in humans. Interestingly, a single bout of night shift work is sufficient to lead to

platelet hypersensitivity, as evidenced by augmented cyclooxygenase-mediated eicosanoid synthesis.[126] Moreover, meta-analysis of 34 unique studies revealed that shift workers have an approximate 25% increased risk of MI (increased stroke risk was also observed).[127] Shift workers exhibit impaired time-of-day fluctuations in PAI-1 levels, presenting with a biphasic pattern (peaks at noon and midnight).[128] Obstructive sleep apnea (OSA) also leads to circadian clock disruption[29, 129, 130]; OSA patients exhibit increased plasma PAI-1 activity along with decreased t-PA activity, coinciding with higher adverse CV event risk.[131] Such observations are consistent with the concept that circadian disruption in humans leads to increased risk of adverse ischemic events, in part through dysregulated clotting. However, shift work and OSA predispose individuals to numerous CVD risk factors, including sleep restriction, dyslipidemia, inflammation, hypertension, obesity, and diabetes mellitus, suggesting that increased adverse ischemic event incidence is likely multifactorial.[33, 132]

In order to directly assess the impact of circadian disruption on clotting, both environmental- and genetic- based animal models have been employed. Genetic disruption of PER2 in mice resulted in decreased levels of pro-platelet formation from megakaryocytes, compromised secretion and aggregation, decreased circulating platelet counts (50% reduction), and longer bleeding times.[133] Moreover, circadian control of hemostatic platelet activity has been shown to be governed by circadian genes, wherein time-of-day fluctuations in platelet activity[134] and clotting time[27] are lost in CLOCK [19] mutant mice. Moreover, BMAL1 knockout mice exhibit a pro-thrombotic phenotype, with increased circulating von Willebrand factor, fibrinogen, and PAI-1 levels[135], as well as shorter clotting time.[27] When mice are forced to consume food during the light (sleep) phase, dyysynchrony occurs between light- and food- based entrainment signals, associated with increased megakaryocyte ploidy levels and cell-cycle activity.[121] Collectively, these observations suggest that circadian disruption adversely impacts clotting potential.

The Ugly.

As highlighted above, increased clotting in the early hours of the morning is hypothetically in anticipation of a greater need for wound healing at this time. However, in individuals with atherosclerosis, circadian governance that once served as a selective advantage may increase risk of adverse ischemic events in the early hours of the morning. In the late 1980's, Muller and colleagues reported an increased incidence of both myocardial infarctions and sudden cardiac death in the morning.[41] When comparing the timing of adverse cardiovascular events with candidate mediators, the investigators developed a model wherein the steep rise in shear stress upon awakening potentially ruptured vulnerable plaques, at a time when platelet aggregability is heightened; temporal synchronization of these risk factors (as well as hypofibrinolysis) in susceptible individuals would create a 'perfect storm' for formation of an occluding thrombus.[41] Circumstances may arise when this temporal synchronization is enhanced. For example, on Monday and Tuesday mornings following the one hour time change during Spring Forward, myocardial infarction risk increases; although increased risk may occur secondary to numerous factors (including sleep deprivation), one possibility is that morning surges in hemodynamic stress (driven primarily by waking) and clotting potential (driven primarily by cell-autonomous clocks, which take a couple of days to reset following the time shift) become better aligned.[136, 137] Conversely, during Fall Back,

myocardial infarction risk decreases for the first few days (perhaps due to temporal misalignment of these risk factors).[136] The endogenous nature of circadian governance over myocardial infarction risk is reinforced by studies investigating adverse cardiovascular events in vacationers; the onset of ischemic events appear to coincide more with the early morning hours in vacationer's home time zones, as opposed to local times.[138] It is noteworthy that additional triggers for increased vulnerable plaque rupture in the morning likely exist, in addition to shear stress, given that the CONVINCE trial (which lowered the morning rise in blood pressure) failed to reduce adverse ischemic events.[139] Taken together, these studies reveal that increased clotting potential in the morning confers a significant risk for adverse cardiovascular events in susceptible patients.

Inflammation

The Good.

Circadian governance applies to various aspects of the immune system, including immune cell function, trafficking and recruitment.[140–142] Time-of-day fluctuations in circulating immune cells are observed in humans and mice. Circulating levels of most innate (monocytes, macrophages, neutrophils) and adaptive (B-cells and CD4+ T-cells) leukocytes peak during the resting phase and decrease during the active period.[142–144] For example, in mice, circulating leukocytes and monocytes peak in the first half of the sleep phase and trough at the start of the active phase.[145, 146] This rhythm is antiphase to monocyte and neutrophil recruitment to tissue (including skeletal muscle and the heart)[142, 146, 147] and the homing of immune cells to the bone marrow and lymph nodes,[141, 142, 146, 148, 149] which peak during the active period. This rhythmicity results from the interplay of several factors, including immune cell circadian clocks, local sympathetic nerve activity and hormonal cues (which are also mediated in part by circadian clocks), retention signals in the bone marrow (*e.g.*, C-X-C motif chemokine ligand 12 [CXCL12]) and lymph nodes (*e.g.*, C-C motif chemokine receptor 21 [CCL21]), as well as pro-migratory signals on endothelial cells (e.g., CCL2 and adhesion molecules).[141, 146, 148–153]

Endogenous molecular clocks have been demonstrated in most innate and adaptive immune cell types.[141] Monocytes and macrophages exhibit a particularly robust clock, with high expression amplitude of clock genes,[145, 154, 155] and temporal gating of several cell-intrinsic functions including toll-like receptor 9 (TLR9) expression,[156] TLR4 responsiveness and activation of nuclear factor(NF)-κB,[155, 157] cytokine and chemokine release,[154, 155, 158–160] and phagocytosis.[161] In contrast, clock gene expression appears to be relatively low in neutrophils, suggesting that other factors may exert greater chronobiological influence (e.g,. autocrine, paracrine, and neuroendocrine factors).[162] Recent studies indicate that key inflammatory genes in macrophages are CCGs, including CCL2, CCL8, interleukin-6 (IL6), matrix metalloproteinase 9 (MMP9) and CX3C-chemokine receptor 1 (CX3CR1), with important mediatory roles for BMAL1 and REV-ERBα/β as drivers of rhythmic expression. [145, 155, 158, 160, 163] REV-ERBα/β impart immunomodulatory (suppressive), in part, by regulating the epigenetic state of DNA enhancer regions and repressing enhancer RNA transcription.[158, 164] This exquisite degree of circadian regulation of both physiological and molecular cell function is hypothesized to confer anticipation of the onset of inflammatory/

infectious insults. More specifically, it appears that the immune system is primed to combat infections at the beginning of the awake period, whereas immune system repair and replenishment peaks at the beginning of the sleep period.[165]

The Bad.

Shift work and circadian misalignment, which confer cardiovascular disease risk in humans, increase systemic inflammation, as reflected by augmented circulating levels of IL-6 and hsCRP.[58, 166] Chronic inflammation is a key pathophysiological factor underlying many cardiovascular diseases; innate immune cell clock disruption may contribute importantly to this response. Interestingly, while dysfunction of the core molecular clock induces dysregulation of inflammatory processes, the influence of distinct clock components on inflammatory and immune mediators appear to be somewhat heterogenous (see[167] for review). Myeloid-restricted loss of *Bmal1* induces a pro-inflammatory phenotype in mice, with loss of time-of-day fluctuations in blood Ly6C^{hi} monocyte levels.[145] *Bmal1* deficient monocytes and macrophages exhibit exaggerated gene expression of monocyte-attractant chemokines (CCL2, CCL8, S100a8) and pro-inflammatory cytokine secretion in response to both acute inflammation and high fat feeding, which increases susceptibility to bacterial infection and chronic cardiometabolic disease.[145] Similarly, genetic loss of cryptochrome (CRY) results in constitutively increased pro-inflammatory cytokine expression and NF-κB activation in macrophages.[159] An analogous heightened systemic and macrophage pro-inflammatory response is also observed after experimental jet lag and circadian disruption in mice.[168]

An augmented inflammatory response accompanying immune cell circadian clock disruption accelerates atherosclerosis. Myeloid BMAL1 deficiency on the ApoE−/− background increased Ly6C^{hi} monocyte trafficking to atherosclerotic lesions, their differentiation to M1 macrophages, and plaque macrophage content and lesion size.[169] Similar atherosclerotic and pro-inflammatory responses were observed upon shRNA-lentiviral hematopoietic cell knockdown of REV-ERBα in LDLR−/− mice.[170] In contrast, macrophage overexpression of REV-ERBα or administration of a REV-ERBα/β agonist, SR9009, induced an anti-inflammatory phenotype, and SR9009 treatment reduced atherosclerotic plaque size in LDLR−/− mice *in vivo*.[170, 171] Human atherosclerotic plaque exhibits reduced CRY1 expression, whereas adenoviral gene transfer of CRY1 to ApoE−/− mice reduced atherosclerotic plaque size and the expression of pro-inflammatory factors.[172] In the absence of genetic modulation, short term manipulation of the light/dark cycle (which causes circadian disruption) increased cardiac macrophage infiltration (but decreased neutrophil infiltration), and concomitantly worsened LV remodeling and function after MI.[109] Collectively, outcomes following distinct strategies for circadian clock modulation (genetic, pharmacologic, and environmental) are consistent with the concept that circadian disruption not only leads to a pro-inflammatory state, but also augments atherosclerotic disease progression and post-MI remodeling in animal models. The impact of immune cell clock dysfunction in other forms of cardiovascular disease (e.g., LV hypertrophy, chronic HF) remains to be studied.

The Ugly.

While circadian disruption in immune cells can promote disease, evidence exists suggesting that circadian control of the immune system may also contribute (in the appropriate context) towards cardiovascular disease pathogenesis. First, normal immune rhythms may increase susceptibility to disease progression during conditions when immune activity is physiologically enhanced. For example, a recent study demonstrated that CCL2-dependent myeloid cell recruitment and adhesion to large vessel atherosclerotic plaque peaks in the latter half of the active phase in mice, and that timed pharmacological CCR2 blockade during the active phase inhibited atherosclerosis.[173] It is conceivable that this pattern may contribute to time-of-day fluctuations in plaque stability and the known bimodal diurnal distribution of MI in the early morning and evening hours.[174] Oscillations in tissue recruitment of myeloid cells to the heart may also significantly impact the intensity of the inflammatory response and tissue damage to myocardial injury. Schloss *et al* demonstrated that neutrophil recruitment to the heart preferentially occurred at the onset of the active period, and that experimentally induced MI at that time resulted in a heightened neutrophil inflammatory response, larger infarct size, worse survival, and exaggerated post-MI LV remodeling and dysfunction.[147] Targeted blockade of neutrophil inflammation at the beginning of the active period improved infarct size and cardiac function. Such time-of-day fluctuations in the immune cell infiltrative response may contribute to reduced cardiac tolerance to adverse ischemic events previously reported at the onset of the awake period in both mice and humans.[41, 111] The full extent to which circadian governance of inflammatory processes plays a role in both the onset (e.g., ischemic events, arrhythmias) and progression (e.g., adverse remodeling following an MI) of cardiovascular disease is an area of translational research worthy of further exploration.

Chronotherapeutic Potential for Cardiovascular Disease

Based on evidence discussed in preceding sections, it is clear that chronobiology plays critical roles not only in cardiovascular physiology, but also in cardiovascular disease (CVD) onset and progression. However, this impact on health and disease extends beyond the examples highlighted already, and includes optimization of diagnostic and treatment strategies.[175] Several routine CVD biomarkers exhibit robust time-of-day fluctuations in patients, including ANP and cTnT, leading to suggestion that the time-of-day at which specific tests are performed may influence diagnostic value (although this does not seem to be the case for cTnT).[176–178] The prognostic value of blood pressure assessment is a topic of heightened awareness.[64, 179] Increased accessibility of ambulatory blood pressure monitoring has highlighted the previously underappreciated incidence of non-dipping hypertension, which is notably higher in certain at risk racial groups (e.g., approximately 2.4-fold higher in African Americans compared to Caucasians).[180] Given the greater risk of adverse cardiac remodeling in individuals with non-dipping hypertension[63], identification of this form of circadian impairment is vital for appropriate therapeutic interventions. The latter includes chronopharmacologic strategies designed to lower blood pressure at night; a large prospective ambulatory blood pressure monitoring study (MAPEC study) reported a reduction in adverse cardiovascular events when nighttime blood pressure was specifically targeted.[181]

Importantly, genes encoding for many common pharmacologic targets exhibit time-of-day fluctuations in multiple cardiovascular tissues.[22] These include targets for aspirin (cyclooxygenase 1) and statins (3-Hydroxy-3-Methylglutaryl-CoA Reductase); both these common drugs have now been shown to elicit greater benefit when taken at bedtime, attenuating platelet aggregability upon awakening (aspirin) and cholesterol synthesis during sleep (statins).[182, 183] The angiotensin-2 receptor is also under circadian governance, such that receptor antagonists appear to exhibit greater efficacy during the evening (a time at which receptor levels are high).[184]

Current and/or future cardiovascular-relevant pharmacologic targets are also likely to benefit from consideration of chronobiology. One example is inflammation; as highlighted in preceding sections, inflammatory processes exhibit profound time-of-day fluctuations, strongly suggesting that strategies for inflammation attenuation/resolution should consider the optimal time for treatment. Pharmacological interventions are not the only treatment modality that is impacted by time-of-day; so too are surgical, lifestyle, and environmental interventions. For example, Montaigne et al recently reported approximate 2-fold better outcomes when aortic valve replacement surgery was performed during the afternoon, relative to the morning (correlating with established time-of-day fluctuations in myocardial ischemic tolerance).[185] With regards to lifestyle, many physicians recommend improvements in diet and physical activity, to reduce CVD risk. What has emerged recently is that time-of-day should be considered, to maximize benefit. Consistent with circadian governance of metabolic homeostasis, improvements in CVD risk factors (such as weight, adiposity, glucose tolerance, and even blood pressure) are observed when the majority of calories are consumed toward the beginning of the day.[186] Moreover, the majority of studies report greater cardiovascular benefits of aerobic exercise in the morning.[187]

Additional behavioral/environmental factors influencing circadian rhythms include sleep and light exposure; appreciation of this knowledge has led to suggestions that some hospital settings may lead to circadian disruption. This is exemplified by the critical care unit, wherein patients are often subject to light-at-night, continual nutrition, and sleep disturbance. When this is modeled in rodents (e.g., MI induction in mice, followed by light/dark disruption), clinically-relevant outcomes (e.g., infarct expansion) are worsened.[109] Such observations suggest that hospital environments should be designed in a 'circadian conscience' manner.[188] A critical question relates to whether circadian clock components are a viable target for CVD treatment. Early pre-clinical studies suggest that the answer may be yes. Indeed, pharmacological activation of REV-ERBα/β attenuates pathogenesis in murine models of atherosclerosis, pressure overload, and MI.[171, 189–191] Moreover, commonly prescribed drugs, such as β-blockers and thiazolidinediones entrain circadian clocks[89, 192], raising the possibility that some therapeutic benefit may be through enhancement of circadian governance.

ACKNOWLEDGEMENTS

ABBREVIATIONS

AKT	protein kinase B
AMP	adenosine monophosphate
AMPK	adenosine monophosphate-activated protein kinase
AngII	angiotensin II
ANP	atrial natriuretic peptide
ApoE	apolipoprotein E
ATP	adenosine triphosphate
BMAL1	brain and muscle ARNT (arylhydrocarbon receptor nuclear translocator)-like
CACNA1C	calcium channel, voltage-dependent, L type, alpha 1C subunit
CACNA1D	calcium channel, voltage-dependent, L type, alpha 1D subunit
CBK	cardiomyocyte-specific BMAL1 knockout
CCG	clock controlled genes
CCL2	C-C motif chemokine ligand 2
CCM	cardiomyocyte specific CLOCK mutant
CCR2	C-C chemokine receptor type 2
CLOCK	circadian locomotor output cycles kaput
CRY	cryptochrome
cTnT	cardiac troponin T
CV	cardiovascular
CVD	cardiovascular disease
CXCL	C-X-C motif chemokine ligand
DGAT2	diacylglycerol acyltransferase 2
DNA	deoxyribonucleic acid
ECG	electrocardiogram
ECM	extracellular matrix
eNOS	endothelial nitric oxide synthase
ER	endoplasmic reticulum

ERK	extracellular signal-regulated kinase
ERK1/2	extracellular signal regulated kinase 1/2
FA	fatty acid
GP	glycoproteins
GSK3β	glycogen synthase kinase β
HRV	heart rate variability
hsCRP	high sensitive c-reactive protein
I/R	ischemia/reperfusion
IL	interleukin
KCHIP2	potassium channel interacting protein 2
KCIP2	potassium voltage-gated channel interacting protein 2
KCNA5	potassium voltage-gated channel subfamily A membrane 5
KCND2	potassium voltage-gated channel subfamily D member 2
KCNH2	potassium voltage-gated channel subfamily H member 2
KCNK3	potassium two pore domain channel subfamily K membrane 3
Klf15	kruppel like factor 15
LDLR	low density lipoprotein receptor
LV	left ventricle
Ly6C	lymphocyte antigen 6 complex
MAPEC	ambulatory blood pressure monitoring in the reduction of cardiovascular events and effects of chronotherapy
MCIP	modulatory calcineurin-interacting protein
MI	myocardial infarction
Mia3	melanoma inhibitory activity family, member 3
miRNAs	micro ribonucleic acid
MMP	matrix metalloproteases
mTOR	mammalian target of rapamycin
NAMPT	nicotinamide phosphoribosyltransferase
NF	nuclear factor

NPAS2	neuronal PAS domain protein 2
OSA	obstructive sleep apnea
PAI-1	plasminogen activator inhibitor-1
Pde4d	phosphodiesterase 4d
Per	period circadian protein
PI3K	phosphoinositide 3-kinase
PKA	protein kinase A
PKB/AKT	protein kinase B
PKC	protein kinase C
PKG	protein kinase G
PPARγ	peroxisome proliferator-activated receptors γ
REV-ERB	nuclear receptor reverse-ERB
RT-PCR	real time – polymerase chain reaction
SCN	suprachiasmatic nucleus
SCN5a	sodium voltage-gated channel alpha subunit 5
SERCA2	sarcoplasmic/endoplasmic reticulum calcium ATPase 2
SMAD2	small mother against decapentaplegic 2
TGFβ	tumor growth factor β
TIMP	tissue inhibitors of metalloprotease
TLR	toll-like receptor
tPA	tissue plasminogen activator
UDP	uridine diphosphate
ULK1	Unc-51 like autophagy activating kinase
Vps33b	vacuolar protein sorting 33 homolog B

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Summary and Future Perspectives

It is fundamentally clear that circadian rhythms critically impact cardiovascular health and disease. Metabolic pathways, signal transduction cascades, transcriptional networks, protein turnover, and more, are all temporally orchestrated, in a manner that is conducive to optimal cellular/organ function (much like a conductor synchronizes symphony players, thereby delivering an audible masterpiece, instead of random noise; The Good [Figure 4]). When circadian governance is impaired, pathology invariably ensues (The Bad). It is also increasingly apparent that persistence and/or augmentation of circadian rhythms can, in susceptible individuals, precipitate and/or intensify CVD (The Ugly). However, many critical questions remain to be addressed in this field. These include: 1) dissection of the molecular mechanisms by which circadian clocks modulate biological processes in cardiovascular-relevant cells (including cardiomyocytes, endothelial cells, smooth muscle cells, myofibroblasts, immune cells); 2) understand how cell-to-cell circadian clock synchrony is achieved in cardiovascular tissues, and whether disruption of this synchrony (e.g., misalignment of phases) contributes to CVD; 3) identify optimal lifestyle behaviors (e.g., timing of eating, exercise, etc.) and environmental conditions (e.g., lighting, temperature) that work in synchrony with circadian governance of cellular processes; and 4) develop therapeutic strategies that specifically target circadian clock components, and/or downstream mediators, for the effective treatment and/or prevention of CVD.
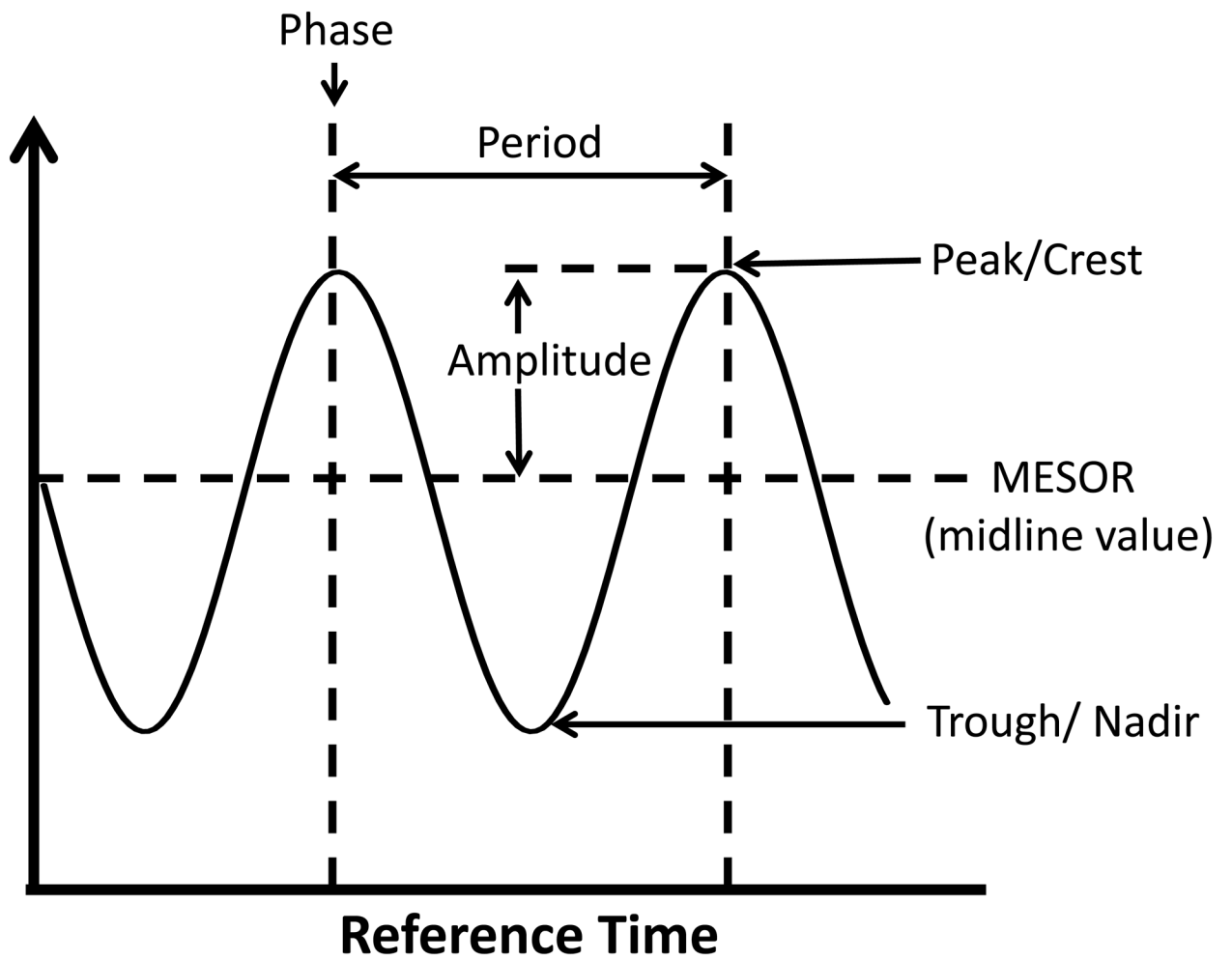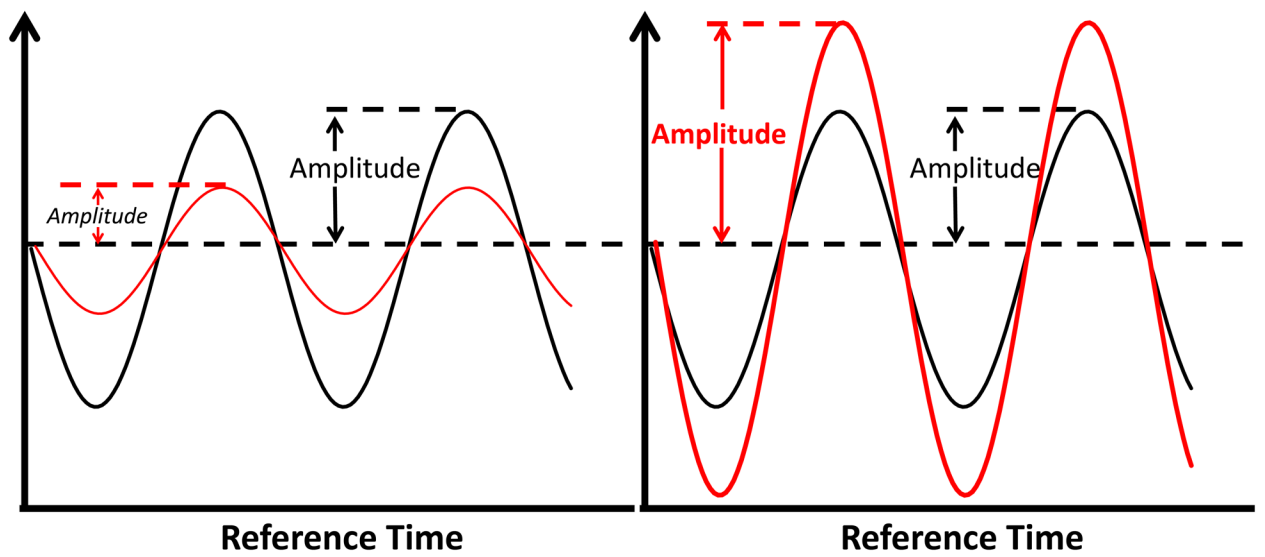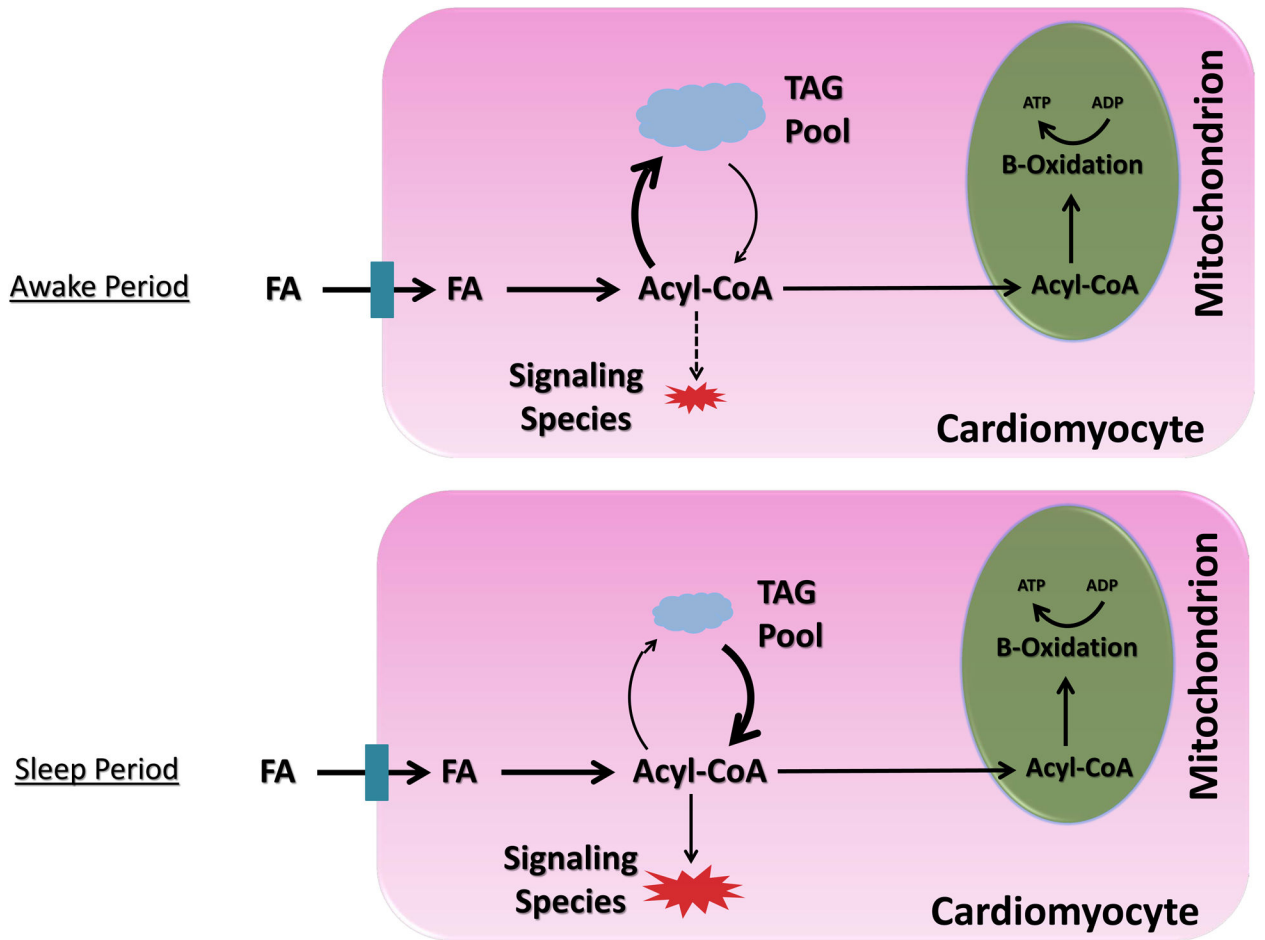
Figure 1.

Factors/processes which fluctuate rhythmically with respect to time that fit to a cosine curve can be defined by the peak (maximal value), trough (minimal value), mesor (midline value), amplitude (peak value minus mesor value), phase (timing of the peak or trough), and periodicity (24-hrs, in the case of circadian rhythms) (A). Alterations in 24-hr fluctuations can manifest at the levels of phase (termed a phase shift), periodicity, mesor, and/or amplitude; the figure illustrates attenuation (i) and augmentation (ii) of amplitude, both of which could hypothetically lead to pathology (B).
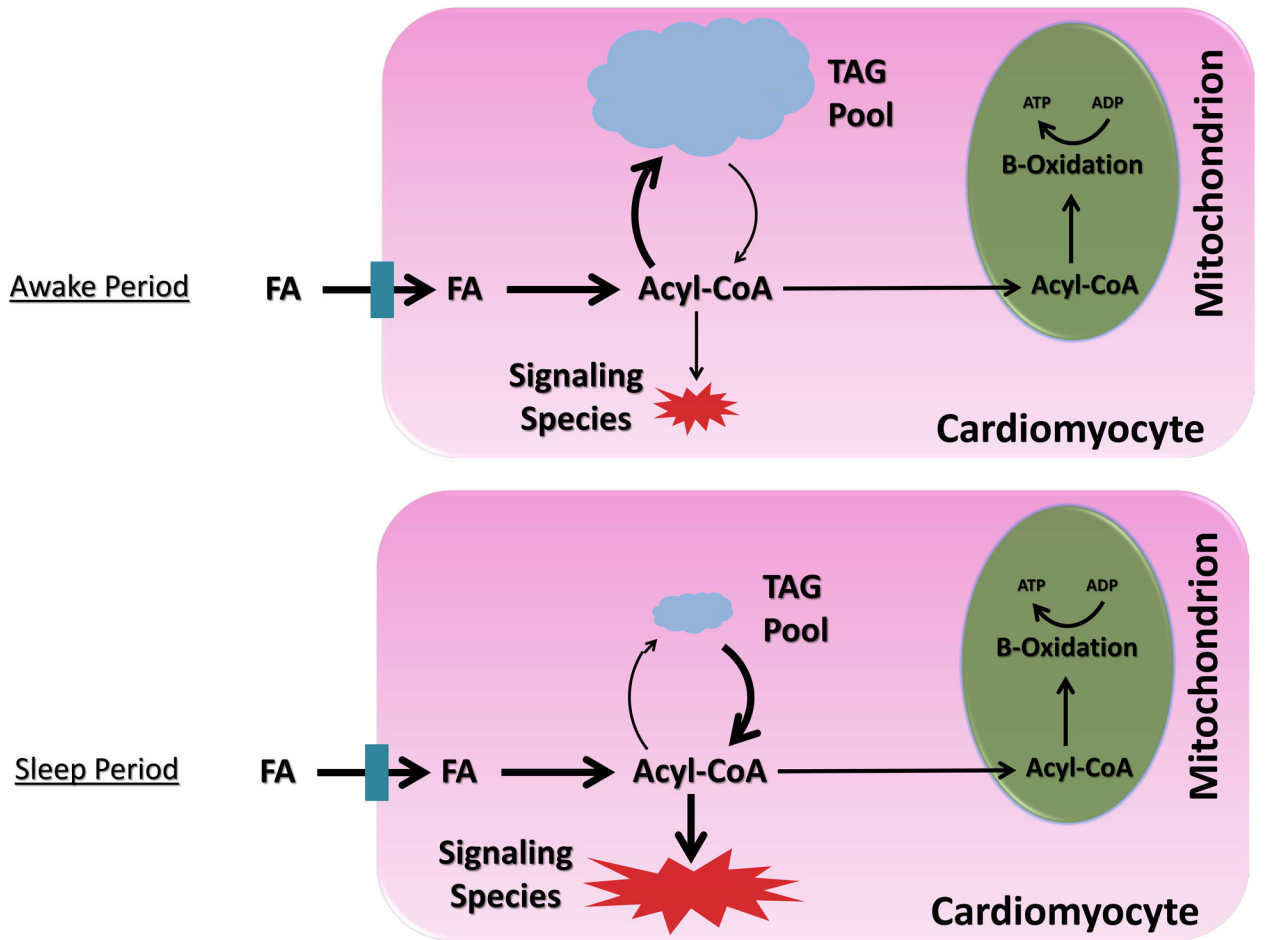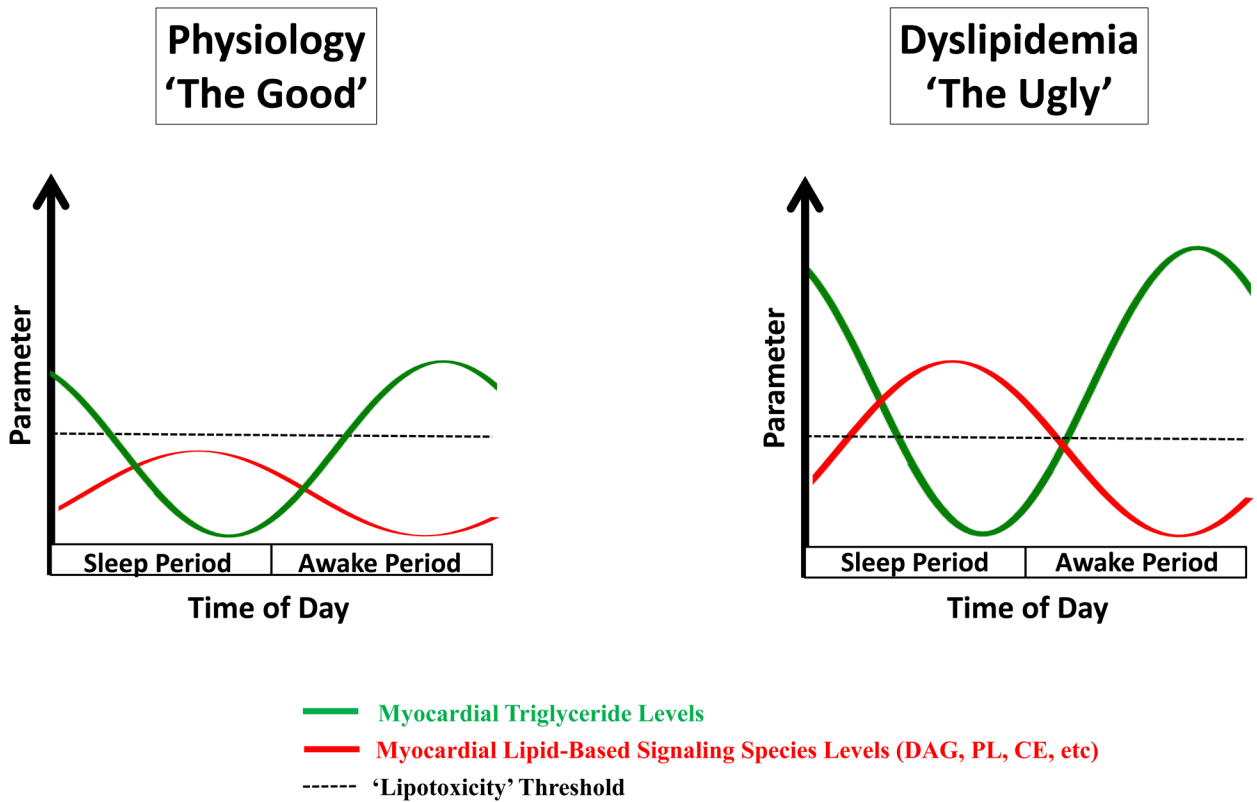
Figure 2.
Evidence-based model for time-of-day fluctuations of myocardial fatty acid metabolism during physiologic (A) and dyslipidemic (B) states. A) Under physiologic conditions, fatty acids (FA) in excess of β-oxidation requirements are utilized cardiomyocyte triglyceride (TAG) synthesis during the awake period, whereas synthesis of FA-derived signaling molecules (e.g., diacylglycerol [DAG], cholesterol esters [CE], phospholipids [PL]) is low at this time; during the sleep period, rates of lipolysis increase, releasing FA from the TAG pool, which are utilized for both β-oxidation and signaling molecule synthesis. B) Under conditions of FA excess (i.e., dyslipidemia), there is a dramatic expansion of the TAG pool during the awake period (due to clock control of TAG synthesis); during the sleep period, lipolysis of the expanded TAG pool occurs, resulting in excessive synthesis of FA-derived signaling molecules, and subsequent cellular dysfunction (i.e., 'lipotoxicity'). Illustration depicting 24-hr fluctuations in myocardial levels TAG and lipid-based signaling species during physiologic and dyslipidemic states (C).
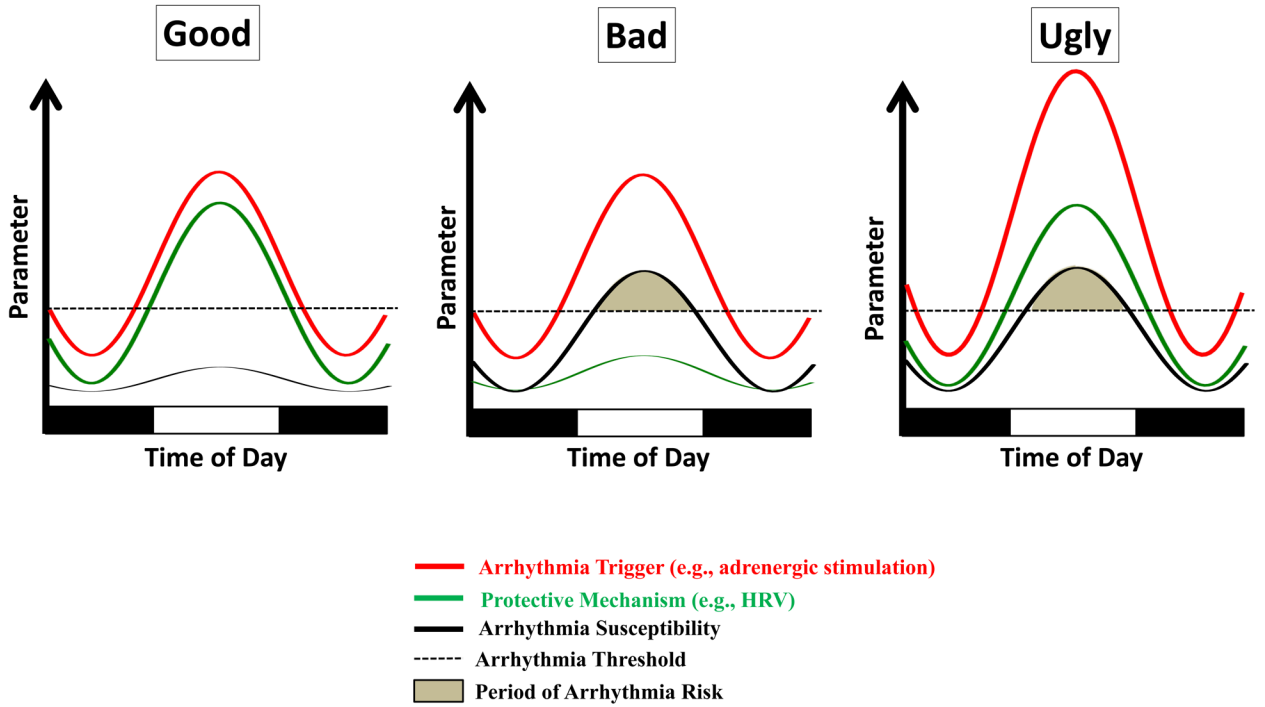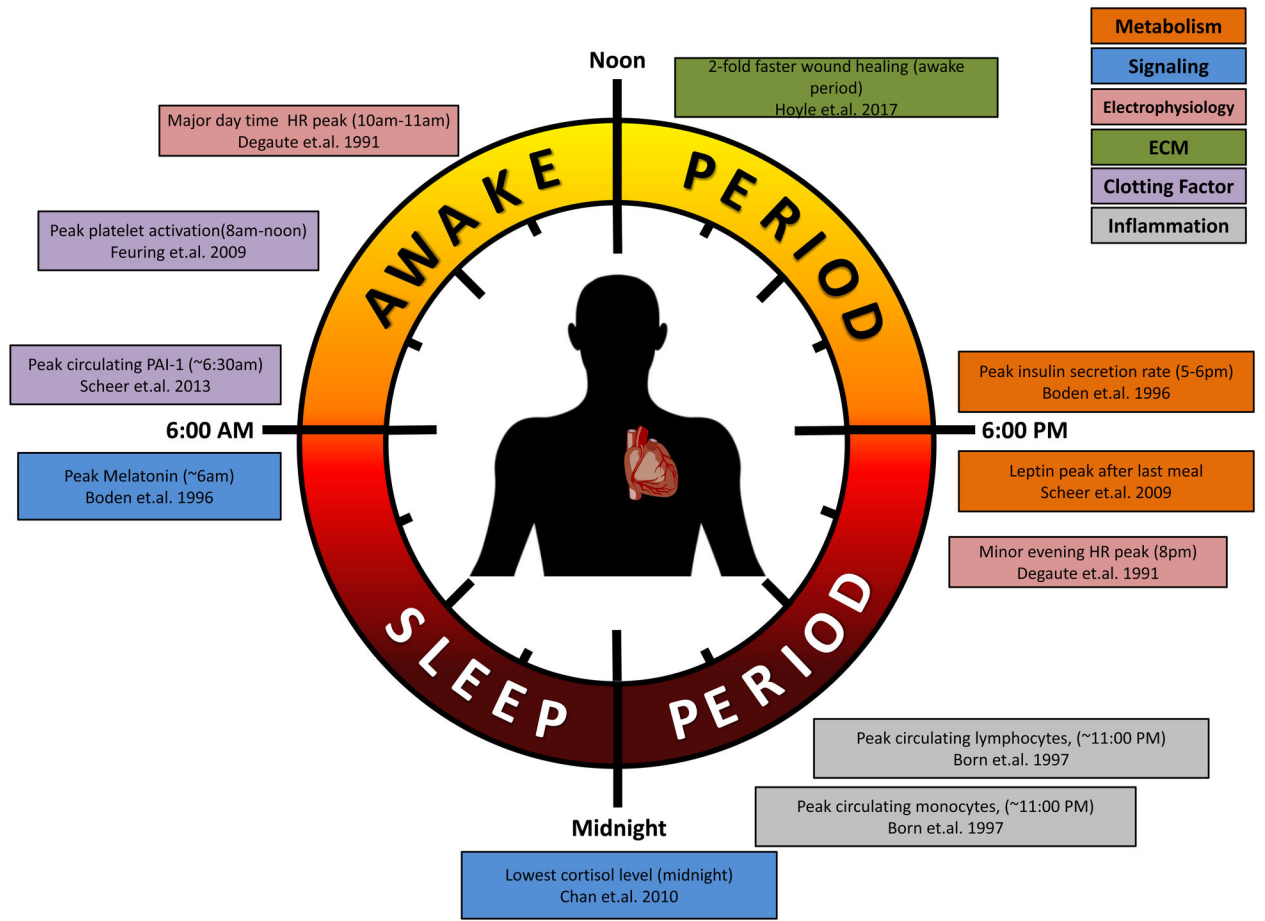
Figure 3.
Hypothetical circadian influences on arrhythmia risk. During physiologic conditions, oscillations in factors that have the potential to trigger arrhythmias (e.g., adrenergic stimulation) are paralleled with oscillations in protective mechanisms (e.g., heart rate variability; HRV), such that arrhythmia susceptibility is low (the 'good'). An attenuation in protective mechanisms (e.g., decreased HRV following myocardial infarction) increases arrhythmia susceptibility above the threshold when arrhythmia triggers are present (the 'bad'). Augmentation of arrhythmia trigger oscillations also have the potential to increase arrhythmia susceptibility above the threshold (the 'ugly').
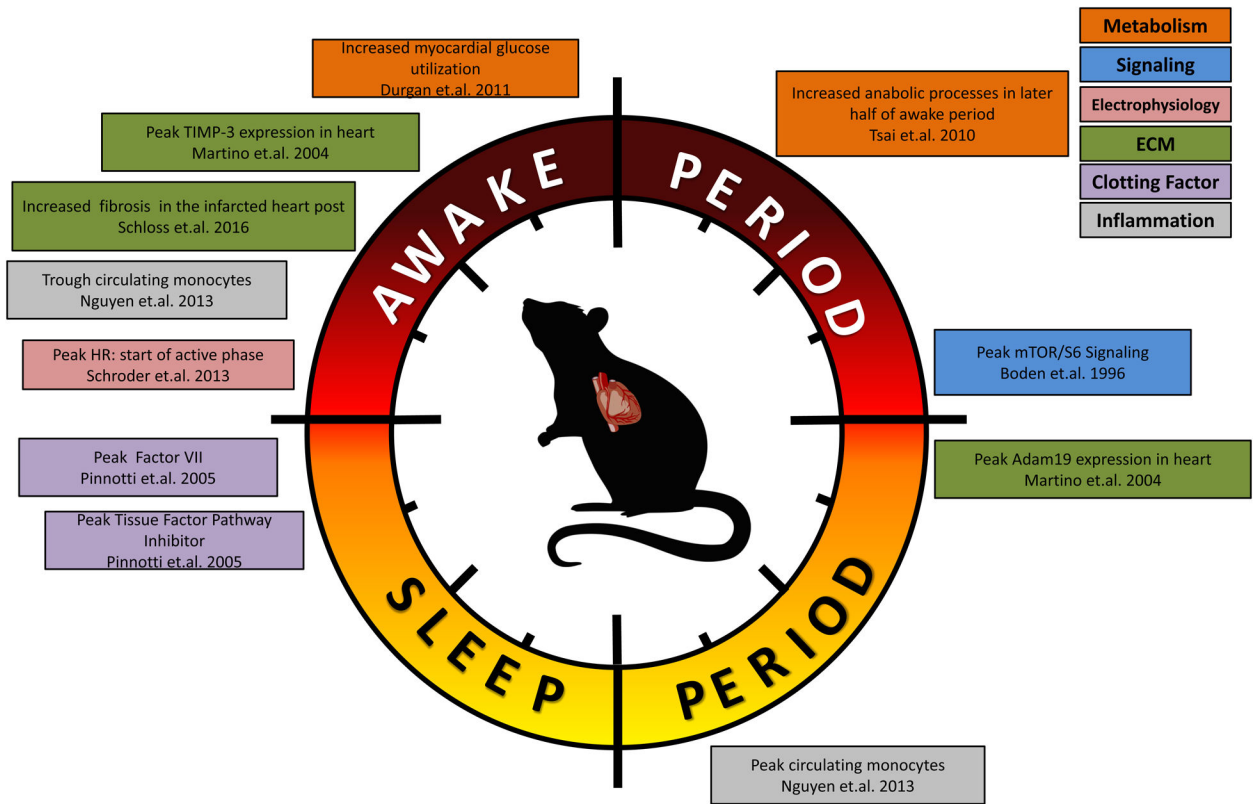
Figure 4.
Summary of circadian influences on cardiovascular-relevant parameters in humans (A) and rodents (B).