Circadian rhythms and the molecular clock in cardiovascular biology and disease

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Abstract | The Earth turns on its axis every 24 h; almost all life on the planet has a mechanism — circadian rhythmicity — to anticipate the daily changes caused by this rotation. The molecular clocks that control circadian rhythms are being revealed as important regulators of physiology and disease. In humans, circadian rhythms have been studied extensively in the cardiovascular system. Many cardiovascular functions, such as endothelial function, thrombus formation, blood pressure and heart rate, are now known to be regulated by the circadian clock. Additionally, the onset of acute myocardial infarction, stroke, arrhythmias and other adverse cardiovascular events show circadian rhythmicity. In this Review, we summarize the role of the circadian clock in all major cardiovascular cell types and organs. Second, we discuss the role of circadian rhythms in cardiovascular physiology and disease. Finally, we postulate how circadian rhythms can serve as a therapeutic target by exploiting or altering molecular time to improve existing therapies and develop novel ones.

Circadian rhythms
Endogenous biorhythms with a period of approximately 24 h; self-sustainable but can be entrained.

Period
Duration of one circadian cycle.

Central or primary clock
Group of neurons in the suprachiasmatic nucleus (part of the hypothalamus) of the brain, which orchestrates rhythmicity within the body.

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In 2017, Jeffrey C. Hall, Michael Rosbash and Michael W. Young received the Nobel Prize in Physiology or Medicine for their discovery of the molecular machinery underpinning the biological clock[1–4] (BOX 1). Their discovery was the start of a period in which the important role of circadian rhythms in physiology and disease was elucidated.

Circadian rhythms are biological rhythms with a period of approximately 24 h that allow organisms to prepare for the daily fluctuations brought on by day–night cycles, aligning internal biological functions with environmental changes. In mammals, circadian rhythms are regulated by circadian clocks. These clocks can be divided into a central or primary clock, comprising around 20,000 neurons located in the suprachiasmatic nucleus of the hypothalamus[5], and peripheral clocks, which can be found in almost every tissue[6]. Light cues, the main clock input signal (synchronizer), are received through the retina and transmitted to the master clock, which consequently synchronizes peripheral clocks throughout the body via various neurohumoral signals[7]. In addition to central clock signalling, peripheral clocks respond to tissue-specific synchronizers (for instance, food intake and exercise)[8–10]. When natural synchronizer input is present, 24-h rhythms are officially referred to as diurnal (or nocturnal) rhythms, and some claim the term circadian should be reserved for processes that persist under constant environmental conditions. In practice, however, almost all diurnal rhythms are found also to be circadian[11]. For the sake of clarity, we use the term circadian in this Review for all 24-h oscillatory processes, regardless of external input.

Circadian rhythms are important regulators of cardiovascular physiology and disease. Peripheral clocks are present in each of the cardiovascular cell types[12–16] (FIG. 1), regulating various (patho)physiological functions, such as endothelial function, blood pressure and heart rate[17,18], as well as the onset of acute myocardial infarction and arrhythmias[19–21]. In this Review, we discuss the role and importance of circadian rhythms in the cardiovascular system and provide an overview of the latest advances in the understanding of circadian cardiovascular physiology. Furthermore, we describe how circadian rhythms are related to cardiovascular disease and elaborate on the importance of maintaining a healthy circadian rhythm. Finally, we discuss chronotherapy and other translational aspects of circadian rhythms, a novel approach to improve existing therapies and develop new ones.

Circadian cardiovascular function

Molecular clock: loops and beyond

Virtually all mammalian cell types have a functional circadian clock[6]. On a molecular level, this clock consists of complex autoregulatory transcription–translation feedback loops whose interplay results in a rhythmic expression of clock-controlled genes, eventually leading to oscillations in proteome and cell function[22]. Although the core clock pathway is preserved among tissues, rhythmic

Key points

- Molecular clocks are found in all cardiovascular cell types.
- Various cardiovascular functions, including endothelial function, thrombus formation, blood pressure and heart rate, are regulated by the circadian clock.
- Disruption of 24-h rhythms leads to cardiovascular disease, including heart failure, myocardial infarction and arrhythmias.
- 24-h rhythms are present in the development, risk factors, incidence and outcome of cardiovascular disease.
- Cardiovascular disease leads to disrupted circadian rhythm and sleep problems.

transcription of clock-controlled genes is tissue-specific[6]. Approximately 10% of genes within each mammalian tissue are regulated by the circadian clock[23,24].

The main feedback loop consists of core clock components: aryl hydrocarbon receptor nuclear translocator-like protein 1 (ARNTL; also known as BMAL1), circadian locomotor output cycles protein kaput (CLOCK), cryptochrome 1 and 2 (CRY1 and CRY2, respectively) and period circadian protein homologue 1, 2 and 3 (PER1, PER2 and PER3, respectively)[25]. Additional components, such as tyrosine-protein kinase transmembrane receptor ROR1 and ROR2 and Rev-erbAα (also known as NR1D1), form secondary feedback loops[26].

At the start of a 24-h cycle, BMAL1 and CLOCK proteins form a complex (heterodimer). BMAL1–CLOCK heterodimers bind to the enhancer box elements (E-boxes) of various clock genes, including *PER* and *CRY*, activating their transcription and translation (positive feedback). PER and CRY accumulate in the cytoplasm, also form a heterodimer and translocate to the nucleus to inhibit the transcriptional activity of BMAL1–CLOCK and, therefore, the transcription of their encoding genes (negative feedback). The whole process results in a negative feedback loop with a period of approximately 24 h (REF.[25]). Core elements of the clock, such as BMAL1 and CLOCK, function not only to keep the clock turning but also to bind to the E-boxes of clock-controlled genes to realize the rhythmic activation of a large part of the genome, consequently leading to 24-h variations in bodily functions.

BMAL1–CLOCK heterodimers also activate ROR and Rev-erbAα. These components compete for the ROR response element (RORE), a binding site located in the promoter region of *BMAL1*. This binding leads to either activation or inhibition of *BMAL1* transcription, depending on the bound protein. ROR1 and ROR2 act as transcriptional activators, whereas Rev-erbAα represses *BMAL1* transcription[27]. This secondary feedback loop is necessary for a rhythmic expression of *BMAL1* (REF.[28]).

Complementary to these feedback loops, circadian rhythms are regulated by various other processes[29]. In brief, on the post-transcriptional and post-translational levels, circadian rhythms are modulated by methylation, polyadenylation, histone modifications and non-coding RNAs, such as antisense RNAs and microRNAs. All these epigenetic and translational processes are necessary for the precision and robustness of the circadian oscillations. Details of their exact role and function are beyond the scope of this Review, but they have been reviewed previously[30].

Peripheral clocks
Molecular mechanism within individual cells that regulates circadian rhythm.

Synchronizer
External or environmental cue (such as light, food intake or exercise) that synchronizes or entrains circadian rhythms; also known as Zeitgeber ('time giver').

24-h rhythms
Patterns re-occurring every 24 h.

Chronotherapy
Scheduling of treatment in relation to 24-h rhythms to increase effectiveness of the therapy and/or reduce adverse effects.

Clock-controlled genes
Genes whose transcription is controlled by the molecular circadian clock.

Core clock genes
Genes that form a basis for generation and regulation of circadian rhythms by encoding BMAL1 , CLOCK, CRY and PER proteins.

Amplitude
Difference between mesor and peak of the sinusoidal-shaped circadian rhythm.

Mesor
Mean value of a (circadian) cycle or rhythm.

Development of the cardiac clock

The developing circadian system can be observed through in utero emergence of circadian rhythms and during differentiation from cultured stem cells to different cellular lineages. The molecular clock develops gradually during differentiation and maturation, with circadian oscillations in the expression of core clock genes starting around the middle to the end of gestation. Maternal synchronizers, mainly melatonin, can pass through the placenta and influence fetal circadian rhythm. After birth, changes in period, amplitude, mesor and phase occur, aligning with the outside environment[31].

Circadian rhythms have also been observed during (stem) cell development and differentiation. Rhythmic expression of clock genes emerges during cardiac differentiation, although oscillation is not present in the undifferentiated embryonic stem cells beforehand[32]. Expression levels of the core clock genes *BMAL1*, *PER2* and *CLOCK* in human embryonic stem cells gradually increase during cardiac differentiation, with robust oscillations in more mature embryonic-stem-cell-derived cardiomyocytes after 45 days in culture. A similar effect was observed for the neuronal lineage[33]; clock genes were expressed in embryonic stem cells but started oscillating only after the addition of retinoic acid, which induced neuronal differentiation. Interestingly, these rhythms were lost and then regained with induction of de-differentiation and re-differentiation, respectively[34]. This finding points to the early development of the clock and its link to the differentiation status of the cell. In contrast to embryonic stem cells, adult stem cells, which can differentiate only into specific cellular lineages, show functional circadian clocks[35]. Overall, the emergence of circadian rhythms on a cellular level can be traced from embryonic and adult stem cells to adult, aged cardiovascular cell types, which are described in the next section.

The clock in adult cardiovascular cells

The molecular clock has specific roles within each type of cardiovascular tissue[36]. Rhythmic activation of clock-controlled genes ultimately leads to an oscillation in various functions of endothelial cells, vascular smooth muscle cells, fibroblasts, cardiomyocytes and cardiac progenitor-like cells.

In blood vessels, the circadian clock was first discovered in mouse aortas, when they were isolated at different time points in a period of 24 h (REF.[37]). These findings were later confirmed in an ex vivo study, in which rhythmic PER1 luciferase activity was measured in veins and arteries cultured from transgenic rats[36]. Subsequent studies showed that all cell types of the major blood vessel layers have a functional circadian clock. In the inner endothelial layer, the existence of circadian rhythms was confirmed by synchronizing haemangioendothelioma and human umbilical vein endothelial cells[13]. Vascular smooth muscle cells, situated in the middle layer of vessels, also showed substantial oscillations of core clock components[38,39]. Finally, robust oscillations were found in cultured fibroblasts, which reside in the outer wall of the vessels[40].

Box 1 | **Discovery of circadian rhythms and the molecular clock**

24-h changes in nature have been studied throughout history. One of the first notions of circadian rhythms dates back to the 18th century when French astronomer d'Ortous de Mairan observed a rhythmic behaviour of *Mimosa pudica*. The plant unfolded its leaves during the day and folded them again at nightfall. Surprisingly at the time, this behaviour continued under conditions of constant darkness. Described and published in the *Proceedings of the Royal Academy of Paris*[165], the observation launched interest and research in the circadian field. Important discoveries that followed involved research in *Drosophila* conducted by Seymour Benzer and Ronald Konopka in the late 1960s. Their main finding resulted in the identification of a gene that, when mutated, led to disruption of the circadian rhythm. The unknown gene was named *period* (*per*) and its existence raised additional questions about the molecular milieu underlying the rhythms[166]. The mystery of *per* began to unfold in the 1980s when Michael Rosbash, Jeff Hall and Michael Young isolated and characterized the gene and its associated protein (Per). They showed the 24-h oscillating pattern of Per levels, with a higher concentration during the night and lower during the morning[1–4]. In 1995, Michael Young discovered another clock component in *Drosophila* encoded by *timeless* (*tim*) and demonstrated a negative feedback loop required for the functioning rhythm[167]. Furthermore, he showed that the double-time (Dbt) protein, encoded by *dbt*, was necessary to tune the 24-h oscillation by delaying the accumulation of Per[168]. Other discoveries followed soon after and painted a clearer picture of clock machinery and its regulatory loops as well as the environmental influences that could synchronize the clock. However, these scientists provided us with crucial and fundamental insights into circadian rhythms and enabled the future research that would ultimately link these rhythms to human physiology and disease.

In addition to the vasculature, circadian rhythms have been found in other cells residing in the heart, including cardiomyocytes, myocardial stromal fibroblasts and cardiac progenitor-like cells. Rat cultured cardiomyocytes have an intrinsic circadian clock mechanism with oscillations that persist for ≥60 h when synchronized with a serum shock, similar to the circadian oscillations seen in the rat intact heart[41]. Further studies found robust, cell-autonomous oscillations in isolated neonatal mice cardiomyocytes[12] as well as in human embryonic stem cell-derived cardiomyocytes[32]. Sato and colleagues investigated the roles of *Bmal1* and *Smad3* in myocardial stromal fibroblasts and found that both genes were expressed in a circadian manner in mouse hearts[42]. Our group also found circadian oscillations in SCA1 (also known as ATXN1)-positive cardiac progenitor-like cells from human fetal and adult hearts[14]. In addition to transcriptional and translational oscillations of core clock elements, substantial circadian variation existed in cell functions, including proliferation, stress tolerance and paracrine factor secretion.

The cardiovascular clock in context

The clock-controlled oscillation of cellular function causes variation in many cardiovascular processes throughout the day. Peripheral clocks have an important role in the cardiovascular system by ensuring daily variation in its physiological functioning. Most knowledge on the circadian clock in cardiac physiology is gathered using animal models with disrupted clock gene expression (TABLE 1).

Within the vasculature, the circadian clock is involved in signalling of residing cells, thrombus formation, and vascular function and tone[13,43]. The most well-known example is blood pressure, with higher values in the wakefulness and activity periods than during sleep or rest. This connection with observed daily fluctuations can be illustrated by the example of diurnal humans and nocturnal rodents, which each have active and inactive phases during different times of the day. In humans, blood pressure rises before wakening early in the morning, peaks in the mid-morning and then decreases towards the night[44], whereas in nocturnal rodents, blood pressure has a diametrically opposite pattern[45]. These observed daily fluctuations are linked not only to sleep and wake cycles but also to daily fluctuations in intrinsic blood vessel properties[46–48].

Moreover, circadian clocks reside in the heart, with confirmed oscillations of core clock components found in rodent[49] and human[50] tissue as well as in cultured cardiomyocytes[41]. Many excellent review articles have been published previously[51–53] that highlight the importance of the physiological roles of the circadian clock in the cardiovascular system. Briefly, heart rate has long been recognized to vary throughout the day[44], which was attributed to central, neurohumorally mediated circadian input. By using cardiomyocyte-specific *Clock*-mutant mice, circadian rhythms in heart rate have also been shown to be regulated by the cardiomyocyte circadian clock[54]. In these mutant mice, circadian rhythmicity is disrupted only in cardiomyocytes, whereas the neurohumoral system and the central clock in the suprachiasmatic nucleus are not affected. Nevertheless, cardiomyocyte-specific *Clock*-mutant mice showed a significant reduction in heart rate compared with wild-type mice. Physiological studies on these mutant mice further showed that cardiac metabolism of non-oxidative fatty acids and glucose, the responsiveness of the heart to fatty acids, contractility and cardiac function (output) are also regulated by the cardiomyocyte circadian clock. In summary, circadian rhythms regulate some of the major features of heart physiology, including heart rate, cardiac metabolism, responsiveness to various extracellular signals, contractility, signalling, and heart growth and regeneration[55].

Circadian cardiovascular disease

Circadian rhythms are an important component of human physiology and are linked to almost all diseases, including cancer[56], infection[57] and those of the nervous system[58]. In cardiovascular disease, knowledge gathered from animal and human studies led to the association between circadian rhythms and the development, incidence and outcome of disease.

From disturbed rhythm to pathologies

Circadian rhythm disruption in animal studies is achieved either by genetically modifying the molecular circadian clock that orchestrates rhythmicity or by desynchronizing external stimuli with the internal clock; for example, housing animals in always-light or continuously changing light conditions.

When circadian rhythms in rodents and their environment are out of synchrony, animals can develop cardiomyopathy, cardiac fibrosis and systolic dysfunction, which can lead to cardiovascular death[59,60]. After the onset of disease, disrupted environmental rhythms lead to further progression of disease and a worse outcome[61]. Disruption of the molecular circadian clock

Serum shock
Acute exposure to a high concentration of serum (50% fetal bovine serum for 2 h) to align desynchronized circadian phases in a multicellular system.

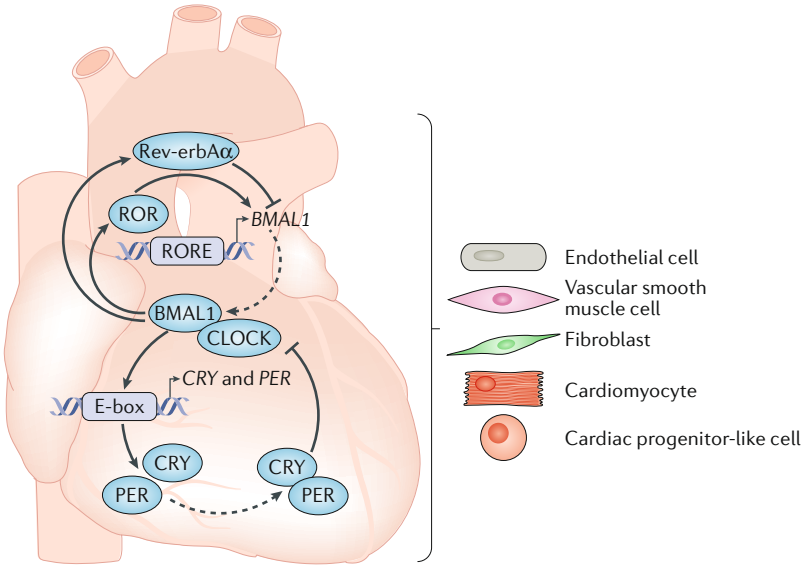

Fig. 1 | **Overview of the core molecular clockwork within the cardiovascular system.** The core clock mechanism consists of intertwined positive and negative feedback loops, present in almost all cell types within the human body. Upon dimerization, aryl hydrocarbon receptor nuclear translocator-like protein 1 (ARNTL; also known as BMAL1) and circadian locomotor output cycles protein kaput (CLOCK) bind to the enhancer box elements (E-boxes) of *CRY* (which encodes cryptochrome) and *PER* (which encodes period circadian protein homologue), thereby initiating their transcription. CRY and PER then form a heterodimer, which inhibits the BMAL1–CLOCK complex[25]. Similarly, BMAL1–CLOCK activates tyrosine-protein kinase transmembrane receptor ROR and Rev-erbAα (also known as NR1D1), which in turn activate or inhibit *BMAL1* transcription, respectively[26]. As a result of these transcriptional–translational interactions, clock-controlled genes display rhythmic activation, which leads to oscillating functions of various cardiovascular cells, including endothelial cells[13], vascular smooth muscle cells[15], fibroblasts[40,42], cardiomyocytes[12] and cardiac progenitor-like cells[14]. RORE, ROR response element.

in rodents shows similar effects and causes cardiomyopathy — specifically, thinning of the myocardial walls, dilatation of the left ventricle, altered sarcomeric structure and decreased cardiac function[62]. Other animal studies link the molecular clock to cardiac arrhythmias. In one study, for example, clock disruption led to a diminished 24-h rhythm in repolarization variation and, as a result, occurrence of ventricular arrhythmias[63].

In addition to the heart, disruption of the molecular clock causes atherosclerosis, insulin resistance, dampening of blood pressure rhythmicity, and a reduced production of vasoactive hormones and neurotransmitters[47,48]. Of note is a study by Martino and colleagues, who studied a combination of environmental and genetic rhythm alterations. Rodents in this study were genetically modified to shorten their molecular clock to a 22-h period, which under normal 24-h light–dark schedules led to cardiomyopathy, extensive cardiac fibrosis and severely impaired cardiac contractility[60]. When the animals with a 22-h clock were housed in a 22-h light–dark schedule, thereby restoring synchrony, no cardiac dysfunction was observed. These data show that both disruption of rhythms and desynchronization of external, central and peripheral clocks lead to cardiovascular disease.

Young and colleagues studied pathological characteristics of circadian dysfunction in the heart in more detail using genetic models. In addition to dampening of the oscillation in physiological functions, cardiomyocyte-specific *Clock*-mutant mice were found to have an altered metabolism, with an increase in oxygen consumption and fatty acid oxidation[54]. Cardiomyocyte-specific *Bmal1*-knockout mice have an even worse phenotype[64]. In addition to metabolic, histological and functional changes, these animals develop severe dilated cardiomyopathy that leads to a reduced lifespan.

The cardiovascular system does not consist only of cardiomyocytes; other cells such as fibroblasts[16], vascular smooth muscle cells[15] and endothelial cells[13] have an important role in cardiovascular physiology. All these cell types have circadian clocks, but tissue-specific circadian knockout models are scarce. One study found signs of atherosclerosis (decreased luminal diameter and increased vessel wall) in mice with a (non-tissue-specific) disrupted circadian clock[65]. Transplantation of the blood vessels of these animals into wild-type littermates did not alter this process, indicating that circadian rhythms in the blood vessels mediate atherosclerotic changes.

Whereas animal studies have provided insights into the mechanisms of circadian rhythms in both health and disease, most of the human studies to date have been observational. These studies demonstrate a clear relationship between circadian disruption and various cardiovascular risk factors and disease and vice versa[66–69]. Given that the molecular circadian clock is a well-preserved mechanism, genetic mutations that lead to complete clock dysfunction (often used in animal models) are very rare in humans. Genome-wide association studies, however, have found several clock gene single nucleotide polymorphisms that are associated with metabolic syndrome, hypertension and diabetes mellitus[70–73].

A much more common cause of circadian disruption in humans is misalignment of external stimuli (day–night cycle) and the intrinsic circadian clock. Artificial light and technology (such as television, computer screens and smartphones) expose people to light in the evening, opposing and desynchronizing intrinsic clocks. Studies show that short-wavelength light (blue light and light-emitting diode (LED) light) is a particularly strong desynchronizer and is associated with the metabolic syndrome, psychiatric disorders, sleepiness and reduced quality of life, especially for particular chronotypes (morning types)[74].

More severe examples of circadian disruption are sleep disorders, jetlag and shift work. Patients with insomnia and sleep disorders have an increased risk of cardiovascular disease[75,76]. Shift work is associated with metabolic syndrome, myocardial infarction, ischaemic stroke and premature death[77–79]. In addition, shift workers experience more psychosocial stress, eat less healthily[80], have a higher blood pressure[81], have a greater incidence of diabetes[82], are more often overweight, smoke more, sleep less and generally have a lower socioeconomic status than their colleagues who do not work shifts[83,84]. Of note, some of these factors might also induce bias in epidemiological studies; therefore,

Chronotypes
Propensity to be active, inactive or asleep at a specific time during a 24-h cycle.

Table 1 | **Clock mechanism disturbance: insights from animal studies**

Method of clock disturbance	Effects	Refs
Bmal1-knockout mice	Loss of physiological rhythms in heart rate and blood pressure	48
Per2-knockout mice	Endothelial dysfunction	47
Csnk1e-mutant mice (rodents have a genetic (circadian) rhythm of 22 h living in 24-h environment)	Cardiomyopathy, cardiac and renal fibrosis, impaired cardiac contractility and renal disease	60
Cardiomyocyte-specific *Clock*-mutant mice (dominant negative overexpression of CLOCK specifically in cardiomyocytes)	Disruption of physiological variation in 10% of the transcriptome; bradycardia, disruption of normal heart rate and rhythm, and mitochondrial dysfunction	54
Bmal1-knockout mice and *Clock*-mutant mice	Vascular injury, endothelial dysfunction and pathological vascular remodelling	171
Dbp$^{-/-}$*Hlf*$^{-/-}$*Tef*$^{-/-}$ mice (knockout of three major transcription factors leading to clock dysfunction)	Cardiomyopathy, cardiac hypertrophy, left ventricular dysfunction and low aldosterone levels	172
Cardiomyocyte-specific *Clock*-mutant mice	Decreased tolerance to ischaemia–reperfusion injury	103
Cardiomyocyte-specific *Clock*-mutant plus cardiomyocyte-specific *Bmal1*-knockout mice	Drug-induced hypertrophic cardiomyopathy	173
Klf15-knockout mice (transcription of *Klf15* is regulated by the molecular clock)	Loss of physiological rhythm in ventricular repolarization duration and increased susceptibility to ventricular arrhythmias	63
Bmal1-knockout mice	Dilated cardiomyopathy (thinning of the myocardial walls, dilatation of the left ventricle and decreased systolic function)	62
Mice with inducible, cardiomyocyte-specific disruption of *Bmal1*	Bradycardia, prolonged QRS duration and ventricular arrhythmias	174
Cardiomyocyte-specific *Clock*-mutant mice	Disruption of physiological variation in 8% of the proteome	116
Cardiomyocyte-specific *Bmal1*-knockout mice	Disruption of physiological variation in 10% of the transcriptome; cardiac metabolism changes, dilated cardiomyopathy and premature death	64
Vascular-smooth-muscle-cell-specific *Bmal1*-knockout mice	Diminished 24-h rhythm in blood pressure and change in timing of blood pressure peak	175

CLOCK, circadian locomotor output cycles protein kaput.

causality is difficult to establish with this method. Another well-studied example of circadian disruption is the intensive care unit. Critically ill patients in the intensive care unit are often exposed to constant circadian input signals, such as parenteral feeding, constant light and no rhythm in activity or social interaction owing to bed rest or sedation. Cardiovascular functions with a 24-h rhythm, such as blood pressure and heart rate, as well as rhythms in core temperature, hormone secretion and activity, are disrupted in these patients[85]. Conversely, a reduction in physiological (cardiovascular) rhythms is a poor prognostic sign associated with organ dysfunction, delirium and an increased risk of death[86,87]. Moreover, an excessive circadian amplitude of blood pressure is independently associated with increased adverse cardiovascular events and could be a prognostic tool for clinical outcomes, regardless of other risk factors or blood pressure mesor[88].

Circadian rhythms in the incidence of disease. Circadian rhythms are involved in physiology and the development of cardiovascular disease. Many of these diseases have a 24-h rhythm in incidence and disease burden. Supraventricular and ventricular arrhythmias, stroke, aortic aneurysm dissection, pulmonary embolism and sudden cardiac death all occur more often in the early morning[89–92]. The incidence of myocardial infarction also has a 24-h pattern[93]. Traditionally, these variations were ascribed to circadian variation in the autonomic nervous system[94]. Indeed, sympathetic activity, shear stress and cardiovascular risk factors such as blood pressure are increased in the morning, potentially triggering the onset of disease[95]. However, animal studies have shown that the peripheral clock has an important role[55]. Platelet aggregation and coagulation, ventricular repolarization abnormalities and many other relevant cardiovascular risk factors that are regulated by cellular (peripheral) circadian clocks logically contribute to peaks in the incidence of cardiovascular events at specific times of the day[63,96–98].

The circadian clock and outcome of disease. Circadian rhythms have an important role in the outcome of disease. A good example is myocardial infarction, in which outcome is related to both the time of onset of the infarction and clock disruption after the event[99–101]. If myocardial infarction occurs in the early morning, the resulting damage and cardiac dysfunction is worse than if a similar infarction occurs in the afternoon, although not all studies demonstrate the same findings[102]. Follow-up

studies in animals showed that the 24-h rhythm in outcome is regulated by the cardiomyocyte circadian clock[102,103]. Young and colleagues linked the variation in outcome to the circadian rhythm in cardiac metabolism, whereas other researchers showed that rhythmicity in the immune response also contributes to the differences found[54,104]. Both time of onset and clock desynchronization after an infarction have a major influence on outcome. Studies from Alibhai and Martino showed that when mice are put in a circadian-disruptive environment after cardiac injury (myocardial infarction and pressure-overload-induced cardiac hypertrophy), outcome severely worsens[61,105]. Finally, myocardial infarction itself is associated with molecular clock alterations and dyssynchrony between the non-ischaemic and scar tissue[106]. A comprehensive summary of the intense interplay between circadian rhythms and myocardial infarction is provided in BOX 2.

From pathologies to disturbed rhythm

Circadian rhythms are involved in the incidence, pathophysiology and outcome of cardiovascular disease. Vice versa, disease leads to disrupted circadian rhythmicity and sleep abnormalities. Development of cardiac hypertrophy in mice, either through a high-salt diet or aortic constriction, leads to decreased variation in molecular clock components including BMAL1, PER and CRY[107,108]. In human studies, cardiovascular risk factors (such as renal dysfunction) and heart failure are associated with blunted circadian rhythms[109,110]. Arterial stiffness, for example, has a physiological circadian rhythm that is blunted in patients with dilated cardiomyopathy[109]. The classic example is sleep apnoea, which is a sleep disorder caused by a combination of obesity and other risk factors for the metabolic syndrome. Patients suffering from sleep apnoea have increased risks of (pulmonary) hypertension, diabetes and cardiovascular disease, such as arrhythmias, stroke, heart failure and cardiovascular death[111]. Treatment of sleep apnoea leads to a reduction in cardiovascular disease[112].

Most studies investigating desynchronization or disruption of normal circadian rhythms in humans are observational. Therefore, causative conclusions about clock disruption and disease cannot be made. These studies do, however, convincingly show that cardiovascular disease is associated with disrupted 24-h rhythms. Conversely, these studies demonstrate that disruption and dyssynchrony of circadian rhythms, either intentional (shift work or jetlag) or as a result of disease, are also associated with cardiovascular disease. These associations are present in the incidence, pathophysiology and outcome of disease. Animals studies teach us that the molecular circadian clock is an important mediator in these rhythms and suggest that the correlations found in human studies might be causal.

Box 2 | **Circadian rhythms and myocardial infarction**

Circadian rhythms are involved in every aspect of myocardial infarction (MI), including its pathogenesis, incidence and outcome. Vice versa, MI leads to disruption of circadian rhythms and sleep problems.

Clock disruption

- Single nucleotide polymorphisms in clock genes are associated with metabolic syndrome, hypertension and diabetes mellitus; these conditions increase the risk of MI[70–73].
- Shift work and insomnia are associated with the incidence of MI (risk ratio 1.23 and HR 1.45, respectively)[76,77].
- Patients exposed to constant synchronizers (such as lights always on or constant feeding) have an increased risk of delirium, which is associated with increased morbidity, incidence of MI and mortality[122].

Incidence

- The incidence of MI follows a 24-h pattern[93].
- Many risk factors for MI, such as elevations in blood pressure, have a 24-h rhythm; disruption of these physiological rhythms is associated with incidence of MI[85].
- Platelets are most active in the early morning, when most MIs occur[138].
- 24-h variation in the cardiac transcriptome and proteome leads to variation in cardiac response to coronary occlusion.

Outcomes

- Neutrophil recruitment to injured tissues is highest in the morning, leading to increased inflammation when an MI occurs in the morning[105].
- Outcome of MI is worse when internal and external rhythms are out of synchrony, such as when patients are exposed to constant light conditions after the coronary occlusion[105].
- Long-term outcomes after MI are substantially worse when MI onset is in the morning[100].
- After MI, cardiac remodelling is associated with disruption of circadian clocks[106]; pharmacological circadian modulators can partially prevent the harmful effects of cardiac remodelling in animal models[169].
- After MI, patients often experience sleep problems, which are associated with a worse outcome[170].

Novel therapeutic approaches

Several strategies have been suggested to prevent clock disruption or desynchronization, to limit negative consequences when circadian rhythm disruption is inevitable and perhaps even to use circadian rhythms to cure patients (BOX 3). A challenging but important first step is to monitor clock disruption or desynchronization in patients. For sleep quality and disruption, polysomnography is the gold standard. However, this technique is time consuming, and results are not easily interpreted. Alternatives, such as electroencephalography monitoring, are being tested but have not yet made it into the clinic for this purpose[113]. Another option is to measure hormones or (epi)genomic, proteomic or metabolic circadian markers that are known to fluctuate throughout the day[114–116]. These fluctuations diminish in disease states or during sleep or circadian disruption. A good example that is already available is melatonin, a hormone that fluctuates throughout the day, which can easily be measured and is a predictor of outcome after myocardial infarction[117]. Furthermore, with technological advances, variables such as activity, heart rate and blood pressure can now be measured noninvasively and automatically over a period of several days, providing useful (personalized) information on circadian disruption and/or response to treatment[118]. However, although these parameters are accessible and easily measured, some might become disturbed in disease states or upon administration of medication, thereby potentially altering the correlation with circadian parameters in other organs. In the near future, a combination of traditional and novel markers

Box 3 | **Circadian rhythm in future cardiovascular research and therapy**

Several aspects of the influence of circadian rhythms on the cardiovascular system should be taken into account in future research and therapy. Circadian rhythms can be observed from several angles — one being a tool to improve existing and to develop new therapies and another being a therapeutic target themselves. When testing possible therapies or utilizing existing ones, the timing of administration can have a crucial effect on efficacy and occurrence of adverse effects[139]. Outcomes of surgical procedures can also be influenced by the time of day at which they are performed[142]. Conversely, targeting the clock could be used as a therapy itself, such as with the use of small-molecule modifiers[145]. Either way, differences between individuals should be taken into account when researching circadian rhythms in cardiovascular disease because various cardiovascular processes have been shown to differ between the sexes[156,157]. Lastly, clock disruption in our everyday lives and hospitals should be avoided and/or minimized when possible. Shift work and jetlag have been shown to have detrimental effects on cardiovascular health[83,84].

will hopefully allow more accurate assessment of the circadian status of patients.

A second step is awareness that timing can be an important factor in the success or failure of therapy. Whereas other patient characteristics, such age, sex and comorbidities, are increasingly taken into account, time is usually forgotten. In many preclinical and clinical cardiovascular studies, information about timing is not collected or reported. The routine collection and reporting of time of therapy as well as time of data collection as parameters in all medical research is necessary to assess potential clinical consequences for patients.

Studies have already shown that awareness of time of day is important in two cardiovascular scenarios. Troponin, a marker of cardiac damage, and corrected QT (QTc) interval, an electrophysiological maker of cardiac repolarization, both have a physiological 24-h rhythm[119,120]. Therefore, a rise or fall in troponin levels several hours after admission might not be indicative of an acute coronary syndrome (if within the normal oscillatory range) but instead might be a simple physiological phenomenon. QTc interval is monitored in patients receiving medication that can prolong QT duration, which would increase the risk of ventricular arrhythmias. However, comparing QTc intervals measured at different times of the day might lead to underestimation or overestimation of the QT-prolonging effect of the drug.

Environmental clock tuning

The most obvious way to deal with clock disruption is to prevent or minimize desynchronization, which can be done by exposing patients to normal 24-h input signals, high light intensity, activity and feeding during the day and darkness in the night. Studies in patients in the intensive care unit show that this strategy improves clinical outcomes. Bright intensive care rooms, especially with windows and visible daylight, reduce delirium and its complications[121,122]. Some studies suggest that critically ill patients might benefit even more from input signals with above-normal intensity, such as bright light in the morning[123]. Other input signals have not been studied thoroughly. Whether other environmental strategies, such as daytime feeding, or rhythmicity in activity or noise also improve outcomes is unknown.

Given that patients are less susceptible to physiological input signals, other strategies were tested to maintain circadian rhythmicity. A synthetic form of melatonin, a hormone produced by the central pacemaker, has produced beneficial results on clock physiology in blind patients[124]. In several clinical settings, melatonin reduced the incidence of delirium, either because of its anti-inflammatory or its clock-restoring effects[125,126].

When clock disruption is evitable, such as during shift work, strategies have been developed to minimize its harmful effects. Short naps during shifts, regular food intake, shift schedule (evening shift before night shift) and enough sleep in between shifts all partially prevent disease, although effects have been mainly studied in the short term in fairly small studies[127,128].

In the cardiovascular field, dialysis is a good example of environmental clock tuning. Many patients with kidney failure experience disruption of physiological rhythms in blood pressure, leading to nocturnal hypertension[129]. The use of nocturnal haemodialysis decreases (nocturnal) blood pressure and reduces left ventricular mass, potentially preventing cardiovascular disease[130].

Targeting the time

Many cardiovascular risk factors vary throughout the day or have an abnormal circadian pattern. An important treatment strategy is, therefore, to decrease these risks at the time of day when they are highest or to restore circadian phase and/or amplitude to normal values. A good example is hypertension, a condition associated with many cardiovascular diseases. Normally, blood pressure varies throughout the day, and studies show that nocturnal hypertension, a condition often present in patients with renal disease, in particular leads to cardiovascular disease[110,131,132]. Conversely, adverse effects of antihypertensive drugs, including orthostatic hypotension, are mainly present during the day. Most diseases associated with hypertension have an increased incidence in the early morning. Therefore, it is remarkable that most once-daily antihypertensive drugs are administered around 08:00 h. Drug plasma levels are highest during the day, when adverse effects are experienced most, and lowest in the early morning, when their desired effect is most needed.

Several studies addressed this issue and analysed the time of day of antihypertensive drug administration. Hermida and colleagues showed that bedtime administration of an angiotensin-converting enzyme inhibitor leads to lower nocturnal blood pressure than morning administration and confirmed that a decrease in nocturnal blood pressure improves cardiovascular outcome[133,134]. Other studies, some performed >2 decades ago, show that other antihypertensive drugs, such as calcium-channel blockers, angiotensin-receptor blockers and β-blockers, also have potential advantages when taken at a specific time of day and can prevent the morning peak in the incidence of cardiovascular disease[135,136]. Nevertheless, large, multicentre, randomized, controlled trials to compare morning and bedtime antihypertensive drug administration on outcome parameters such as cardiovascular death or all-cause mortality are currently lacking[137].

A second cardiovascular drug for which time-of-administration data are available is the classic platelet aggregation inhibitor aspirin. Studies show that thrombocytes are most active in the morning, when the risk of plaque rupture and myocardial infarction is highest[138]. In a study from 2015, Bonten and colleagues demonstrated that bedtime administration of aspirin leads to reduced morning platelet activity compared with morning administration[139]. As with antihypertensive drugs, large trials investigating major end points are not available. Of note, however, is that no studies indicate that morning administration of either aspirin or antihypertensive drugs is better than evening administration.

In addition to time-of-day administration of therapy, which entails lowering of the average blood pressure, the results of several studies suggest that restoring circadian amplitude to normal levels might be of greater importance[118,140,141]. Even in the absence of mesor hypertension, exceeding a threshold of circadian amplitude contributes to increased risk of cardiovascular disease[118]. The results of a small chronobiological trial involving 30 patients, in which the optimal circadian stage of administering losartan and hydrochlorothiazide was assessed, point to personalized treatment of blood pressure in which not only mesor but also circadian phase and amplitude should be taken into account[140]. In the future, ascertaining optimal treatment strategies is likely to include individual circadian blood pressure pattern (for example, reversed rhythm with high blood pressure during the night or excessive amplitude with high blood pressure during the day) together with frequency and timing of the medication[141].

The importance of the time of day of therapy is not limited to cardiovascular drugs. Montaigne and colleagues, for example, compared the outcome of surgical aortic valve replacement[142]. A total of 600 patients were paired on the basis of preoperative and perioperative characteristics, the only difference being their time of operation (morning or afternoon). Morning operation was associated with a twofold higher rate of major complications in the short and long term. In a second, randomized study, the researchers confirmed that morning operations lead to higher perioperative myocardial damage. Observed outcomes are in accordance with the established tendency of major adverse cardiovascular events, such as myocardial infarction, to occur in the morning.

Time-specific therapy has also been introduced in stem-cell-based cardiac repair, a therapy that aims to cure the failing heart. In the clinic, both cardiac and non-cardiac patient-derived (multipotent) stem cells have been tested for their potential regenerative and paracrine effects[143]. In animal studies, cardiomyocytes derived from pluripotent stem cells (embryonic or genetically modified or induced) showed promising results for cardiac repair[144]. These stem cells have circadian clocks and clear 24-h rhythms in function and paracrine effects[14]. However, whether the use of stem cells at a specific time during the day improves patient outcomes remains to be investigated.

Targeting the clock

Another therapeutic option might be to change the phase of the circadian clock to a time-of-day setting or specific phase in physiological rhythms that is the most beneficial in a given situation. Myocardial infarction and aortic valve replacement, as described above, have a worse outcome when they occur in the morning because of reduced ischaemia tolerance. Manipulating the molecular and functional situation of the heart to resemble that in the afternoon or evening might, therefore, be an attractive strategy. Animal studies show that genetic clock changes influence infarct size[99,103,105]. Targeting the molecular clock in humans is more complicated, especially in the case of acute, unexpected disease when interventions can be initiated only after the index event, but some studies show promising results. Small-molecule modifiers, such as Rev-erbAα inhibitors, can change the molecular circadian clock to a beneficial state preventing cardiac damage in animal models[142,145]. Many compounds that influence phase, amplitude and period of the circadian rhythm are available, hopefully allowing clock-targeting therapies in the near future[146].

Are we all ticking in the same way?

In cardiovascular disease and circadian research, major differences are known to exist on the basis of sex, age and ethnicity. Large epidemiological studies show that African-American individuals have a shorter free-running circadian period than European-American individuals, whereas Chinese-American and Latin-American individuals reportedly sleep less[147,148]. Second, desynchronization of external and internal rhythms leads to more cognitive dysfunction in elderly individuals and in women than in young men, possibly caused by a different response of the autonomic nervous system[149,150]. Changes in circadian rhythms with age can be observed on different levels: a shift towards a morning chronotype as well as a preference for 'morningness' in terms of cognitive skill performance and alertness; worsened sleep quality and quantity; dampened capacity to accommodate light–dark schedule change; reduced amplitude and peak shift in hormones such as cortisol and melatonin; and progressive dampening of many metabolic rhythms, which can lead to diabetes, dyslipidaemia and hypertension, which are all risk factors for cardiovascular diseases[151].

These differences affect cardiovascular disease. The onset of myocardial infarction has a different time-of-day pattern in white European individuals in Spain from that in white European or Asian European individuals in the UK[152]. Second, risk factors might differ between ethnic groups. In African-American individuals, increased sleep duration is associated with obesity, whereas European-American individuals who sleep for a long time are less obese[153]. African-American individuals also experience more nocturnal hypertension (non-dippers) than European-American individuals and have a greater incidence of cardiovascular disease[154].

The incidence of cardiovascular disease is similar in men and women[155]. However, sex-specific differences in circadian rhythms and cardiovascular disease have not been studied thoroughly. Given that many

Acrophase
Time at which the peak of the rhythm occurs.

cardiovascular processes such as cardiac metabolism differ between the sexes and that women are protected from heart disease in both animal and human studies, sex and cardiovascular diseases related to circadian disruption are likely to be linked[156,157]. Alibhai and colleagues did a pioneering study and indeed found that female mice with disrupted circadian clocks are protected from the development of metabolic changes and cardiomyopathy[158]. This protection is likely to be mediated by ovarian hormones.

In terms of diagnosis and treatment, taking into account individual differences is equally important, even among patients with the same sex, age or ethnicity[159]. A one-size-fits-all approach to blood pressure reduction might be detrimental in some cases. Currently used reference values for blood pressure might not reveal all the vascular variability anomalies that are associated with an increased risk of adverse cardiovascular events, whereas time-specified, individualized reference values obtained by long-term monitoring might do so. With a proper diagnosis based on circadian amplitude and acrophase instead of only mesor, a simple adjustment in timing of the daily drug administration might be sufficient to treat and prevent further vascular variability anomalies.

Conclusions

The broad range of cardiovascular (patho)physiologies are subject to circadian oscillations. Circadian clocks have been found in all cell types in the heart and vasculature, and many important biological processes, such as heart rate, body temperature, blood pressure, metabolism and hormone levels, show daily fluctuations. In addition, the incidence, development and outcome of disease are linked to the circadian clock. Better understanding of molecular mechanisms underlying variability in cardiovascular disease might lead to new treatment strategies and improvement of existing approaches.

What happens when knowledge of circadian rhythms is used as a tool? Chronotherapy immediately emerges as an obvious application, given its usage for the timing of administration of several commonly used medications[160,161], such as calcium-channel blockers, angiotensin-converting enzyme inhibitors and aspirin. However, not only have the pharmacokinetics and pharmacodynamics of various drugs shown circadian variability but choosing different times of the day or phase of marker rhythms (such as blood pressure or a symptom) at which to perform some medical procedures might also be beneficial to patients[142]. In addition to cardiovascular conditions, diseases such as asthma, acute and chronic inflammations, allergies and cancer show daily fluctuations in symptoms and occurrence[162]. Accordingly, administration of treatments and analysis of the outcome for these conditions can be tailored to particular times of day[163,164].

In conclusion, time is a crucial consideration in the treatment of cardiovascular diseases. Translational application of circadian knowledge benefits patients, both by timing of therapy to maximize the desired effect and/or to minimize adverse effects and by avoiding clock disruption in our everyday lives and disease (such as in intensive care units). The use of circadian rhythms in research and in the clinic (BOX 3) will hopefully improve the treatment and survival of patients with cardiovascular disease.

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