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REVIEWS

Review

Impact of Circadian Disruption on Cardiovascular Function and Disease

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The circadian system, that is ubiquitous across species, generates ~24 h rhythms in virtually all biological processes, and allows them to anticipate and adapt to the 24 h day/night cycle, thus ensuring optimal physiological function. Epidemiological studies show time-of-day variations in adverse cardiovascular (CV) events, and controlled laboratory studies demonstrate a circadian influence on key markers of CV function and risk. Furthermore, circadian misalignment, that is typically experienced by shift workers as well as by individuals who experience late eating, (social) jet lag, or circadian rhythm sleep–wake disturbances, increases CV risk factors. Therefore, understanding the mechanisms by which the circadian system regulates CV function, and which of these are affected by circadian disruption, may help to develop intervention strategies to mitigate CV risk.

Highlights

Adverse CV events, including myocardial infarction, arrhythmias, and stroke, show time-of-day variations. Underlying factors may include circadian system control over a plethora of markers associated with CV function.

Our modern lifestyle, which includes shiftwork, jet lag, and disturbed sleep, has been associated with increased CV risk.

Misalignment of the endogenous circadian timing system and behavioral/environmental cycles can adversely impact on CV function in both animal models and human studies. These mechanistic insights may help to explain why some aspects of our modern lifestyle can increase CV risk.

Circadian disruption may play a role in the onset and development of CV disease, and treatments aimed at mitigating circadian disruption may diminish CV risk.

Why Is Time of Day Important for CV Risk?

Adverse CV events, including myocardial infarction, stroke, and ventricular arrhythmias, are worldwide leading causes of death [1]. Epidemiological evidence supports a heightened risk of these adverse CV events during the 'vulnerable morning hours' (6:00 am–12:00 pm) [2,3]. Importantly, these time-of-day variations are not fully explained by daily rhythms in behavioral triggers such as physical activity [4], fasting/feeding, and sleep schedules. They may instead be due to the influence of the circadian system, as well as its interaction with behavioral triggers, on specific CV risk factors. These daily rhythms in acute events are superimposed on the slower rise in overall CV risk during the development and progression of CV disease. Such long-term CV risk is associated with modifiable risk factors such as smoking, unhealthy diet, sedentarism, and excessive alcohol consumption [5]. Beyond these established risk factors, growing epidemiological evidence indicates that **shiftwork** (see Glossary) is an important risk factor for CV diseases including hypertension, ischemic stroke, coronary heart disease, and sudden cardiac death [6–11]. Similarly, social jet lag ('jet lag' between weekdays and weekends [12]) may be associated with an increased CV risk, as indicated by decreased high-density lipoprotein-cholesterol levels, higher triglyceride levels, and decreased insulin sensitivity [13]. Chronotype (a measure of preferred timing of sleep and wake-related activity) may be linked to CV disease mortality, with evening types being at a higher risk [14]. Even on a shorter timescale, the transition to daylight-saving time is associated with incidence of myocardial infarction and ischemic stroke hospitalizations [15]. Common to these conditions is the disruption of circadian alignment with behavioral/environmental rhythms (Figure 1), which means that the endogenous circadian timing system loses its optimal alignment with behavioral/environmental cycles (i.e., sleep/wake, light/dark, fasting/feeding). Collectively, these findings have raised the hypothesis that this **circadian misalignment** may increase CV risk. Indeed, growing evidence from animal models [16–19] and experimental human studies [20–24] now provides physiological mechanistic insights into the **circadian disruption**/misalignment effects on CV risk, and suggests that mitigating circadian disruption may benefit vulnerable populations such as shift workers. In this review we present the current state of knowledge regarding the role of the circadian system in CV function, potential physiological mechanisms underlying the association between shiftwork and CV risk factors, implications for CV disease, and novel targeted cardioprotective treatments. This review focuses primarily on human studies, supplemented by data from animal models.

Circadian Rhythms in CV Risk

In humans, there are **day/night rhythms** in key aspects of CV function, including blood pressure (BP; higher levels during the day and lower levels at night [25]), and **platelet aggregability** (higher levels in the morning [26,27]). Epidemiologic studies report a morning peak in adverse CV events

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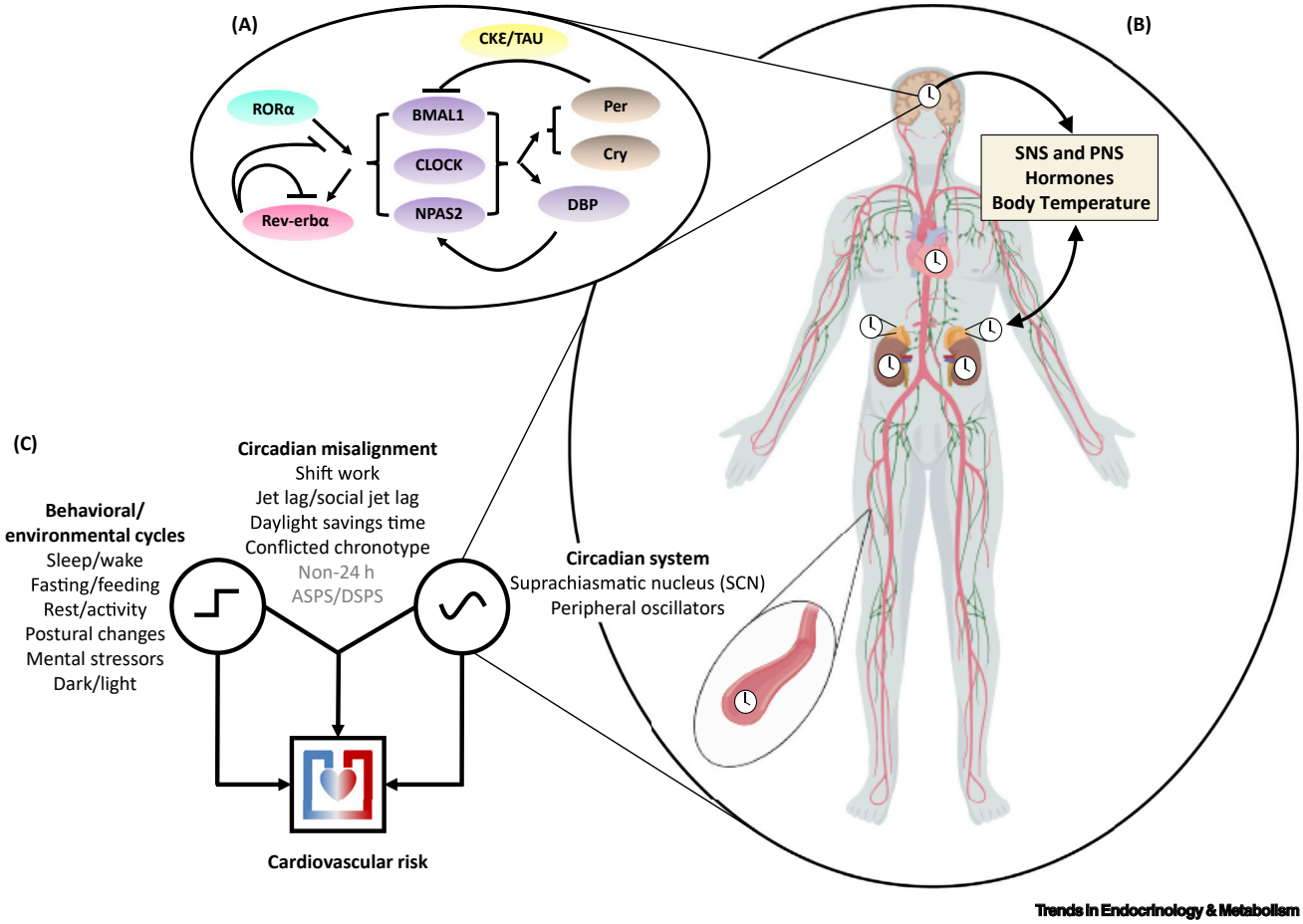

Figure 1. Conceptual Framework of Circadian Misalignment Effects on Cardiovascular (CV) Risk.

(A) Circadian rhythms are driven by molecular clocks involving core clock genes such as *BMAL1*, *CLOCK*, and *NPAS2* that regulate rhythms of their own transcription/expression via negative (and positive) feedback loops (top left inset for schematic; [70] for review). (B) These molecular clocks are present in virtually every cell in the body, including cardiac/vascular tissues, adrenal glands, kidneys, the immune system, and, importantly, the 'master clock' in the suprachiasmatic nucleus (SCN). The SCN clock can affect CV function independently of peripheral clocks (e.g., via autonomic nervous system outflow/endocrine outflow) and by synchronizing peripheral clocks (e.g., via circadian rhythms in body temperature/endocrine outflow). Peripheral circadian clocks provide feedback to each other and the SCN clock via their modulatory effects on CV and endocrine physiology. Endogenous circadian rhythms also influence daily patterns in behavior/exposure to environmental factors, which in turn also impact CV physiology. (C) The circadian system can influence CV function. Disruptions to the circadian system can impact CV risk without necessarily disrupting behavioral/environmental cycles (e.g., via tissue-specific circadian clock-gene mutations). Behavioral/environmental cycles can impact CV risk even when these rhythms are entrained/aligned to the endogenous circadian cycle (e.g., via disrupted sleep in individuals who maintain regular schedules). However, in the most common/epidemiologically relevant scenarios, behavioral/environmental cycles become misaligned relative to the endogenous circadian cycle, and it is this circadian misalignment that impacts CV risk. Known risk contributors are shown in black, putative contributors in grey. Abbreviations and definitions: ASPS/DSPS, advanced sleep phase syndrome/delayed sleep phase syndrome; non-24 h, any scenario with individuals living on non-24 h schedules; PNS, parasympathetic nervous system; SNS, sympathetic nervous system.

[2,28]. However, such data collected during regular sleep/wake cycles cannot determine whether this temporal pattern is caused by the circadian system or by behavioral and environmental factors. **Circadian rhythms** correspond to biological processes that display endogenous, entrainable oscillations of ~24 h that continue even in the absence of external time cues [29]. The distinction between day/night rhythms and circadian rhythms is of crucial mechanistic and clinical relevance for several reasons. One is that understanding the mechanisms will be necessary to develop targeted preventative and therapeutic strategies to adequately address time-of-day effects in CV

disease. Furthermore, distinguishing the limits of 'clock time' versus circadian time is important because of the large inter-individual variability in sleep/wake timing [30] and in the **phase angle of entrainment** (each with a range ~5 h, adding up to ~10 h) [90]. When considering individuals with comorbidities, medication use, circadian sleep/wake disturbances, extreme chronotypes, and/or shiftwork exposure, these differences are likely to be much larger. Collectively, these findings suggest that differences between clock time and circadian time differ greatly across individuals, and thus day/night rhythms based on clock time cannot be generalized across the population. Therefore, there is substantial benefit in determining the endogenous circadian phase for disease processes and the risk factors that are under circadian influence. In view of well-validated assessments of the phase of the central circadian pacemaker by **dim light melatonin onset** (DMLO) [31], as well as the advent of biomarkers for the assessment of circadian phase from one or two blood samples [32], these assessments are expected to be within the reach of clinical implementation in the future.

To adequately assess the role of the circadian system in CV function, stringently controlled circadian protocols such as constant routine (CR) or forced desynchrony (FD) protocols are needed to determine the effects of the circadian system, both independent from and interacting with behavioral and environmental factors. The CR protocol is a study paradigm whereby participants remain awake under dim light (typically <5 lux to minimize light effects on the circadian system), under semirecumbent posture, and with isocaloric, evenly spaced meals, all for more than 24 h [31]. The FD protocol is designed to uncouple circadian cycles from daily behavioral/environmental rhythms, which enables the assessment of the circadian effects, exactly as for the CR, but also allows assessment of their interaction with behaviors. This uncoupling is achieved by maintaining participants on a non-24 h behavioral sleep/wake cycle (often 20 h or 28 h cycles) in dim light, thus out of the range of entrainment of the human circadian system. Therefore, the circadian system 'runs free' at its inherent rate of ~24 h, which allows scheduled behaviors to be uniformly distributed across the circadian cycle. Using these carefully controlled paradigms, circadian rhythms have been observed in BP [21,33], heart rate (HR) [34], heartbeat dynamics [35], circulating **epinephrine** and **norepinephrine** [21,36], cardiac vagal modulation [37], platelet aggregability [38], **plasminogen activator inhibitor 1** (PAI-1) [39], and immune responses [40]. Collectively, the circadian system influences CV risk factors, with possible contributions to the morning peak of CV events (Figure 2; Box 1 overviews the molecular mechanisms underlying the effects of the central circadian clock and peripheral clocks on CV function).

Behaviors (i.e., physical activity, posture, mental stress) impact on CV function both directly and by interacting with the circadian system. One FD protocol where participants underwent standardized tilt-table tests (passive head-up tilt) to assess CV responses to an orthostatic challenge across all circadian phases [37] revealed a circadian rhythm in the risk of **presyncopal events**, with a robust peak during the biological night. Furthermore, the circadian system modulates the reactivity of numerous CV risk markers to standardized exercise, as tested across all circadian phases during a FD protocol [21], with peaks in catecholamine reactivity in the endogenous circadian phase typically equivalent to ~9:00 am, and a maximum decrease in cardiac vagal modulation at ~9:00 am. The combination of the maximum increase in catecholamine reactivity and the maximum decrease in cardiac vagal modulation simultaneously at a circadian phase equivalent to 9:00 am may contribute to the increased risk of serious CV events, as observed in epidemiological studies. Recently, using a FD protocol, we showed additive effects of mental stress and the circadian system on hemodynamic function, **sympathovagal balance**, epinephrine, and cardiac vagal modulation [36]. A key environmental factor that impacts on CV function is light exposure. Light is the most important input to the suprachiasmatic nucleus (SCN) and has robust effects on the circadian system. Moreover, the acute effects of light on CV function potentially occur through mechanisms independent from phase shifting of the circadian system (reviewed in [41]). Light exposure shows maximal effects on HR, preejection period, and relative sympathetic tone during the night and morning hours [41]. Collectively, the translational value of studies assessing the interaction of circadian and behavioral/environmental effects on CV function is in identifying the time of day when increased CV risk is associated with common behaviors.

Glossary

Circadian disruption: disruption of endogenous circadian rhythms. This can occur from the level of the molecular clock mutations to misalignment between the circadian system with behavioral and/or environmental cycles.
Circadian misalignment: misalignment between the endogenous circadian timing system and behavioral/environmental cycles (i.e., sleep/wake, light/dark, fasting/feeding), or between components of the circadian system.
Circadian rhythm: a biological process with an endogenous, entrainable oscillation of ~24 h that persists under constant environmental and behavioral conditions. Circadian rhythms can be synchronized to the environmental cycle by the light/dark (LD) cycle.
Day/night rhythms: a rhythm in physiology or behavior over the 24 h LD cycle. When environmental and behavioral changes are present (e.g., LD cycle), it is virtually impossible to tease apart whether day/night rhythms are endogenously generated or whether they are a consequence of behavioral/environmental changes.
Dim light melatonin onset (DLMO): the onset of melatonin secretion when individuals are exposed to dim light (typically <5 lux). DLMO is useful for determining whether an individual is entrained (synchronized) to a 24 h LD cycle, and for assessing the phase angle of entrainment (see definition below) in entrained individuals.
Epinephrine: a hormone, also known as adrenaline, that is secreted by the adrenal medulla upon stimulation by the central nervous system in response to stress, that is under strong circadian control, and acts to increase heart rate (HR), blood pressure (BP), cardiac output, and carbohydrate metabolism.
Heart-rate (HR) variability: the variation in the time interval between heartbeats.
LF/HF power ratio: the ratio of low-frequency (LF) to high-frequency (HF) power, which allows an approximation of the ratio between sympathetic nervous system (SNS) and parasympathetic

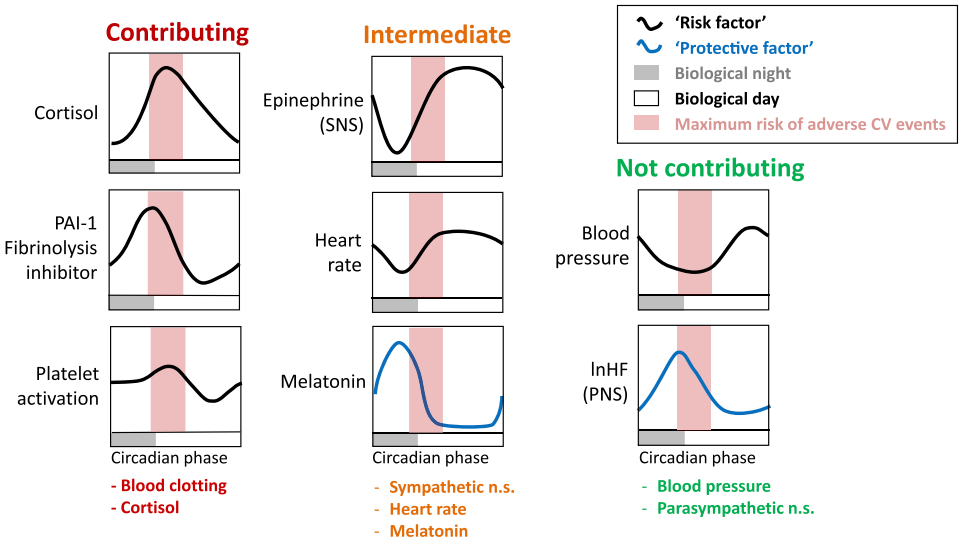

Figure 2. Circadian System Influences Cardiovascular (CV) Risk Factors: Possible Contribution to the Morning Peak of CV Events.

Displayed here are CV risk factors shown to be under endogenous circadian control. They are grouped according to the temporal alignment with the window of increased CV vulnerability in the morning hours. Risk factors (black lines) are those whose elevation may be associated with increased acute risk of adverse CV events, whereas an elevation of protective factors (blue lines) is considered to decrease the acute risk of adverse CV events. Risk factors are considered to be contributing if the peak in their circadian rhythm occurs during the vulnerable window (biological morning), intermediate if they rise during the vulnerable window, and not contributing if they are at their minimum during the vulnerable window. Protective factors are considered to be not contributing if the peak in their circadian rhythm occurs during the vulnerable window (biological morning), and intermediate if they fall during the vulnerable window. Abbreviations and definitions: epinephrine (SNS), epinephrine is used here as a marker of SNS activation; lnHF (PNS), the natural log of high-frequency power in the electrocardiogram, used here as a marker of PNS activation/cardiac vagal modulation; n.s., nervous system; PAI-1, plasminogen activating factor 1; PNS, parasympathetic nervous system; SNS, sympathetic nervous system. This a qualitative summary of quantitative data collected during circadian unmasking (forced desynchrony) experiments in human participants [21,33,37–39]. These studies suggest that rhythms of the hemostatic (blood clotting) system and of cortisol contribute to the morning peak of adverse CV events, that rhythms in blood pressure and lnHF (PNS) do not contribute, and that circadian rhythms in melatonin, heart rate, and epinephrine (SNS) may contribute to an intermediate extent.

Impact of Circadian Misalignment on CV Risk

If circadian rhythms contribute to CV health [42], then circadian disruption/misalignment may influence CV risk. Circadian misalignment effects on circadian factors and CV risk factors in humans (Figure 3) can be determined using controlled in-laboratory studies, including FD and simulated shiftwork protocols [43]. In a FD protocol, where participants lived for seven 28 h 'days' under dim light [20], waketime BP was higher during circadian misalignment. These misalignment-induced effects on BP did not seem to be explained by changes in waketime HR (unchanged), cardiac vagal modulation (unchanged), or sympathoadrenal measures (urinary epinephrine decreased and norepinephrine unchanged). The effects on epinephrine were likely due to the inhibitory role of the circadian system on epinephrine during the biological night (when participants were awake because they were misaligned by 12 h) [21]. Moreover, circadian misalignment did not impact on the 24 h average of plasma cortisol. Although FD protocols provide experimental evidence for the adverse effects of circadian misalignment on CV function, their translational value may be limited because shift workers do not live on 28 h days in dim light. Therefore, carefully controlled laboratory shiftwork simulation protocols provide a closer approximation to what shift workers typically experience, while still controlling for behavioral/environmental factors. Using a simulated shiftwork protocol, healthy non-shift workers underwent

nervous system (PNS) activity, although the validity of the measure depends on the conditions.
Norepinephrine: a hormone, also known as noradrenaline, that is secreted by the adrenal medulla. Norepinephrine release is lowest during sleep, rises during wakefulness, with higher levels during situations of stress or danger.
Phase angle of entrainment: the relationship between the timing of two entrained oscillations. In chronobiology, this is often used to describe the relative time difference between the central circadian clock (often estimated in humans by DMLO) and the timing of an external time cue (e.g., light) or behavior (e.g., bedtime).
Plasminogen activator inhibitor 1 (PAI-1): the primary inhibitor of tissue plasminogen activator, and thereby an inhibitor of fibrinolysis (breakdown of blood clots). PAI-1 is associated with increased risk of developing occlusive thrombi (blood clots in the vascular system).
Platelet aggregability: the likelihood or ability of blood platelets to clump together as part of the sequence of events leading to the formation of a thrombus.
Presyncopal events: a symptom or prodrome to the sudden and transient loss of consciousness owing to transient global cerebral hypoperfusion which is associated with hypotension and/or bradycardia.
Shiftwork: a work schedule that differs from the traditional 9:00 am–5:00 pm day. It can involve evening or night shifts, early morning shifts, or rotating shifts.
Sympathovagal balance: reflects the autonomic state resulting from the sympathetic and parasympathetic influences.

Box 1. Effects of Molecular Clocks and Their Mutations on CV Physiology and Risk Factors

The SCN clock modulates CV function through two mechanisms: (i) directly via the autonomic nervous system [78,79] and endocrine outflow (e.g., glucocorticoid rhythms) that act primarily on the cardiac muscle/blood vessels via receptors on the myocardium/vascular endothelium [80], and (ii) indirectly via synchronization of relevant peripheral clocks, including robust local clocks in cardiac tissues [81]. In rats, cardiomyocytes in culture show rhythmic clock gene expression, including *Bmal1*, *Nr1d1* (Rev-Erbα), *Per2*, and *Dbp* [74]. *In vivo* studies of the cardiac transcriptome indicate that ~10% of transcribed genes are regulated by the cardiomyocyte circadian clock [57]. Furthermore, *in vivo* and *in vitro* studies show rhythmic oscillations in circadian clock gene transcripts (e.g., mRNA transcripts for *Per2*, *Bmal1*, and *Dbp*) in mouse aorta/vascular tissue [50,82,83]. Importantly, cardiomyocyte-specific *Bmal1* knockout mice exhibit disrupted systolic function (fractional shortening and ejection fraction) with increased age, and ultimately, decreased lifespan [57].

In mice, cardiac ion-channel expression and QT-interval duration (an index of myocardial repolarization) show circadian rhythms, and both are under the control of a clock-dependent oscillator that is responsible for rhythmic expression of the transient outward potassium current [71]. The circadian molecular machinery, that is present in the vascular system including endothelial cells, plays a central role in vascular function [84], and potentially in vascular dysfunction, including blood clotting. Because the kidney is central to maintaining fluid and ion homeostasis, circadian rhythms in kidney tissue may help to explain circadian rhythms in BP [85]. Mice lacking functional *Per1* and *Per2* genes show dramatic alteration to normal rhythms in mRNA for renal αENaC (α subunit of the epithelial sodium channel, linked to renal absorption of sodium). Moreover, mice lacking the circadian *Cry1* and *Cry2* genes show salt-sensitive hypertension that is potentially driven by high aldosterone synthesis [76]. Aldosterone (and corticosterone) also modulate the levels of *Cry1*, *Per1*, *Per2*, and *Nr1d1* gene expression in the heart over the 24 h day [86].

The circadian timing system may also impact CV risk through modulation of immune function [87,88] or potentially via Rev-ErbA-sensitive cholesterol pathways and bile acid regulation [89]. Virtually all immune system measures show rhythmic oscillations, and the circadian system may act on inflammatory responses by restraining the expression of inflammatory genes to specific times within the circadian cycle [88].

circadian alignment ('dayshift work') or circadian misalignment ('nightshift work') for 3 days [22]. Consistent with the FD results, circadian misalignment increased waketime BP, as well as 24 h average systolic and diastolic BP, and dampened the healthy sleep-associated systolic BP dipping effect linked to increased CV risk and mortality [44]. Moreover, circadian misalignment decreased waketime cardiac vagal modulation [26], where vagal (parasympathetic) activity is typically considered to be cardioprotective [45]. By contrast, 24 h average urinary epinephrine excretion rate decreased, whereas no effects were observed for norepinephrine, suggesting that parasympathetic changes rather than sympathetic changes may contribute to increased CV risk. However, the specific roles of the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS) in CV risk under circadian misalignment cannot be fully teased apart because the techniques used under circadian misalignment conditions are typically derived from non-invasive measures (i.e., electrocardiogram-based HR and **HR variability**, urinary catecholamine levels). The techniques that best mirror SNS and PNS activity are invasive and are not often utilized in clinical research (i.e., microneurography, norepinephrine spillover). Therefore, the specificity and reproducibility in capturing the heterogeneous and often regionalized responses of SNS and PNS activity under circadian misalignment conditions should be viewed with caution. Furthermore, circadian misalignment has been shown to increase serum levels of inflammatory markers [including interleukin-6 (IL-6), high-sensitivity C-reactive protein (hsCRP), resistin, and tumor necrosis factor α (TNF-α)] in this study. A limitation of this study is that non-shift workers were studied, raising the question of whether these effects of circadian misalignment translate to shift workers. When the latter were exposed to two 3 day laboratory protocols under circadian alignment or misalignment, profound misalignment effects were observed for markers of CV risk [23]. Circadian misalignment in these real-life shift workers also increased hsCRP, a marker of systemic inflammation. Furthermore, waketime systolic BP increased, and diastolic BP increased, predominantly during sleep. These findings show that shift workers may not be necessarily resilient against the adverse CV effects of circadian misalignment.

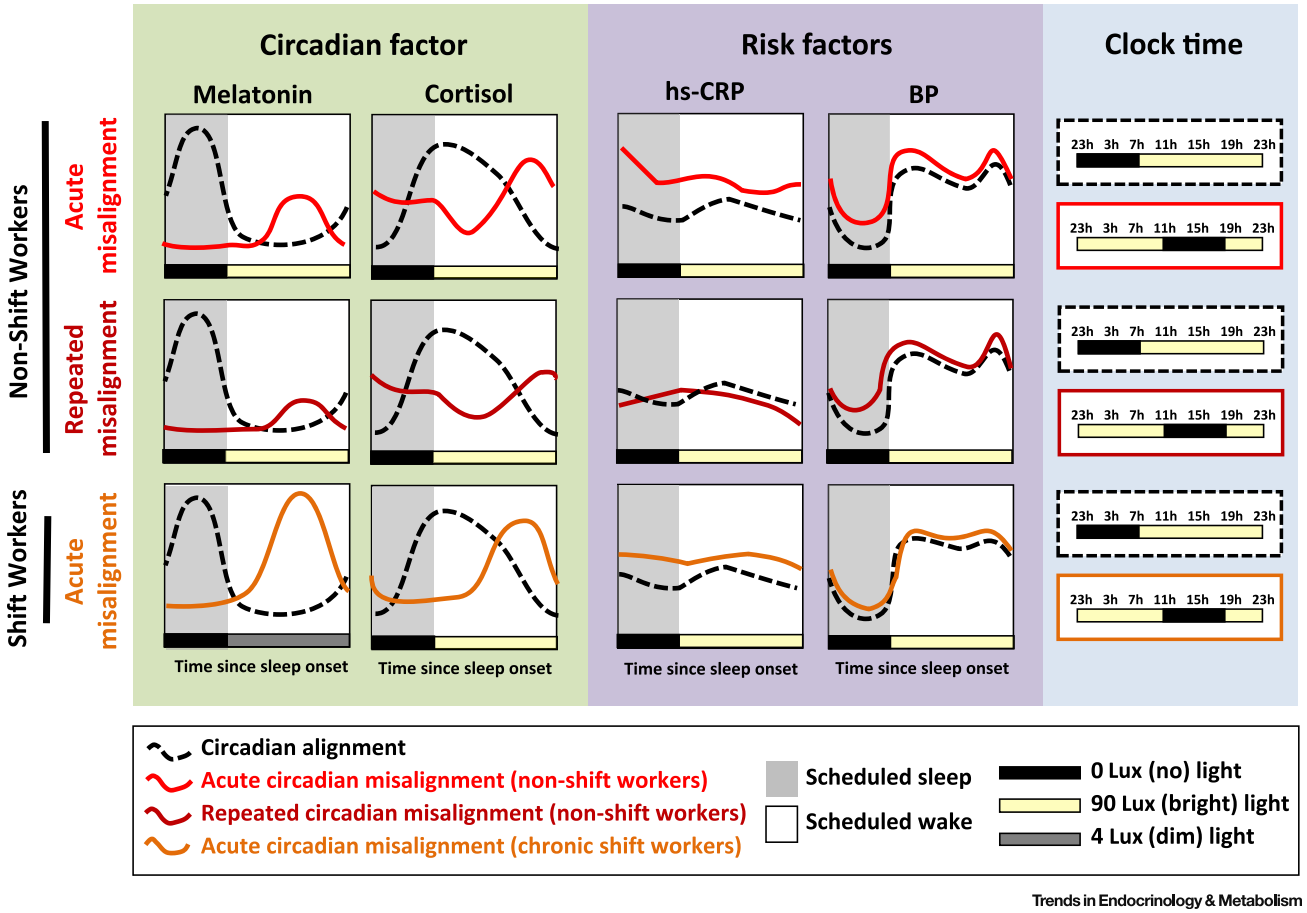

Figure 3. Effects of Circadian Misalignment on Circadian and Cardiovascular (CV) Risk Markers.
The figure shows a visual, qualitative summary of quantitative data collected during laboratory circadian misalignment (nightshift work simulation) experiments in human participants [22,23] (blue panel on the right for clarification of the inverted light/dark schedule, and note that all other panels are plotted by elapsed time since scheduled lights-off). In the green box (left) we show the effect of acute/repeated circadian misalignment on two classic phase markers of the central circadian pacemaker: melatonin and cortisol. In addition to dampened amplitude of cortisol/melatonin (in part owing the ~90 lux light exposure), acute and short-term repeated misalignment leads to these two phase markers being ~12 h temporally offset relative to the sleep/wake cycle as a result of the inertia of the circadian system. In the purple box (middle) we show the effects of acute/recurrent circadian misalignment on two markers of CV risk: high-sensitivity C-reactive protein (hsCRP) and blood pressure (BP). Daily rhythms in these two profiles are primarily driven by behavior (sleep/wake); thus, unlike melatonin and cortisol, their rhythms are not offset by 12 h during misalignment. However, during misalignment hsCRP and BP are elevated (in both non-shift workers and shift workers). These observations may help to elucidate the underlying mechanisms by which shiftwork (or other forms of recurrent circadian misalignment) increases CV risk.

Circadian misalignment causes disrupted sleep because sleeping during the biological day and being awake at night is at odds with the endogenous circadian system [46]. Disrupted sleep is associated with increased risk of CV disease, including atherosclerosis [47], and sleep disruption may change HR and BP, ANS function, and fluid homeostasis (reviewed in [48]). Therefore, nightshift workers, who typically experience the effects of circadian misalignment, may also be exposed to the effects of sleep disruption. Laboratory studies that induced circadian misalignment and further experimentally restricted their sleep [24,49] indicate that their combination increased 24 h urinary norepinephrine. During scheduled wake, sleep restriction increased HR following both circadian alignment and misalignment conditions. Similarly, during scheduled sleep, preceding circadian misalignment increased HR and **LF/HF power ratio** (a measure of sympathovagal balance), and reduced cardiac vagal modulation, although no changes were observed for BP [24]. Circadian misalignment combined

with sleep restriction [49] has also been shown to increase the levels of inflammatory markers relative to circadian alignment and sleep restriction. Consistently, evidence from circadian misalignment studies show that either there were no correlations between sleep polysonmography and/or actigraphy-determined total sleep duration and cardiovascular outcome measures, or if there were, that the effects of circadian misalignment were sustained after adjusting for total sleep duration [22,23]. Collectively, these findings demonstrate that changes in sleep cannot fully explain the changes observed with circadian misalignment, and that circadian misalignment has effects above and beyond those of sleep disruption.

Molecular Mechanisms of Circadian Misalignment Effects on CV Risk

Animal experimental paradigms to test how circadian disruption adversely affects CV function include models of light/dark (LD) cycle manipulation, meal timing schedules, genetic mutations with a circadian period phenotype (changing circadian cycle length of the whole animal or a specific tissue), circadian rhythm ablation mutations (complete molecular knockout of the clock in a whole animal or tissue), and SCN lesion studies [50]. In a LD cycle manipulation model, mice were randomized to conditions with either a 20 h cycle (LD cycle of 10:10h) or a typical 24 h cycle (LD cycle of 12:12h) to induce circadian disruption [51]. Accordingly, only mice under a 20 h cycle (circadian disruption) had decreased levels of cardiomyocytes and vascular smooth muscle cell hypertrophy, as well as compensatory reduced expression of key genes in cardiac hypertrophic pathways (*Anf*, *Bnp*, *Ace*, and *Col*). When the mice were subsequently exposed to a typical 24 h rhythm, there was a rescue (reversal/attenuation) of abnormal cardiac pathophysiology [51]. Furthermore, circadian disruption induced by a genetic mutation causing a shortening of the circadian period (22 h) in hamsters kept on a typical 24 h LD cycle resulted in extensive cardiac fibrosis, impaired contractility, renal disease, and early death as a result of cardiomyopathy [16]. Intriguingly, when placed on an LD cycle matching their genotype (i.e., 22 h), their circadian rhythm patterns, as well as cardiac and renal structure and function, were normalized [4]. These observations [16,51] support the notion that circadian synchrony ('resonance' of internal and external cycles) is integral to compensatory hypertrophy and normal remodeling. Moreover, an experimental 'shiftwork' paradigm in mice (under 22.5 h, 24 h, and 27 h LD cycles for 17 weeks) showed that mice synchronized at an adverse circadian phase angle (i.e., misaligned) under 22.5 h and 27 h LD cycles had decreased metabolic efficiency and disrupted cardiac function compared to mice under a 24 h LD cycle [52]. This suggests that 'environmental misalignment' [43] may have adverse CV health consequences even if the animals remain synchronized, but at an abnormal phase angle. As mentioned previously, clear links between low-grade inflammation and CV risk have been shown [53], which raises the question of whether immune function changes may serve as causal mechanisms contributing to CV risk during circadian disruption. In a circadian disruption paradigm with mice exposed to a once-a-week 6 h phase advance of the LD cycle for 4 weeks, immune responses changed dramatically [54]. Endotoxemic shock induced by an immune change [lipopolysaccharide (LPS) from *Escherichia coli]* was magnified, leading to a ~90% mortality rate compared to ~20% mortality in unshifted control mice. These immune responses were likely driven by increased release of proinflammatory cytokines (i.e., IL-1β, IL-12, IL-13, IL-6) in response to LPS challenge in shifted mice. Proinflammatory cytokines mediate immune responses, and increased levels are associated with endothelial dysfunction in patients with coronary artery disease and/or heart failure [55]. Clock genetic mutations in mice models also impact on CV function. Whole-animal deletion or mutation of core clock genes (i.e., *Bmal1*, *Clock*, and *Npas2*) lowered mean arterial pressure and disrupted sympathoadrenal responses (norepinephrine and epinephrine) to stress [56]. Furthermore, cardiomyocyte-specific *Bmal1* knockout attenuated glucose utilization, accelerated dilated cardiomyopathy, and reduceds longevity [57]. Cardiomyocyte-specific *Bmal1* knockout or *Clock* mutation also disrupted gene and protein expression of insulin signaling components in cardiac tissues [18]. Collectively, these mechanistic insights into circadian disruption from animal experimental work, including LD cycle manipulations [51,52,54], circadian period mutations (by causing period dissonance) [4,16], SCN lesioning ([58]), and whole-animal [56] or cardiomyocyte-specific clock gene disruption [18,57,59], highlight the profound effects of circadian disruption on CV and immune function, consistent with human misalignment data.

Clinical Implications of Circadian Misalignment for CV Disease

Human physiological mechanistic work provides translational evidence for the involvement of the circadian system in daily rhythms in adverse CV events [21,33–39], as well as the contribution of circadian disruption (e.g., misalignment) to increasing CV risk factors [20,22–24]. Mechanistic insights from preclinical experimental work have shed light on the existence of circadian clocks throughout the CV system, and that circadian clocks in myocardial tissue become altered in CV disease (Box 1). Based on these insights, the effects of the circadian system and of its disruption on CV risk may have direct clinical implications. In humans, a randomized trial showed that the timing of aortic valve surgery was associated with adverse cardiac events up to 500 days following surgery, with less events and less perioperative cardiac troponin T release with afternoon surgery than with morning surgery [60]. *Ex vivo* analyses of the myocardium [Rev-Erbα (*NR1D1*) gene deletion and antagonism] indicated alterations in circadian gene expression such that nuclear receptor Rev-Erbα was higher in the morning surgery group, which may be connected to the increased CV risk at that time. Importantly, nightshift workers are repeatedly exposed to the effects of circadian misalignment, and may be at higher CV risk owing to the progressive increase in BP over time [23]. Together with epidemiological data that show time-of-day variations in adverse CV events [2,3], the abovementioned studies provide mounting evidence in favor of a role of circadian disruption/misalignment in CV risk (Figure 4).

Although it does not address the question of cause and effect, there is evidence for decreased neurotransmitter content (at both the mRNA and protein levels) in the SCN of hypertensive patients [61] that relate to changes in corticotropin-releasing hormone-expressing neurons in the paraventricular nucleus of the hypothalamus [62]. Initial suggestions of a potential beneficial effect of interventions that can influence circadian function have come from studies using melatonin in clinical populations with increased CV risk [63,64] because melatonin affects the circadian system, although additional or alternative mechanisms such as antioxidant and/or immunomodulatory effects may be involved [65]. Repeated use of 2.5 mg of melatonin for 3 weeks in a randomized clinical trial reduced BP during

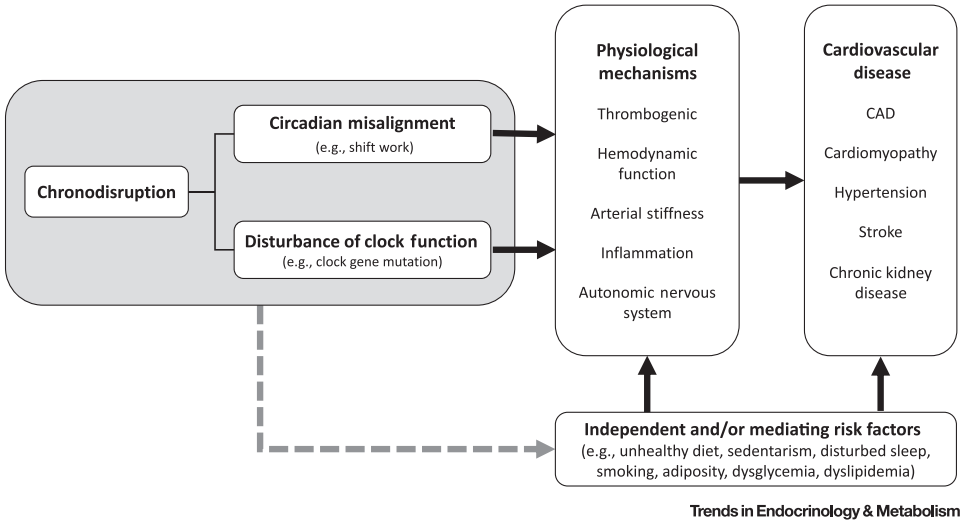

Figure 4. Linking Chronodisruption to Cardiovascular (CV) Disease.
Chronodisruption (left panel) can take the form of circadian misalignment or disturbance of circadian clock function *per se*, either of which can lead to increased CV risk. Chronodisruption leads to physiological changes which are associated with and often precede CV disease (middle panel), including adverse effects on thrombogenic pathways [22,23], hemodynamic function [22,52,57,71,72], arterial stiffness [73], inflammation [22,23,49,54,74], and autonomic nervous system function [24,37,56]. Furthermore, chronodisruption has also been directly linked to many forms of CV disease (right panel), including coronary artery disease (CAD) [75], cardiomyopathy [16,18,51,57], hypertension [76], stroke [15,59], and chronic kidney disease [16,77]. Chronodisruption may also contribute to these physiological changes and/or CV disease via other classes of risk factors (e.g., alterations to behavioral patterns; bottom panel).

sleep, with an increase in the day/night amplitudes of BP rhythms in hypertensive patients [63]. More recently, data on patients with type 2 diabetes (T2D) and essential hypertension who took melatonin (3 mg or 5 mg) in the evening for 4 weeks indicate that ~30% of non-dippers had an improvement in night BP and night average BP dipping, whereas no effects were observed in normal dippers with melatonin or in the control groups without melatonin [64]. Although the data are limited with respect to other CV diseases, there is some evidence that melatonin supplementation (10 mg nightly) for 12 weeks reduced BP and serum hsCRP in patients with T2D and coronary heart disease [66]. Taken together, these studies raise the question of whether normalizing circadian rhythms in patients with CV disease may offer a new approach in risk reduction (Figure 5). Treatment interventions to the morning peak in adverse CV events (Figure 5) could include (i) pharmacological chronotherapy,

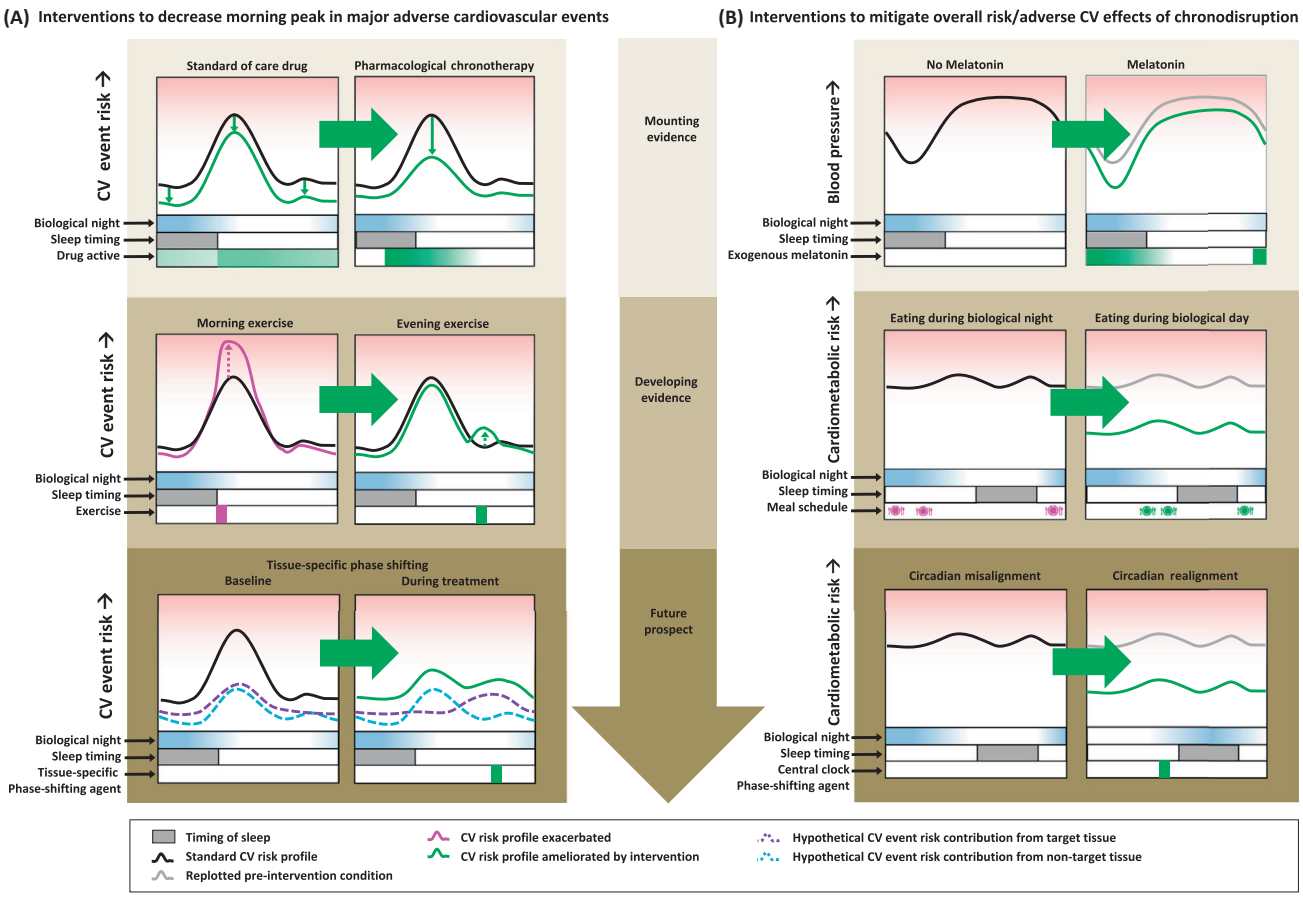

Figure 5. Prospects for Circadian Interventions to Mitigate Cardiovascular (CV) Risk.

(A) Interventions to decrease the morning peak of major adverse CV events. (Top panel) Pharmacological chronotherapy here refers to timing CV medication (via timing of administration and/or delayed/slow-release formulations) aimed at maximizing the dose during the vulnerable time while minimizing the dose at other times to decrease adverse side effects. (Middle panel) Although exercise lowers overall/long-term CV risk, it may carry increased acute risk of an adverse event for patients with CV disease, which could be mitigated by optimal timing of exercise within the biological day. (Bottom panel) There may be a multiplicative effect of CV event risk factors produced by some tissues in the body at a given circadian phase (e.g., the circadian morning peak in PAI-1 and the coinciding peak in platelet activation). If so, tissue-specific phase-shifting of peripheral clocks in one (but not the other) tissue could serve to blunt overall CV event risk during the vulnerable time-window. (B) Interventions to mitigate overall risk/adverse CV effects of chronodisruption. (Top panel) Mounting evidence suggests that optimally timed exogenous melatonin may decrease blood pressure. (Middle panel) During circadian misalignment, evidence is emerging that restricting meals to the biological day may decrease metabolic/glucoregulatory consequences of circadian misalignment. (Bottom panel) A long-term goal of treating circadian misalignment is the development of therapeutics (e.g., small molecules) that accelerate re-entrainment of the central and/or peripheral clocks to shifted behavioral and environmental cycles, for example in shift workers, jet lag, and circadian rhythm sleep/wake disorders.

wherein medication would be targeted before the time of highest CV risk (morning), as in the case of β-blockers and aspirin [67]; (ii) modifying the timing of behavioral triggers to minimize their impact on CV risk (e.g., when to exercise [21]); and (iii) the use of small molecules to impact on clocks to accelerate entrainment in shiftwork, jet lag, and circadian rhythm sleep disturbances including extreme chronotypes [68].

Concluding Remarks and Future Perspectives

Time-of-day patterns in the risk of acute myocardial infarction were first described in 1963 [69]. Since then, numerous studies have reported time-of-day variations of system-level markers of CV function, and in molecular and gene control in the heart, vasculature, and kidneys, which may play a role in the onset and development of CV diseases. Central to these time-of-day fluctuations is the modulatory role of the circadian timing system, as well as its interaction with behavioral/environmental triggers. Epidemiological data show an association between shiftwork and CV disease [6], and controlled experimental human studies indicate that circadian misalignment (typical in night shift workers) adversely affects CV risk factors. These studies strengthen the argument that the circadian system and the deleterious effects of circadian disruption/misalignment need to be considered in the assessment of CV risk. Despite these advances, there are important gaps in our knowledge (see Outstanding Questions). Human studies have focused on measures of system-level CV function, including HR, BP, autonomic nervous system (ANS), cortisol, and measures of fibrinolysis, platelet activation, and cytokines and chemokines, many of which are the typical systemic measures assessed under CV challenges (e.g., exercise tolerance testing). Additional measures to be considered include for example ion channel involvement, as well as cardiac electrical conduction and repolarization, which focus on cardiac electrophysiology and are particularly suited for assessing the mechanisms underlying ventricular arrhythmia. Furthermore, omics approaches (genomic, transcriptomic, proteomic, metabolomic) may help to identify biomarkers of circadian disruption or subset susceptibility associated with CV risk [67], while tissue-specific omics (heart, endothelial function, vasculature smooth muscle, kidneys) may offer in-depth mechanistic insights. Although animal models of circadian disruption provide important insights into clock gene expression in the heart and vasculature, these effects remain to be fully established across cardiometabolic tissues, and their relevance needs to be established in humans. Furthermore, in humans it remains unknown which specific exposures underlie the adverse effects of circadian misalignment. In other words, is it the sleep disruption, and/or the mistiming of food intake, physical activity, posture, and/or the LD cycle? Addressing this question will aid in the identification of which facets of misalignment would impact on CV risk and help to guide preventative and therapeutic interventions. Furthermore, most studies to date have assessed fundamental basic physiological processes in humans, but there have been few (if any) preventative studies on circadian disruption in vulnerable populations. Future studies in humans will be necessary to establish whether potential circadian therapeutic interventions (Figure 5), such as appropriately timed light exposure, meal timing, exercise, sleep strategies, melatonin supplementation, and/or timing of medications, could provide cardioprotective strategies to minimize CV risk in shift workers and populations with CV disease.

Outstanding Questions

Which metabolic, gene, and protein pathways that are disrupted by circadian misalignment contribute to increased CV risk in humans? Systems biology approaches integrating 'omics' techniques, such as transcriptomics, proteomics, metabolomics, and genomics, are currently being applied to sleep loss and more recently to circadian misalignment paradigms, but mechanistic insights into increased CV risk are limited.

How does circadian disruption affect different organ systems associated with cardiometabolic control, and what are their relative contributions to the increased CV risk? Animal models using circadian disruption paradigms have provided key insights into molecular and clock gene expression in heart tissues. However, these effects remain to be fully established across cardiometabolic tissues.

Which behavioral/environmental exposures underlie the adverse consequences of circadian misalignment: is it the sleep disruption, the mistiming of food intake, of physical activity, of posture, or LD cycle? Addressing this question will help to develop targeted behavioral, environmental, and pharmacological approaches to mitigate CV risk associated with circadian misalignment.

How can we mitigate or prevent the adverse consequences of circadian disruption/misalignment in individuals susceptible to CV risk? Can we use individualized recommendations using chronotherapeutic approaches, such as light therapy, time of food intake, sleep strategies (e.g., naps), physical activity, and/or timing of medication intake (i.e., melatonin supplementation, modafinil, caffeine, sleep aids)?

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