npj | biological timing and sleep

Review

https://doi.org/10.1038/s44323-024-00007-z

Chronobiotic and cytoprotective activity of melatonin in the cardiovascular system. Doses matter

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A circadian disruption, manifested by disturbed sleep and low-grade inflammation, is commonly seen in cardiovascular diseases (CVDs). The decline in plasma melatonin, which is a conserved phylogenetic molecule across all known aerobic creatures, is also a common feature in CVDs. The daily evening pineal melatonin surge synchronizes both the central pacemaker located in the hypothalamic suprachiasmatic nuclei and myriads of cellular clocks in the periphery ("chronobiotic effect"). Melatonin also has cytoprotective properties, acting primarily not only as an antioxidant by buffering free radicals but also by regulating inflammation. In CVDs, exogenous melatonin administration decreases nocturnal hypertension, improves systolic and diastolic blood pressure, reduces the pulsatility index in the internal carotid artery, decreases platelet aggregation, and reduces serum catecholamine levels. Melatonin evokes an increase in parasympathetic activity in the heart. Allometric calculations based on animal research show that melatonin's cytoprotective benefits in CVDs may require high doses to be fully manifested (in the 100–200 mg/day range). If melatonin is expected to improve health in CVDs, the low doses currently used in clinical trials (i.e., 2–10 mg) are presumably insufficient.

Cardiovascular diseases (CVDs) account for the majority of deaths worldwide[1,2]. In 2022, age-standardized CVD mortality rates by region ranged from 73.6 per 100,000 in high-income Asia Pacific to 32.3 per 100,000 in Eastern Europe, while in Southern Latin America, age-standardized CVD mortality rates among countries ranged from 114.9 to 73.6 per 100,0002. Although the mortality rate is now being reduced, the prevalence of CVDs still remains very high and is strongly linked to impairment, dependency, and the need for long-term care, particularly in the elderly.

Chronic CVDs are characterized by a persistent low-grade pro-inflammatory state called metainflammation or "inflammaging"[3–5]. Meta-flammation refers to a metabolically orchestrated, chronic, non-resolving, low-grade inflammation that occurs in critical metabolic organs. This condition plays a central role in impaired metabolic homeostasis and sets the stage for various pathologies associated with obesity, aging, and other chronic metabolic diseases. Some of the consequences of metaflammation include chronic inflammation, dyslipidemia, insulin resistance, diabetes, fatty liver disease, cardiovascular issues, respiratory problems, and even cancer. Essentially, it's an inflammatory cascade that contributes to a variety of metabolic disorders[3–5].

Metainflammation involves the alteration of several pro- and anti-inflammatory pathways. The nuclear factor (NF)-κB and cyclooxygenase pathways are major pro-inflammatory pathways, whereas the hypothalamic/pituitary-adrenal axis, the inflammatory resolution pathway (specialized pro-resolving mediators), and the melatonin/endocannabinoid/angiotensin 1,7 axis are major anti-inflammatory pathways[6–8]. Circadian disruption plays a major role in the dysregulation of pro- and anti-inflammatory pathways[9]. As a comorbidity of inflammaging, sleep/wake cycle disruption causes a slew of pathophysiological changes that hasten CVDs.

Melatonin is a methoxyindole displaying pleiotropic functions, being effective in the treatment of both daily rhythm dysregulation and inflammation. Melatonin acts as a chronobiotic (i.e., by causing synchronization, phase-shifting, and amplitude enhancement of daily rhythms)[10]. It also acts as a direct and indirect antioxidant, immunological modulator, and mitochondrial protector and modulator[11] Melatonin levels decline with age, and they are even lower in people with CVDs[12]. Melatonin reduces inflammatory responses and inflammation progression. This review examines the several activities of melatonin as a chronobiotic and cytoprotector in the context of CVDs, with emphasis in range of doses involved in human and animal studies.

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The circadian apparatus

Biological functions intensify and attenuate in cycles of daily and annual periodicity. This situation does not reflect the simple passive response of the organism to cyclic environmental changes. On the contrary, they are the result of endogenous rhythms, that is, an intrinsic capacity to detect and predict temporal changes in order to optimize adaptation[13,14].

As the Earth rotates on its axis, it presents two well-defined environments: light and darkness. Because the Earth's axis of rotation is tilted, the relative length of the light and dark periods changes during the year. As a consequence of the process of evolution, living beings have responded to these two situations by developing specific prediction mechanisms to adapt successfully. This is the origin of biological rhythms that repeat every 24 hours and annual rhythms, which oscillate with the seasons of the year[13,14].

When animals show diurnal, nocturnal, or seasonal modes of behavior, they are not merely responding to external light/dark (LD) conditions. On the contrary, they respond to signals generated by an internal pacemaker, synchronized with the Earth's rotation cycle, which anticipates the transitions between day and night and triggers physiological and behavioral changes in accordance with them[15]. In this way, the brain pacemaker creates a "day" and a "night" within the body, as a mirror of the outside world. The influence of the lunar cycle (approximately 29.5 days) on the sleep/wake rhythm may also be relevant[16,17].

The rationality of the temporal structure in living organisms is related to homeostasis, that is, to the mechanisms that enable the body to maintain the balance of the internal environment in response to environmental variations[18]. Such variations affect the physiological balance and thus generate adaptive responses that restore balance and cover a wide range from genomic to behavioral. This complete repertoire of adaptive responses is called "reactive homeostasis"[19].

There are, in addition to unforeseen modifications of the environment, other repetitive ones that can thus be anticipated, such as the length of day and night. These periodic environmental cues generate endogenous signals that anticipate expected variation and facilitate the speed and efficiency of adaptation. It constitutes another type of homeostasis, i.e. "predictive homeostasis"[19,20]. Let's give an example: every day, a few hours before waking up, our cardiovascular system begins to prepare for the important change in body position that will occur hours later. Thus, the vascular regulation changes, even though no stimulus has been triggered to justify its response[18].

Instead of being related to external factors, the sleep-wake rhythm is regulated by an internal pacemaker, or "biological clock." The existence of this biological clock is demonstrated in situations such as isolation. In the absence of environmental temporal cues (a clock, noise, TV, etc.) the sleep-wake rhythm still persists, with a distribution of approximately 2/3 of the day awake and 1/3 asleep. The main difference is that in isolation the human rhythm period is no longer exactly 24 hours but becomes close to 25 hours. This is a "circadian" rhythm (from the Latin "circa", around 24 hours, and "dies", day)[21].

The mammalian circadian timing system comprises tissue-specific cellular clocks. To generate appropriate physiological and behavioral responses, the phases of this multitude of body clocks are controlled by a master circadian oscillator found in the anterior hypothalamic suprachiasmatic nuclei (SCN)[22]. Natural light is the most pervasive and prominent synchronizer among photic (e.g., natural LD cycle) as well as nonphotic cues, e.g., food, behavioral arousal, etc. (time givers or "*zeitgebers*"). The retinohypothalamic tract entrains the SCN via messenger neurotransmitters, controlling the differential expression of clock genes and clock-controlled genes within SCN cells and influencing observable output in the form of physiology and behavior[23].

Circadian clocks are based on the so-called clock genes, with key genes encoding proteins that feedback and repress transcription at the molecular level. These oscillators consist of interconnected transcriptional and post-translational feedback loops that are regulated by a small number of core clock genes[24]. Transgenic gene deletion technology in rats and mice was used to study the negative and positive transcriptional/translational feedback loops that comprise the core clockwork. The 24-h oscillation in clock gene expression is caused by a delay in the feedback loops, regulated in part by phosphorylation of the clock proteins, affecting their stability, nuclear re-entry, and transcription complex formation[25].

Several overlapping feedback loops comprise the mammalian circadian clock (Fig. 1). As the *Clock* and *Bmal1* genes in mammals are transcribed, the transcription factors CLOCK and BMAL1 are produced, which then form dimers via basic helix-loop-helix domains. The dimers subsequently stimulate the transcription of two additional genes, *Per* and *Cry*, resulting in the creation of the proteins PER and CRY, which dimerize and inhibit the expression of CLOCK and BMAL1. The cycle is restarted as PER and CRY decline over time[25].

Per and *Cry* mRNA levels in the SCN peak in the middle to late afternoon in both nocturnal and diurnal mammals[23]. *Bmal1* mRNA levels rise around midnight, but *Clock* mRNA is present in the SCN at a constant concentration all day[26]. Production of PER and CRY is limited by binding to the E-box element of the promoter regions of *Bmal1*, *Clock*, *Rev-Erb* and other clock-controlled genes via the CLOCK/BMAL1 complex. Casein kinase 1 δ/ε phosphorylates PER and CRY, which are then translocated to the nucleus[26].

The master oscillation is modulated by four secondary regulatory loops (Fig. 1). One of them involves the nuclear receptors REV-ERB and ROR (retinoid-related orphan receptor). REV-ERB suppresses *Bmal1*, whereas ROR promotes it via attaching to the RORE (response element-binding site) sequence in *Bmal1*'s promoter region[27,28]. A second regulatory loop is given by the protein ROR that also binds to the RORE element in the promotor of CLOCK, and to NFIL3 (nuclear factor, interleukin 3 regulated), resulting in their transcription. NR1D1 (nuclear receptor subfamily 1 group D member 1) and possibly other proteins from this family inhibit ROR binding to the RORE element. A third regulatory loop includes DBP (D-box binding PAR b ZIP transcription factor), a protein whose expression is controlled by BMAL1:CLOCK from the first loop binds that binds to the D-box in the promotor region of PER. NFIL3 from the second loop regulates this binding negatively[25]. CRY1, like PER1/2, is regulated by a combinatorial mechanism involving both E-box and RORE, resulting in a distinct phase from DBP and REV-ERB. A fourth regulatory loop is given by DEC (AKA Basic helix-loop-helix family member e40 loop) as an ancillary circadian loop characterized by the expression of DEC and other circadian-controlled genes that are controlled by BMAL1:CLOCK. DEC, in turn, inhibits BMAL1:CLOCK binding to the E-box element, regulating its expression[29]. Phosphorylation and ubiquitylation by the E3 ligase complex regulate PER and CRY stability, leading to proteasomal degradation.

The sleep/wake cycle as the most conspicuous 24-hour circadian rhythm

Four stages of sleep are distinguished depending on the type of brain activity shown in the electroencephalogram (EEG). Stages N1 to N3 correspond to the progressive slowing of brain waves. The 4th stage, R, called "paradoxical sleep" or rapid eye movement sleep ("REM sleep"), corresponds to a desynchronized EEG, similar, although not identical, to that of wakefulness.

Three mechanisms have been identified as responsible for the sleep phenomenon[30].

- A process called "S" (for sleep), determined by the individual's previous sleep and wake history. The "S" process is manifested by the increased sleep propensity observable following sleep deprivation (accumulative homeostatic drive).
- A process called "C" (for circadian), controlled by the endogenous biological clock and independent of the previous history of sleep and wakefulness. Process "C" includes the tendency of sleep to begin in the phase of falling body temperature (first part of the night) and ending sleep during the phase of increasing temperature (second part of the night). It is due to this "C" process that after a night awake we are more sleepy ~4–5 in the morning than about 2 or 3 hours later (despite

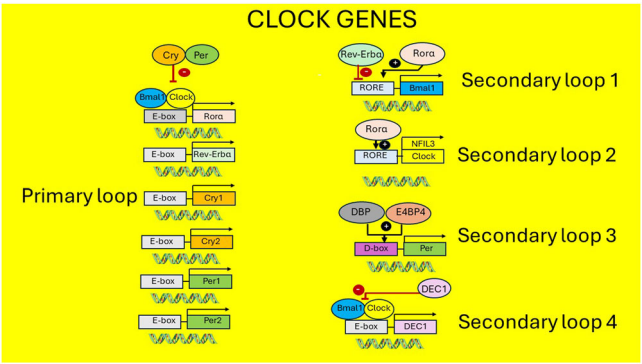

Fig. 1 | The primary master circadian oscillation (left) is modulated by four secondary autoregulatory loops. Primary loop: Bmal1 heterodimerizes with Clock and activate the transcription of CCGs including *Cry* and *Per*. Once accumulating to a high level, CRY and PER proteins inhibit the activity of Bmal1/Clock complex. Secondary loop 1: *Rorα γ* and *Rev-erbα* are target genes of Bmal1/Clock. *Rorα* activate, whereas *Rev-erbα* repress *Bmal1* transcription by binding to RORE. Secondary loop 2: the transcription of BMAL1 and NFIL3 triggered when *RORα* binds to the RORE element in their promoter region. This binding of ROR to RORE is inhibited by NR1D1. Secondary loop 3: DBP and E4BP4 synergistically regulate the expression of *Per* via actions on D-box. This binding is negatively regulated by NFIL3 (Loop 2). Secondary loop 4 (or DEC loop): the DEC protein is responsible for its own oscillatory expression by inhibiting the binding of BMAL1:CLOCK to E-box elements in its promoter.

having accumulated more sleep debt due to the accumulative homeostatic drive). The sleep is thus similar to a bank: if we want to pay the "bank debt" (process S) when the "bank window" (process C) is "closed" we will not be able to do so and we will have to wait for the "bank to open".

- A periodicity component of 90–120 min (ultradian), perceptible both within sleep (alternance of REM sleep and slow-wave sleep with this periodicity) as in wakefulness (periodicity of ~90–120 mins in maximum and minimum of attention and wakefulness). We know from experience that attention cannot be maintained on a task, for example, listening to a lecture, for more than 60–90 min[31]. Melatonin plays a fundamental role in triggering the sleep process and is the physiological signal that "opens the doors of sleep"[32].

Melatonin is a potent chronobiotic with mild hypnotic properties. Daily dosages of 2–5 mg melatonin, timed to advance the phase of the internal clock through interaction with MT1 receptors in the SCN, sustain circadian rhythm synchronization to a 24-h cycle in sighted people who live in situations that are prone to produce a free-running rhythm[33]. Melatonin synchronizes a person's circadian rhythm after a brief time of free running. By administering melatonin to blind subjects showing free-running rhythms, researchers were able to stabilize or entrain the sleep/wake cycle to 24 hours, resulting in improved sleep and mood[34]. In a month-long crossover research, 24 healthy older individuals were given a placebo for two weeks and then either 0.3 mg or 5 mg of melatonin 30 min before going to bed. The 5 mg melatonin dosage considerably boosted sleep efficiency throughout both biological day and night, mostly by extending the duration of Stage 2 non-REM (NREM) sleep and somewhat reducing awakenings[35].

Melatonin therapy helps to minimize variance in sleep start time in normal aged adults and dementia patients with disrupted synchronization of the sleep/wake cycle. Melatonin's phase-shifting effects are also adequate to explain its efficacy as a therapy for circadian-related sleep disorders such as jet lag or delayed phase sleep syndrome[36,37].

A common mistake is to identify sleep as an exclusive phenomenon of the CNS. In reality, it is a complete physiological program, different from wakefulness and comprising two very different physiological states of organs and systems: slow or NREM sleep, and REM sleep. From the autonomic nervous system point of view, wakefulness is characterized by a sympathetic predominance while NREM sleep exhibits a parasympathetic predominance[38]. REM sleep is accompanied by a profound loss of muscle tone (fortunately with few exceptions such as the diaphragm, the main respiratory muscle, so our breathing is not interrupted, or the cricopharyngeus muscle whose contraction closes off the entrance to the esophagus). REM sleep coexists with increased variability in blood pressure (BP) and heart rate, and with a tendency to lose control of body temperature. The most complex mechanisms of cardiocirculatory, respiratory and thermal control temporarily stop functioning during REM sleep, with only the basic autonomic reflexes of the spinal cord persisting. Thus, wake, slow sleep and REM sleep are three different homeostatic programs that must necessarily occur in harmony to ensure health[18].

According to the Centers for Disease Control and Prevention (CDC), the age-adjusted prevalence of adults reporting short sleep duration (less than 7 hours) ranged between 30% and 40% from 2013 to 2020 (CDC Behavioral Risk Factor Surveillance System, 2020. https://www.cdc.gov/sleep/data-and-statistics/Adults.html) We thus live in a sleep-deprived Society, where the sympathicotonic catabolic configuration of wakefulness has become predominant at the expense of the parasympathicotonic anabolic configuration of slow-wave sleep.

Within the physiological program of sleep and wakefulness, the activity of the cardiovascular system presents clear characteristics that define them[39,40]. We will analyze some fundamental aspects of the relationship among the daily rhythms, melatonin and the cardiovascular system.

Reactive and predictive homeostatic processes in cardiovascular function

As mentioned, reactive homeostasis are the set of reactions needed when faced with unexpected changes in physiological variables necessary for life. The different mechanisms of hormonal counterregulation to sudden changes in physiologic equilibrium are examples of reactive homeostasis[19,20]. For its part, predictive homeostasis comprises the anticipatory mechanisms that precede a temporally predictable environmental phenomenon and that facilitate better physiological adaptation to them. For any species to have a time control system integrated into the organism itself, which allows temporal prediction without having to depend on reading external signals, is extremely useful. The circadian clock is ideal for fulfilling this function: we could have a sufficiently precise idea of the time of day just by analyzing our periodic biological structure and without consulting our wristwatch. That is, a "day" and a "night" have been created within the organism that allows to optimize adaptation[13].

A conspicuous expression of circadian clock genes in human heart and aorta has been detected[41,42]. Ex vivo experiments demonstrate that various functions of the mouse heart and aorta are dependent on the time at which the tissues are harvested[13]. In murine circadian gene knockout models, it has been observed that deletion of *Bmal1* in cardiomyocytes results in an abnormal electrocardiogram with prolonged RR and QRS intervals[43–45]. *Bmal1* removal in cardiomyocytes can reduce cardiac arrhythmias probably by a decreased propensity to intracellular calcium overload[46]. Cardiomyocyte-specific deletion of *Bmal1* in mice leads to age-dependent dilated cardiomyopathy and decreased lifespan[47,48]. BMAL1 is critical for normal mitochondrial activities in cardiomyocytes, its knockout reducing BNIP3 protein levels, and compromising mitophagy[49]. Other studies have revealed that deletion of *Bmal1* in endothelial cells or vascular smooth muscle cells alters the diurnal variation of BP[41]. Another study has shown that mice lacking the *Bmal1* gene exhibit a significant decrease in the expression of genes associated with the fatty acid oxidative pathway, the tricarboxylic acid cycle, and the mitochondrial respiratory chain in the heart[50]. This can lead to severe progressive heart failure with age. It is important to note that there has been some debate in the scientific community about the role of the *Bmal1* gene in regulating circadian rhythms. Some studies have reported that tissues continued to follow a 24-hour rhythm even in the absence of the *Bmal1* gene[50].

Disruption of normal day-night cycles, such as jet lag or shift work, leads to desynchronization between central and peripheral clocks and deregulation of cardiovascular clock genes[24,50]. For example, using a mouse model of induced cardiac hypertrophy, it has been found that exposure to 10 h L: 10 h D cycles adversely affects cardiac structure and function reflected in the altered expression of clock genes and remodeling genes[51]. Thus, restoring a normal diurnal rhythm could rescue these changes, indicating that a normal daily rhythm is crucial for cardiovascular health[52]. Indeed, chronic jet lag and shift work have been linked to CVD disease[53]. The use of chronotherapies, such as melatonin and/or timely light exposure, to modulate the molecular elements of daily rhythms to alleviate these negative effects is beginning to gain traction in the scientific literature.

BP and heart rate during sleep

BP decreases during slow sleep and becomes variable in REM sleep[54]. In this period, transient increases of up to 40 mmHg occur that coincide with the phasic events of REM sleep in conjunction with vasoconstriction in the skeletal muscles during these events. Pulmonary artery pressure remains stable. Sleep-related BP variation can be described by a square wave function with changes at sleep onset and offset and relatively constant values over the course of sleep. This drop in BP during sleep is important for cardiovascular health[52].

Systolic pressure values fall by 15 mmHg or more and are strongly influenced by Process S. At the beginning of sleep, there is an initial drop in BP due to postural change and darkness (~7 mmHg), a period of instability when sleep is unstable sleep (stage N1 of sleep) and an abrupt drop once stable sleep (stages N2-N3 of sleep) is achieved (~7 mmHg). Within each sleep phase, the BP is constant; in NREM sleep, the values are lower than in wakefulness, and in REM sleep, they resemble relaxed wakefulness. BP at the end of sleep shows an increase largely due to postural changes. The magnitude of the changes is greater if the awakening is in the N2 stage of sleep compared to the REM stage[54].

In the case of heart rate, the closest approximation is to a sinusoidal pattern compatible with the influence of Process C (circadian). The lowest point of the oscillation occurs around the middle of the sleep cycle. As for BP, at the beginning of sleep there is a drop in heart rate with two components (1. preparation for sleep; 2. when sleep becomes stable). There is a close relationship between heart rate and metabolic heat production as a consequence of their circadian dependence. Heart rate is higher during REM, with transient tachycardia related to REM phasic events.

Brief awakenings are a characteristic of normal sleep[55]. They occur with high frequency (HF, >15–20 per night) and are a normal situation in which an increase in heart rate ≥8 beats per min) and BP (<15 mmHg), and peripheral vasoconstriction are observed. Awakenings occur in both NREM and REM sleep. There are two components related to awakenings. The first component is a transient peak in heart rate and BP that occurs within 3 to 6 s of the event. The second component is dependent on the previous wakefulness. Most awakenings are brief, and this second component is absent[55].

Heart rate variability in the study of circadian cardiovascular regulation

Cardiac autonomic activity can be studied through heart rate variability (HRV) analysis. Time domain indexes provide a measure of RR intervals variation over time. Among these, RRm (mean duration of RR intervals) quantifies the mean heart rate, SDNN (standard deviation of RR intervals) measures global variability, and RMSSD (square root of the mean squared differences of successive normal RR) represents short-term heart rate variations. Frequency domain variables provide a measure of the amplitude of the frequencies underlying HRV signal. Its HF component (0.15–0.4 Hz) is related to respiratory sinus arrhythmia and mediated by parasympathetic activity and its low-frequency (LF) component (0.04–0.15 Hz) is related to baroreflex control and relies on both sympathetic and parasympathetic influences. A very low-frequency (VLF) component (0.003–0.04 Hz) has been attributed to humoral factors and to thermoregulatory fluctuations and was attributed to parasympathetic regulation. Thus, parasympathetic predominance is usually reflected by an increase in the HF and RMSSD indexes, either isolated or accompanied by increases in LF and VLF, while sympathetic predominance is reflected by a relative increase in LF[56,57].

Using HRV as an indirect measure of autonomic activity, it was shown that the sleep-wake cycle exhibits distinct autonomic patterns. During wakefulness, different reflex arcs including the baroreflex, the respiratory sinus arrhythmia and the chemoreflex, which encompass spinal and central centers as well as the central autonomic network, contribute to cardiac activity leading to an increased heart rate, increased sympathetic and decreased parasympathetic activity. During NREM sleep, the decrease in brain activity in certain cortical and subcortical areas would determine a predominance of the contribution of the reflex arcs over the central autonomic activity, leading to a decrease in heart rate, with parasympathetic predominance and decreased sympathetic modulation. HRV studies during have revealed that there is a decrease in the LF component and an increase in the HF component in relation to wakefulness, consistent with the characteristic parasympathetic prevalence[58]. During REM sleep, cardiac autonomic regulation is shared between central control related to amygdala activity and the homeostatic control of the cardiovascular system through reflex arcs, leading to an increase in heart rate, with sympathetic predominance and a decrease in parasympathetic activity. Consistently, the degree of network connectivity and the strength of physiological interactions among different central and peripheral systems are intermediate between slow-wave sleep and wakefulness. During this stage, a pattern of increased variability is observed, with reports of maxima for the LF component and null values for the HF component[58].

In addition to variations in cardiac autonomic activity in relation to sleep stages, the existence of an endogenous circadian rhythm in heart rate and HRV has been demonstrated, in the absence of the masking effects of sleep, general activity, postural changes and light[59]. The results suggest that circadian control of heart rate is not entirely mediated by the sleep-wake cycle and that autonomic modulations are influenced by the circadian system. While heart rate peaks during late wakefulness, all HRV measures reach maximum values in the latter part of the sleep period[59].

Melatonin as a chronobiotic

Daily rhythms in both the synthesis and secretion of pineal melatonin are tightly tied to the sleep/wake cycle rhythm not only in normal but also in blind human subjects[60]. The onset of nightly melatonin secretion begins around two hours prior to an individual's habitual bedtime and has been connected to the onset of evening weariness. Several studies have documented that sleep proclivity is the physiologic outcome of an increase in endogenous melatonin[61–63]. Melatonin plays an important role in the coordination of circadian rhythmicity. Melatonin secretion is a "hand" of the biological clock that responds to SCN signals so that the timing of the melatonin rhythm reveals the phase of the clock (i.e., internal clock time relative to external clock time) as well as the rhythm amplitude[64]. Melatonin, in another sense, is a chemical signal of the darkness: the longer the night, the greater the duration of its secretion. In most mammalian species, this secretion pattern also serves as a timing cue for seasonal rhythms[65,66].

The sympathetic nervous system regulates pineal melatonin production through a neural pathway that travels from the hypothalamic paraventricular nucleus and ends at the upper level of the thoracic spinal cord[64]. The superior cervical ganglion postganglionic sympathetic nerve terminals release norepinephrine into the pineal gland, where it interacts with β- (primarily) and α-adrenoceptors on pineal cell membranes to activate melatonin synthesis. Melatonin is not stored in the pineal and is released as soon as it is produced[67].

Vertebrate species produce pineal melatonin exclusively during the dark phase of the LD cycle. Melatonin is generated constantly during the night, regardless of the species' day activity/rest cycle, and is closely related to the external photoperiod. It should be emphasized that melatonin is produced at night providing that there is no light present[64].

The effects of melatonin as an internal *zeitgeber* of the circadian clock, like the effects of the external *zeitgeber* light, are time-dependent. Melatonin alters the phase of the circadian clock in rats, which could explain how melatonin affects sleep in humans[64] Clinical trials with melatonin in blind subjects (who have free-running daily rhythms) provide indirect support for such a physiological involvement[34]. It has been shown that the phase response curve for melatonin was opposite (i.e., ~180 degrees out of phase) to that of light, providing more concrete evidence for this theory[33].

Melatonin receptors are present in both the CNS and the periphery[68]. The G-protein coupled (GPCR) families of MT1 and MT2 receptors have been cloned. GPR50, a new melatonin receptor subfamily member, was identified[69]. GPR50 has a structure similar to MT1 and MT2, however it does not bind to melatonin or any other known ligand. Nevertheless, these receptors' capacity to form homo- and heteromers with other GPCRs, such as the serotonin 5-HT 2 C receptor, may alter receptor activity.

While melatonin's main physiological role is to control daily and seasonal rhythmicity, its actions are not confined to receptor-rich areas. Melatonin has effects on mitochondria including modulating electron flux, the permeability transition pore, and organelle´s biogenesis, as well as having anti-excitatory properties, immunomodulation (including pro- and anti-inflammatory properties), antioxidant actions, and energy metabolism[70]. Most of these actions are not mediated by receptors.

Melatonin is loosely linked to albumin in human blood[71] and is metabolized by hydroxylation in the liver before conjugation with sulfate or glucuronide[72]. 6-Sulphatoxymelatonin is the primary metabolite in human urine. In the brain, melatonin is converted to kynurenine metabolites, some of which (e.g., N^1-acetyl-5-methoxykynuramine) share its well-documented antioxidant effects. Additional antioxidant metabolites of melatonin are cyclic 3-hydroxymelatonin and N^1-acetyl-N^2-formyl-5-methoxykynuramine. Thus, melatonin administration to experimental animals and humans triggers an antioxidant cascade[73].

The pineal gland produces nearly all circulating melatonin in mammals. However, a significant portion of total body melatonin is produced locally in cells, organs, and tissues including lymphocytes, bone marrow, the thymus, the gastrointestinal tract, the skin, and the eyes, where it can play an autocrine or paracrine role[74]. Indeed, melatonin is known to be synthesized in every animal cell with mitochondria, where it can play a functional role[70]. Although it is widely accepted that natural melatonin's chronobiotic influence is mediated by MT receptors, a chronobiotic effect can also be observed when pharmaceutical quantities of fast-release melatonin (that saturate receptors) are utilized. Even at such high doses, melatonin ingested as a fast-release preparation at a single time of day (bedtime) maintains chronobiotic effects[75].

Despite the antiphase circadian rhythms in humans and rodents, melatonin serves as an important zeitgeber, or time cue, for the endogenous circadian system. It coordinates the night adaptive physiology through immediate effects and primes the day adaptive responses through prospective effects that will only appear at daytime, when melatonin is absent[76]. This means that even though melatonin levels increase during the awake period in rodents, it still plays a crucial role in regulating their sleep patterns[77]. Thus, melatonin triggers changes characteristic of the dark period for each species: increased activity and wakefulness in nocturnal animals, and rest and sleep in diurnal animals. This is further backed by melatonin's ability to promote rest in diurnal animals like birds, monkeys, humans, and some rodents, and to boost motor activity in nocturnal rodents[78]. Therefore, melatonin signals darkness and reinforces nighttime physiology, including timing of the sleep–wake cycle and other circadian rhythms[64]. In vitro, studies in mice show two distinct effects of melatonin on SCN: a phase-shifting effect on the rhythm in electrical activity and an acute inhibitory effect on neuronal firing[64].

The phase dependent effect of melatonin, which influences the circadian rhythm of neuronal SCN firing and other measured outputs, is mediated via MT1 and MT2 receptors[63]. A single injection of melatonin administered to rats kept in constant darkness caused a phase-advance in *Rev-Erb* and *Bmal1* mRNA expression in the SCN during the first subjective night after the melatonin administration[79].

Melatonin injections did not immediately influence levels of clock genes in the rat SCN after a single injection[80]. Because of this finding, the authors concluded that transcription of clock gene mRNAs was not the immediate target of melatonin, in situations in which melatonin-induced phase advances of circadian rhythms associated with the SCN. A possible post-translation mechanism could involve melatonin inhibition of the ubiquitin–proteasome system[81]. The effects of melatonin on clock gene expression are much more pronounced in the pars tuberalis of the anterior pituitary, whose physiological role consists in the control of reproduction of photoperiodic animals. Melatonin, via abundantly expressed MT1 receptors, influences specific cells in the pars tuberalis, affects clock gene expression, and by inducing prolactin stimulating factor(s) promotes prolactin secretion[82].

The effects of melatonin on clock gene expression in the heart of control and genetically hypertensive (mRen2)27 rats was examined[83]. Melatonin administered in drinking water during the dark phase for 6 weeks affected expression of *Per1*, *Per2* and *Bmal1* in the left ventricle of control as well as hypertensive rats. Expression of *Per2* was increased after the melatonin treatment during the light phase and decreased during the dark phase of LD cycle in both groups of rats. Expression of *Bmal1* was decreased during the light phase and increased during the dark phase after the melatonin treatment. The effect of melatonin on *Bmal1* expression was observed only in controls[83]. However, a recent observation do not support an effect of melatonin on stress- or glucocorticoid-induced phase shifts of Per2 rhythm in lung, pituitary or kidney[84].

The interplay among melatonin, sirtuins and the circadian clock, particularly focusing on sirtuin 1 (SIRT1), will be discussed below in the context of cardioprotection[85]. Overall, the studies provide valuable insights into the role of melatonin in regulating circadian rhythms and its potential therapeutic applications. However, more research is needed to fully understand the mechanisms and implications of these effects.

Since the phase of the SCN is the same in animals of different chronotypes (e.g., increased firing during the day), differences in activity rhythms must be controlled downstream by distinct brain responses to the same stimuli. These regions include the olivary pretectal nucleus, intergeniculate leaflet, and ventral geniculate nucleus, and possibly the ventral subparaventricular zone as a circadian modulator[86].

In both humans and rodents, melatonin has a sleep-promoting effect. In humans, it helps to regulate sleep-wake cycles and improves sleep quality[77]. In rodents, melatonin also promotes sleep, but since rodents are nocturnal, their sleep-wake cycles are opposite to those of humans[87]. As melatonin exhibits the same phase in both diurnal and nocturnal animals it could be suggested that melatonin is not acting on sleep by itself, but rather regulates the expression of MT1 and MT2, located in NREM (including reticular thalamus) or REM areas (including locus coeruleus and lateral hypothalamus). In diurnal animals, the nocturnal overexpression of MT2 receptors increases the sleep drive by activating neurons that promote NREM sleep. In nocturnal animals, the rise in melatonin would downregulate MT2 receptors while upregulating MT1 and other receptors involved in wakefulness, including monoamines and orexin receptors[62].

The hypnotic effect of exogenous melatonin in nocturnal rodents not only depends on the time of day and concentration but is also influenced by the lighting conditions. In a recent study carried out in pigmented male Brown Norway rats under both LD and constant dark (DD) conditions[88], melatonin was administered i.p. at either 1 h after lights on under LD condition or 1 h after the activity offset under DD condition. Only the rats under DD conditions showed a significant reduction in NREM sleep latency but sleep power spectrum remained unaffected. Under LD condition, melatonin did not alter NREM sleep latency. Regardless of lighting conditions, melatonin administration resulted in less, but longer episodes for all vigilance states suggesting increased vigilance state consolidation[88]. Thus, in nocturnal animals, melatonin may consolidate sleep-wake rhythm and through that enhance sleep quality. Indeed, increased melatonin levels during the awake period in rodents do not contradict its role in sleep regulation, but rather align with their nocturnal lifestyle[87].

Overall, endogenous melatonin acts, directly or indirectly, via other internal cues, as an internal circadian synchronizer, consolidating the sleep-wake cycle either in nocturnal or diurnal animals. In nocturnal rodents, exogenous melatonin, entrains body temperature or general locomotor activity rhythms. In humans, exogenous melatonin has a wide range of effects, including a phase-shifting effect and a sleep-promoting effect, justifying the use of melatonin as a drug for sleep disorders[89]. The role of melatonin in promoting sleep and regulating sleep patterns offers valuable therapeutic potential.

Melatonin as a cytoprotector

In the context of CVDs, melatonin has been shown to exert, besides its chronobiotic action, a complex cytoprotective activity that includes antioxidant, anti-inflammatory and epigenetic regulatory functions[90]. It plays a crucial role in several cardiovascular functions, e.g. melatonin decreases the cardiotoxicity mediated by doxorubicin through maintaining Yes-associated protein levels, leading to lower apoptosis and oxidative stress[91]. Among these cytoprotective actions, melatonin's role in lowering inflammation has gotten a lot of attention recently, especially when it comes to therapeutic possibilities for those individuals with low endogenous melatonin levels[92].

Melatonin is a methoxyindole that has been shown to decrease with age and, more importantly, in various age-related non-communicable diseases[93,94]. Low melatonin levels are found in people with chronic diseases, notably coronary heart disease, metabolic syndrome, and type 2 diabetes mellitus[95–99]. Moreover, polymorphisms found in human melatonin receptor genes in prediabetes, type 2 diabetes, elevated cholesterol, and coronary heart disease suggest that melatonergic signaling deviations may favor the development of these disorders. In mice, knocking out the melatonin receptor MT1 resulted in insulin resistance[100].

Melatonin exerts cytoprotective properties via several physiopathogenic mechanisms. One of these is metabolic dysregulation repair, which includes preventing insulin resistance, an inflammation-promoting change that is associated with the metabolic syndrome[101,102]. Melatonin is effective in reducing insulin resistance in a variety of animals and tissues, and with different induction methods. The reduction of serine phosphorylation of insulin receptor substrate 1 (IRS-1) is a key effect, which is frequently followed by an increase in IRS-1 expression[103]. Melatonin and the melatonergic agonist piromelatine are known to reverse the inhibition of insulin signal transduction[104].

Another useful action of melatonin is to avoid processes that promote or lead to inflammation. Calcium overload, excessive nitric oxide (NO) release, which results in the formation of peroxynitrite, peroxynitrite-derived free radicals, and, eventually, tyrosine nitration, are all examples, as is a mitochondrial dysfunction due to oxidative stress[11,105]. Many of these alterations have been connected to low-grade inflammation in a range of organs and are associated with ageing. Melatonin has been found in animal models to protect against these damaging processes by acting as an anti-excitatory agent, protecting mitochondria and lowering peroxynitrite-related damage.

The immunological effects of melatonin are a third significant aspect relevant to melatonin's cytoprotection, Melatonin's many immunomodulatory functions include both pro-inflammatory and anti-inflammatory effects, which result in either pro-oxidant or antioxidant disequilibrium[106–108]. In immunocompromised people, melatonin is often pro-inflammatory. The exact mechanisms by which melatonin acts pro- or anti-inflammatory are unknown, although inflammation intensity and the temporal sequence of initiation and healing processes are certainly involved. The anti-inflammatory effects of melatonin become increasingly essential as people age.

Melatonin decreases pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6 and it increases anti-inflammatory cytokine IL-10 in elderly, ovariectomized female rats[109]. Corresponding findings in the hippocampal dentate gyrus occur, as was an increase in sirtuin (SIRT)1, a protein with strong anti-inflammatory properties[110]. TNF-α and IL-1 levels are lower in the liver, pancreas, and heart of the senescence-accelerated mouse strain SAMP8, whereas IL-10 levels were higher[111–113]. Melatonin has been shown to have anti-inflammatory properties in brain injury, ischemia/reperfusion (I/R) lesions, hemorrhagic shock, and many forms of high-grade inflammation, including endotoxemia and sepsis. For example, the use of melatonin as a SARS-CoV-2 antidote has been advocated[114,115].

In several human diseases melatonin treatment strongly attenuates the increase of circulating cytokine levels. This was documented in patients with diabetes mellitus and periodontitis[116] and in severe multiple sclerosis[117]. Likewise, in the acute phase of inflammation seen during reperfusion of the coronary artery[118], surgical stress[119], or cerebral reperfusion[120] melatonin treatment decreased the levels of pro-inflammatory cytokines. The available experimental information indicates that melatonin has a preventive effect against sepsis-induced kidney damage and septic cardiomyopathy[121,122]. Melatonin has also been reported as beneficial in patients with hypertensive heart disease, cardiomyopathy, pulmonary hypertension, and myocardial infarction[91,123–128].

Distinguishing between direct and indirect effects of melatonin via changes in the phase or amplitude of local circadian oscillators is not always possible[129–131]. Melatonin has been demonstrated to influence various metabolic sensing factors such as peroxisome proliferator-activated receptor coactivator 1 α (PGC-1α), phosphoinositide 3-kinase, protein kinase B, and the accessory oscillator components AMP kinase, NAMPT, and SIRT1. It has been reported that melatonin will induce the induction of antioxidant enzymes in the rat liver and pancreas under inflammatory conditions including the expression and nuclear translocation of nuclear factor erythroid 2-related factor 2 (Nrf2), which mediates the upregulation of protective enzymes[132]. Additionally, melatonin reduces NF-κB production by attracting a histone deacetylase (HDAC) to its promoter, reducing pro-inflammatory molecules such as TNF-, IL-1, and iNOS.

SIRT1 and SIRT3 are two sirtuins that are particularly important for the anti-inflammatory properties of melatonin[133]. SIRT1 is also involved in signaling pathways that participate in the development of cognitive impairment, heart disease, ageing, cancer, and energy homeostasis, including lipid and glucose homeostasis[134]. SIRT1 has been linked to increased longevity and the prevention of neurodegenerative diseases. SIRT1 overexpression in Alzheimer's disease reduces the increase in amyloid-β (Aβ) deposition[135]. SIRT1 overexpression is also beneficial in Parkinson's disease because it reduces acetylation of SIRT1 substrate and inhibits α-synuclein aggregation by preventing protein misfolding[136]. SIRT1, like melatonin, has been shown in numerous studies to have antioxidant and anti-inflammatory properties[133]. This includes inhibiting TLR4 (toll-like receptor 4) signaling, suppressing NF-κB activation, upregulating Nrf2, and suppressing NLRP3 inflammasome activation. TLR4 activation is dependent on the inflammatory signaling protein HMGB1 (high mobility group box-1), which is secreted by monocytes and macrophages[107]. SIRT1 deacetylates HMGB1, preventing its nucleo-cytoplasmic transfer and release. Importantly, HMGB1 promotes the polarization of macrophages and microglia towards the pro-inflammatory M1 type. Melatonin has also been shown to have anti-inflammatory properties through HMGB1 inhibition[133].

Examples of sirtuin-mediated suppression by melatonin were discovered in more severe inflammation. This was observed in normal and diabetic rats with cardiac I/R, H9C2 cardiomyocytes with endoplasmic reticulum stress, LPS-treated microglial cell lines, and mice with cecal ligation/puncture-induced brain injury[107]. SIRT3 regulates the pyruvate dehydrogenase complex (PDH) and participates in ATP synthesis, making it an important factor in mitochondrial function.

Several studies have discovered that melatonin acts at the mitochondrial level via SIRT3[133], switching from cytosolic aerobic glycolysis to oxidative phosphorylation, thus enhancing ATP biosynthesis, ATP production-coupled oxygen consumption rate, and lactic acid secretion. Melatonin, through its impacts on SIRT3 and PDH, boosted the mitochondrial membrane potential and the activity of complexes I and IV in the electron transport chain. Melatonin significantly improves mitochondrial energy metabolism by reversing the Warburg effect and stimulating SIRT3[137]. Melatonin induces the transformation of pro-inflammatory glycolytic M1

macrophages into anti-inflammatory M2 macrophages[138]. Melatonin stimulates mitochondrial pyruvate metabolism, the tricarboxylic acid cycle, oxidative phosphorylation, and ROS production via down-regulating hypoxia-inducible factor 1 (HIF-1), resulting in PDH disinhibition. Melatonin and its metabolites are especially efficient direct scavengers of partly reduced derivatives of oxygen under these circumstances, in addition to decreasing mitochondrial ROS generation.

Sirtuins and circadian clock components collaborate to control oxidative metabolism through NAD^+ and NADH responses[139,140]. The CLOCK-BMAL1 heterodimer not only activates the clock genes *Per* and *Cry*, as well as other clock-controlled genes, but it also regulates the activity of the gene *Nampt*, which encodes the rate-limiting enzyme nicotinamide phosphoribosyl transferase (NAmPRTase or NAMPT), the metabolite of which is NAD^+. The synthesis of NAD^+ has a distinct daily cycle due to oscillations in NAMPT levels. The distribution of NAD^+ in the cytosol, nucleus, and mitochondria maintains the cellular redox status, which is required for the normal functioning of the bioenergetic enzymatic machinery[139,140]. The findings suggest that a complex set of regulators, including SIRT1, stabilizes the molecular circadian clock via multiple mechanisms. Melatonin regulates SIRT1 activity, which may be central to the cytoprotective and chronobiotic effects of methoxyindole[141–144].

SIRT1 signaling in antioxidative response pathways mediates melatonin's cardioprotective action during I/R. The antioxidant enzymes manganese superoxide dismutase (MnSOD) and catalase are produced when SIRT1 is deacetylated. Acetylated FOXO1 (Ac-FOXO1) increases apoptosis. SIRT1 and Ac-FOXO1 expression were drastically enhanced and lowered in melatonin-treated myocardial I/R rats, respectively. In the I/R plus vehicle group, SIRT1 expression was lowered whereas Ac-FOXO1 expression was dramatically elevated[145]. Melatonin therapy boosted antiapoptotic gene Bcl-2 expression by upregulating SIRT1 and thereby reducing Ac-FOXO1.

Because it reduces oxidative stress, regulates inflammatory responses, and inhibits apoptotic pathways, SIRT1 is also the effector responsible for melatonin's protective role in kidney function in severely burned rats[146,147]. SIRT1 contributes to melatonin's protective role after cecal ligation and puncture in a C57BL/6 J mouse model of sepsis[148]. Melatonin alleviates the neuroinflammatory and oxidative stress caused by septic encephalopathy[149]. A SIRT1 inhibitor reduced this benefit, implying that melatonin's beneficial effect is mediated by SIRT1[148]. The activation of the NLRP3 inflammasome in several systems, under various conditions, and the effects of melatonin have been reviewed[150–152]. Melatonin's modulation of NF-κB signaling, which is vital in the protection of oxidative damage, was connected to these findings. Additionally, NF-κB has been found to promote pyroptosis in adipose tissue, which melatonin inhibits. TLR4 activation via the IFN-adaptor protein, a toll-receptor-associated activator of interferon (TRIF), is another pro-inflammatory pathway[153,154]. Melatonin has been demonstrated to block TRIF and TLR4 and hence lower the production of pro-inflammatory cytokines such as TNF- α, IL-1, IL-6, and IL-8. Melatonin's effects on this pathway are likely to be more widespread because TLR4 also causes prooxidant actions via NF-κB. Overall, melatonin exhibits cytoprotective activity in CVDs through several mechanisms, including repair of metabolic dysregulation, reduction of oxidative stress, inhibition of inflammatory processes, and modulation of immune responses.

Melatonin and heart rate variability

Few studies examined the relation between melatonin and autonomic nervous system (ANS) activity measured by HRV in humans. The utilization of HRV serves as a valuable indicator of autonomic control but requires careful interpretation. Increased Root Mean Square of the Successive Differences (RMSSD) and HF HRV indexes are commonly construed as indicators of vagal tone. Meanwhile, LF components may reflect a combination of sympathetic and parasympathetic activities at varying ratios. Furthermore, it is important to note that numerous HRV metrics are directly influenced by the absolute heart rate, which is governed by multiple factors beyond autonomic signaling alone. That being stated, results show that the administration of melatonin (1.5 mg) can advance the endogenous circadian rhythm phase of heart rate and RMSSD and HF[59]. Compared with placebo, melatonin (2 mg) administration increased R-R interval, the RMSSD, HF power, and LF power of HRV and decreased the LF/HF and BP in the supine position. Plasma norepinephrine and dopamine levels in the supine position 60 min after melatonin administration were lower compared with placebo. These findings indicated that melatonin administration increased cardiac vagal tone in the supine position in awake men[155]. Another study assessed the effects of 5 mg melatonin before sleep in patients with coronary artery disease and with an abnormal circadian pattern of BP on changes in circadian BP profile and heart rate variability. It was observed a decrease in nighttime BP and an increase in daytime BP in the treatment but not in the control group, with no significant changes in HRV patterns[156]. Also, it was studied the effects of melatonin (3 mg) replacement therapy on cardiac autonomic modulation in pinealectomized patients. Melatonin treatment increased vagal-dominated HRV indices including RMSSD and HF power. These HRV indices returned to pretreatment values when melatonin treatment was discontinued[157]. Finally, in an exploratory observational study in patients with mild cognitive impairment, we reported that those under melatonin treatment (3–50 mg daily) presented greater parasympathetic activity (RRm, SDNN, VLF, LF, HF) during sleep and in sleep-wake differences than patients not taking melatonin[56].

The exact mechanisms by which melatonin elicits vagal predominance are not well-defined but are possibly related with the inhibition of sympathetic or stimulation of parasympathetic system through bidirectional pathways within the central autonomic network[158]. Additionally, as discussed above, melatonin intimately participates in regulating the sleep-wake cycle, which in turn determines the prevalence of nocturnal parasympathetic and diurnal sympathetic activity.

Melatonin's therapeutic value in CVDs

The therapeutic value of melatonin in CVDs is remarkable. The methoxyindole significantly decreases nocturnal systolic, diastolic, and mean BP[159]. This effect is inversely related to the day-night difference in BP. The nocturnal decline of BP is almost coincident with the elevation of circulating melatonin, which may exert vasodilating and hypotensive effects. Melatonin also reduces the pulsatility index (PI) of the internal carotid artery in young women[160]. The PI is an index of resistance distal to the sampling site, and a decrease in PI suggests a reduction in vasoconstriction.

The effects of melatonin on BP may also involve the reduction of inflammation and oxidative damage, as well as the promotion of endothelial function[161]. It has been described that melatonin lowers angiotensin II and endothelin levels while elevating NO and endothelial nitric oxide synthase (eNOS)[162]. Additionally, melatonin leads to a decrease in asymmetric dimethylarginine (ADMA) (which inhibits nitric oxide production), increases the arginine-to-ADMA ratio, and reduces the amount of 8-hydroxydeoxyguanosine immunostaining, a marker of DNA oxidative damage[163]. Furthermore, melatonin appears to play a role in epigenetic modification by preventing cellular programs that contribute to hypertension[164].

Several mechanisms may explain the role of melatonin in preventing in ischemia-reperfusion injury and myocardial damage. Melatonin has been shown to reduce platelet aggregation[165], and also has the ability to attach to calcium-binding proteins such as calmodulin, thereby inhibiting the activation of myosin light-chain kinase, which in turn will decrease the contractile response in different smooth muscles[166]. Melatonin may act by stimulating the central inhibitory adrenergic pathways, thereby diminishing the basal tone of the peripheral sympathetic nervous system and serum catecholamine levels[18]. Myocardium melatonin membrane receptors regulate numerous survival signaling pathways, such as SIRT1[145] and Notch1/Hairy and enhancer of split 1 (Hes1)[167]. Melatonin could ameliorate oxidative stress, reduce apoptosis, and restore cardiac function by regulating cyclic guanosine monophosphate (cGMP)–protein kinase GI α(PKGIα) signaling, Mitogen-activated protein kinase (MAPK) cascade and Nrf2/Heme oxygenase 1 (HO-1) axes[168], as well as Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) signaling pathway, all

involved in in ischemic myocardium and reperfusion injury[169]. After a myocardial infarction or reperfusion injury, reducing mitochondrial fission can ameliorate damage, while promoting mitochondrial fusion can stimulate the mitochondria and allow damaged mitochondria to repair themselves. Research has shown that melatonin increases levels of Unc-51-like autophagy-activating kinase 1 (ULK1), Ras-related in brain GTPases 9 (Rab9), and P-ULK1, while decreasing levels of dynamin-related protein (pDrp1) and the mitochondrial P/t Drp1 ratio. This suggests that melatonin may inhibit mitochondrial fission[170]. Additionally, melatonin has been shown to preserve myocardial function, decrease the infarct area, and reduce cardiac myocyte death in response to cardiac reperfusion stress by increasing the expression of Optic atrophy GTPase 1 (OPA1) via the Yes-associated protein (Yap)–Hpo kinase (Hippo) signaling pathway, which largely restores mitochondrial fusion[171]. Ischemia-reperfusion injury is caused by Ca^{2+} excess, which leads to cardiomyocyte death by apoptosis and necroptosis. There is evidence that melatonin may protect the heart from reperfusion damage by enhancing calcium handling in cardiomyocytes through an antioxidant mechanism[172], inhibiting the Receptor-interacting protein kinase (Ripk3)/Phosphoglycerate mutase family member 5 (PGAM5)/Cyclophilin D (CypD)/Mitochondrial permeability transition pore (mPTP) cascade[173] and regulating the cardiac proteins Inositol 1,4,5-trisphosphate receptor (IP3R) and Sarco/endoplasmic reticulum calcium ATPase 2a (SERCA2a) to decrease calcium overload, via the Extracellular signal-regulated kinase 1 (ERK1) pathway[174].

In atherosclerosis, melatonin inhibits the Toll-like receptor 4 (TLR4)/NF-κB pathway, improving lipid metabolism, vascular endothelial dysfunction, and inflammation, as well as slowing the progression of the disorder[175]. Long-term administration of melatonin in hypercholesterolemic rats reduces fatty infiltration in the intima and alters the plasma fatty acid content[176]. It also suppress atherosclerosis triggered by cigarette smoke by affecting the Nrf2/ROS/NLRP3 axis, possibly involved in nicotine-induced endothelial cell pyroptosis[177]. Melatonin may reduce plaque formation and may stabilize plaques through several mechanisms. It suppresses inflammation inside the plaque by preventing plaque macrophages from differentiating into the M1 phenotype by affecting the retinoic acid receptor-related orphan receptor alpha (RORα). and can modify the macrophage phenotype through RORα and by affecting the AMP-activated protein kinase alpha (AMPKα)/STATs axis[178]. In addition, melatonin stabilizes plaques by upregulating Prolyl 4-hydroxylase alpha (P4Hα1)[179]. Finally, it has been shown to activate the hepatocyte growth factor (HGF)/HGF receptor (c-Met) axis, which prevents macrophage infiltration and improve plaque stability[180].

Several experimental studies report the beneficial effects of melatonin treatment in various heart failure (HF) models, addressing significant pathogenic processes associated with HF, including oxidative stress, apoptosis, necrosis, necroptosis, fibrosis, autophagy, inflammation, and pathological remodeling and dysfunction[181]. In patients with HF with reduced ejection fraction melatonin administration normalizes the BP circadian rhythm, reduces cardiomyocyte loss, and improves the left ventricular function[182]. Melatonin may improve ischemic HF by reducing oxidative stress-induced damage to cardiac tissue, enhancing activities of cardiac Na^+,K^+-ATPase and SERCA, and decreasing levels of myeloperoxidase (MPO), caspase-3 expression, caveolin-3, GSH, and MDA in the cardiac tissue[183]. Additionally, melatonin activates Nrf2 in cardiomyocytes, leading to the secretion of C1q/tumor necrosis factor-related protein 3 (CTRP3) by adipose tissue. Deficiency of CTRP3 is associated with increased oxidative stress and cell apoptosis in cardiomyocytes, potentially mitigating obesity-related heart failure with preserved ejection fraction[184]. In isoproterenol-induced heart failure models, melatonin demonstrates anti-remodeling effects, reduces mortality, and increases survival time. These effects are associated with melatonin's ability to inhibit oxidative stress development, reduce levels of insoluble and total collagen, and prevent beta-tubulin alteration in the left ventricle[185]. Melatonin also protects against mitochondrial dysfunction in cardiomyocytes by maintaining 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNPase) content, which is elevated during acute heart failure to prevent mitochondrial permeability transition pore opening[185]. Moreover, melatonin modulates extracellular matrix homeostasis in the left ventricular myocardium by improving the balance between Matrix metalloproteinase-1 (MMP-1) and T-cell immunoglobulin and mucin domain-1 (TIM-1) protein expression[186].

Melatonin has also shown value in drug-induced cardiotoxicity, stem cell therapy for heart regeneration, cardiac arrhythmias, septic cardiomyopathy, cardiorenal syndrome, myocarditis, and Chagas' disease[187]. It is important to note that while these effects have been observed in various studies, the exact mechanisms are still being researched and may vary depending on the dosage and on individual factors such as age and health status.

It is important to note that while these effects have been observed in various studies, the exact mechanisms are still being researched and may vary depending on the dosage and on individual factors such as age and health status. Indeed, CVDs consistently co-exist with reduced circulating melatonin levels and a modest number of clinical trials with melatonin doses ranging from 3 to 24 mg/day yielded partially favorable results. The reader is addressed to excellent review articles that have been published in the last years on melatonin activity in CVDs[91,123–128] A scheme of the different mechanisms involved in melatonin activity on CVDs is shown in Fig. 2.

A major caveat on melatonin activity in CVDs concerns to the dosage employed. Tables 1 and 2 summarize published data on the effectivity of melatonin in reducing symptomatology in animal models of non-ischemic and ischemic cardiac damage.

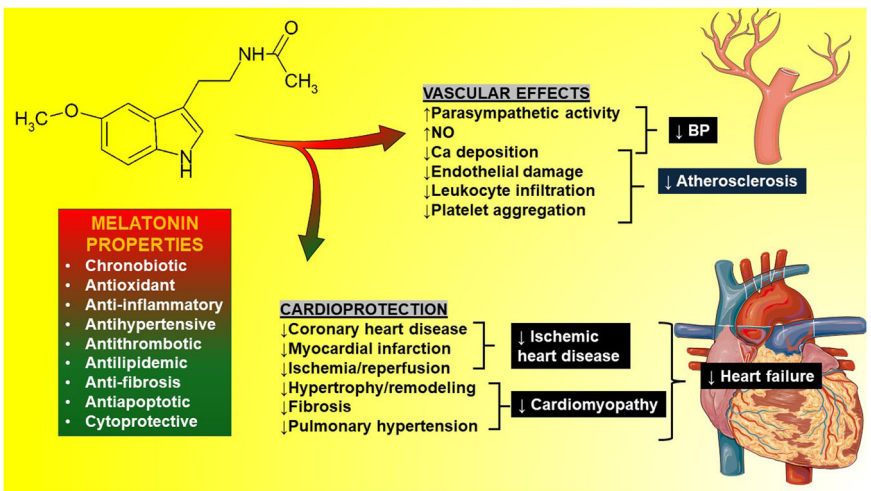

Fig. 2 | Cardiovascular protection by melatonin. The detailed mechanisms are explained in the text.

Table 1 | Effect of systemically administered melatonin on animal models of ischemic heart damage

Findings	Daily melatonin dose	HED for a 75 kg adult	Ref.
In a rat model of myocardial infarction melatonin produced cardioprotection with more than 80% reduction of area and number of lesioned myocardium	6 mg/kg p.o.	75 mg	197
In a rat model of myocardial I/R injury protective effects of melatonin on myocardial infarction size and heart oxidative changes were reported	10 mg/kg i.p.	120 mg	198
In cardiomyopathic hamsters melatonin prevented microvascular injury and ventricular arrhythmia	6 mg/kg i.p.	60 mg	199
In a rat model of myocardial I/R injury melatonin-Induced cardioprotection	50 mg/kg i.p.	600 mg	200
In a rat model of isoproterenol-induced myocardial infarction melatonin decreased cardiac injury markers and augmented cardiac antioxidant defense system	10 mg/kg i.p.	120 mg	181
Melatonin ameliorated vascular endothelial dysfunction, inflammation, and atherosclerosis by suppressing the TLR4/NF-κB system in high-fat-fed rabbits	10 mg/kg p.o.	220 mg	175
In a rat model of myocardial infarction melatonin caused cardioprotection by augmenting cardiac Na^+, K^+-ATPase and SERCA activities, glutathione content and caveolin-3 levels and preventing heart oxidative damage	10 mg/kg i.p.	120 mg	183
Melatonin alleviated myosin light-chain kinase expression and activity via the mitogen-activated protein kinase pathway during atherosclerosis in rabbits	20 mg/kg i.p.	440 mg	201
In a model of myocardial I/R injury in type 2 diabetic rats melatonin ameliorated reperfusion-induced oxidative stress and improved cardiac function	20 mg/kg p.o.	240 mg	202
In a murine model of myocardial infarction melatonin produced cardioprotection via melatonin receptors	10 mg/kg i.p.	60 mg	203
In a murine model of myocardial I/R injury melatonin reduced myocardial apoptosis and oxidative stress	12 mg/kg i.p.	72 mg	204
In a murine model of post-infarction cardiac remodeling and dysfunction, melatonin reversed adverse left ventricle remodeling, increased autophagy, and reduced apoptosis and mitochondrial dysfunction via MST1 inhibition	20 mg/kg p.o.	120 mg	205
In a murine model of myocardial infarction melatonin decreases post-myocardial infarction damage, by preserving mitochondria integrity via Tom70 expression	10 mg/kg i.p.	60 mg	206
In a murine model of myocardial I/R injury melatonin protects the heart through SIRT3-dependent regulation of oxidative stress and apoptosis	20 mg/kg i.p.	120 mg	207
In a murine model of myocardial I/R injury melatonin suppressed platelet activation and reduced heart damage via PPARγ/FUNDC1/mitophagy pathways	20 mg/kg i.p.	120 mg	208
In a rat model of myocardial I/R injury melatonin attenuated injury by inhibiting autophagy Via AMPK/mTOR signaling pathway	20 mg/kg i.p.	240 mg	209
In a murine model of myocardial I/R injury melatonin protected against myocardial injury by elevating SIRT3 expression and Mn superoxide dismutase activity	10 mg/kg i.p.	60 mg	210
In a rat model of myocardial I/R injury melatonin-Induced cardioprotection	20 mg/kg i.p.	240 mg	174
Melatonin ameliorated the progression of atherosclerosis via mitophagy activation and NLRP3 inflammasome inhibition	20 mg/kg i.p.	120 mg	211
In a rat model of diabetic I/R injury melatonin ameliorated heart function and reduced cardiac cell apoptosis and oxidative stress	10 mg/kg i.p.	120 mg	212
In a murine model of myocardial I/R injury melatonin decreased endothelial necroptosis	20 mg/kg i.p.	120 mg	
In a murine model of rupture-prone vulnerable atherosclerotic plaques melatonin stabilized them via regulating macrophage polarization in a circadian receptor RORα-dependent manner	10 mg/kg p.o.	60 mg	178
In a rat model of myocardial I/R injury melatonin improved the protective remote ischemic preconditioning against injury	10 mg/kg i.p.	120 mg	213
In a rat model of myocardial I/R injury melatonin protected heart via the JAK2/STAT3 signaling pathway	10 mg/kg i.p.	120 mg	169
In apolipoprotein E knockout murine model melatonin enhanced atherosclerotic plaque stability by inducing prolyl-4-hydroxylase α1 expression	30 mg/kg i.p.	180 mg	179
Melatonin inhibited in smooth muscle cell inflammation and proliferation in apolipoprotein E-deficient mice	30 mg/kg p.o.	60 mg	214
In a murine model of myocardial I/R injury melatonin protected the heart via improving mitochondrial fusion/mitophagy and activating the AMPK-OPA1 signaling pathways	20 mg/kg i.p.	120 mg	215
In atherosclerotic rabbits melatonin promoted plaque stabilization by upregulating anti-inflammatory mechanisms	10 mg/kg p.o.	220 mg	180
In a rat model of cigarette smoke-induced vascular injury melatonin reduced injury by activating the Nrf2 pathway via NLRP3 inflammasomes in endothelial cells	10 mg/kg i.p.	120 mg	177
In hypercholesterolemic rats melatonin improved atherosclerosis-induced endothelial dysfunction	5 or 10 mg/kg p.o.	60 or 120 mg	216
In a rat model of myocardial I/R injury, melatonin inhibited myocardial apoptosis during IR and protected mitochondrial structure and function	10 mg/kg i.p.	120 mg	217

ATP Adenosine triphosphate, *AMPK* AMP-activated protein kinase, *FUNDC1* FUN14 domain containing 1, I/R ischemia/reperfusion, *JAK2* Janus kinase 2, *MST1* macrophage-stimulating 1, *mTOR* mammalian target of rapamycin, *NF* Nuclear factor, *NLRP3* NLR family pyrin domain containing 3, *Nrf2* Nuclear factor erythroid 2-related factor 2, *OPA1* optic atrophy 1, *PPARγ* peroxisome proliferator-activated receptor γ, *RORα* RAR-related orphan receptor alpha, *SERCA* sarcoplasmic/endoplasmic reticulum Ca2+-ATPase, *SIRT* Sirtuin, *STAT3* signal transducer and transcription activator 3, *TLR4* Toll-like receptor 4.

Table 2 | Effect of systemically administered melatonin in animal models of non-ischemic heart damage

Findings	Daily Melatonin Dose	HED for a 75 kg adult	Ref.
In a rat model of doxorubicin-induced cardiotoxicity, melatonin protected the heart from oxidative damage	4 mg/kg i.p.	48 mg	218
In a murine model of doxorubicin cardiotoxicity melatonin protected against heart damage and increased antitumor activity	5 mg/kg i.p.	30 mg	219
In a rat model of doxorubicin-induced cardiotoxicity, melatonin protected the heart from oxidative damage	50 µg/kg i.p.	0.6 mg	220
In a murine model of doxorubicin cardiotoxicity melatonin protected against heart oxidative damage	6 mg/kg i.p.	36 mg	221
In a rat model of doxorubicin-induced cardiotoxicity, melatonin protected the heart from oxidative damage	10 mg/kg s.c.	120 mg	222
In a murine model of doxorubicin-induced cardiomyopathy melatonin protected against cardiotoxicity without interfering with antitumor effect	10 mg/kg p.o.	60 mg	223
In a rat model of doxorubicin-induced cardiotoxicity melatonin reduced oxidative stress in cardiac cells	5 mg/kg i.p.	60 mg	224
In a rat model of severe obstructive sleep apnea, melatonin augmented right ventricular function and reduced oxidative stress and cardiac interstitial fibrosis	10 mg/kg i.p.	120 mg	225
In a rat model of doxorubicin-induced cardiotoxicity, melatonin protected the heart from oxidative damage	4 mg/kg i.p.	48 mg	226
In a rat model of doxorubicin -induced cardiotoxicity melatonin prevented oxidative damage and augmented antitumor activity	10 mg/kg s.c.	120 mg	227
In a rat model of sepsis melatonin protected against cardiac injury as a free radical scavenger and antioxidant	10 mg/kg i.p.	120 mg	228
In a rat model of cyclosporin -induced cardiotoxicity melatonin Increased heart antioxidant enzymes and normalize d cardiac morphology	1 mg/kg i.p.	12 mg	229
In a rat model of doxorubicin-induced cardiotoxicity melatonin prevented lipid peroxidation and myocardial lesions	10 mg/kg i.p.	120 mg	230
In a murine model of septic cardiomyopathy melatonin suppressed iNOS/imt NOS activity triggered by sepsis and restored mitochondrial function	30 mg/kg i.p. and s.c.	180 mg	231
In a rat model of epirubicin-induced cardiotoxicity melatonin prevented heart nitrosative damage	200 µg/kg	2 mg	232
In a rat model of cardiotoxicity caused by doxorubicin plus trastuzumab administration melatonin reversed oxidative stress markers in heart	20 mg/kg	120 mg	233
In a murine model of doxorubicin toxicity melatonin inhibited drug-induced lipid peroxidation in heart, liver and kidney	5 mg/kg i.p.	30 mg	234
Melatonin improved rat cardiac mitochondria and survival rate in septic heart injury	30 mg/kg i.p. and s.c.	360 mg	235
In a rat model of doxorubicin-induced cardiotoxicity melatonin prevented oxidative damage	10 mg/kg i.p.	60 mg	236
In a rat model of doxorubicin-induced cardiotoxicity melatonin Inhibited cardiac damage	10 mg/kg i.p.	60 mg	237
In a rat model of pulmonary hypertension due to chronic intermittent hypoxia, melatonin reduced cardiac fibrosis and oxidative stress	15 mg/kg i.p.	180 mg	238
In a murine model of septic cardiomyopathy melatonin suppressed iNOS/imt NOS activity, enhanced mitochondrial function and nNOS/c-mtNOS	30 mg/kg i.p. and s.c.	180 mg	239
In a rat heart failure model induced by isoproterenol, melatonin decreased cardiac fibrosis, oxidative stress and insoluble and total collagen	10 mg/kg p.o.	120 mg	240
In a model of hypertension in rats induced by continuous light, melatonin reduced cardiac fibrosis and oxidative stress, but did not affect left ventricle hypertrophy	10 mg/kg p.o.	120 mg	241
In a murine model of diabetic cardiomyopathy melatonin ameliorated metabolic risk factors, modulates apoptotic proteins, and protects the rat heart against diabetes-induced apoptosis	10 mg/kg i.p.	60 mg	242
In a rat model of monocrotaline-induced pulmonary hypertension, melatonin decreased right ventricular hypertrophy, systemic oxidative stress and cardiac interstitial fibrosis	6 mg/kg p.o.	72 mg	243
In a rat model of septic cardiomyopathy melatonin attenuated sepsis-induced cardiac dysfunction via a PI3K/Akt-dependent mechanism	30 mg/kg i.p.	360 mg	244
In a murine model of septic cardiomyopathy melatonin prevented sepsis-dependent mitochondrial injury and improved mitochondrial respiration	30 mg/kg i.p.	180 mg	245
In a rat model of doxorubicin-induced cardiotoxicity, melatonin improved heart contractility and hemodynamics	10 mg/kg i.p.	120 mg	246
In a murine model of septic cardiomyopathy melatonin decreased NLRP3 and inhibited caspase-1 and IL-1β	30 mg/kg i.p. and s.c.	180 mg	152
In a model of murine cardiac hypertrophy induced by transverse aortic constriction, melatonin reduced body weight gain, left ventricular fibrosis and diastolic dysfunction, decreased cardiac oxidative stress and inflammation and augmented insulin signaling	20 mg/kg p.o.	120 mg	247
Melatonin protects against diabetic cardiomyopathy through Mst1/SIRT3 signaling	20 mg/kg i.p.	120 mg	248
In a murine model of diabetic cardiomyopathy melatonin prevented mitochondrial fission through SIRT1-PGC-1α pathway	10 mg/kg i.p.	60 mg	249
In a rat model of doxorubicin-induced cardiotoxicity, melatonin improved cardiac and mitochondrial function through SIRT1-PGC-1α pathway	6 mg/kg p.o.	72 mg	250
In a murine model of doxorubicin cardiotoxicity melatonin attenuated mitochondrial oxidative damage and apoptosis	20 mg/kg p.o.	120 mg	251

Table 2 (continued) | Effect of systemically administered melatonin in animal models of non-ischemic heart damage

Findings	Daily Melatonin Dose	HED for a 75 kg adult	Ref.
In a murine model of septic cardiomyopathy melatonin balanced the autophagy and apoptosis via regulation of UCP2	30 mg/kg i.p.	180 mg	252
In a mouse model of coxsackie virus B3-infected myocarditis, melatonin counteracted myocardial injury	14.4 mg/kg i.p.	87 mg	253
In a murine model of cardiomyopathy melatonin activated Parkin translocation and rescues the impaired mitophagy activity through Mst1 inhibition	20 mg/kg p.o.	120 mg	254
In a rat model of ventricular hypertrophy induced by abdominal aortic constriction, melatonin prevented changes in cardiac fibrosis and gene expressions of HDAC1, HDAC2, HDAC3, HDAC4 in cardiomyocytes	10 mg/kg i.p.	120 mg	255
In a rat model of diabetic cardiomyopathy, melatonin ameliorates myocardial apoptosis by suppressing endoplasmic reticulum stress	10 mg/kg p.o.	120 mg	256
In a murine chronic pain model, melatonin attenuated susceptibility by inhibiting RIP3-MLKL/CaMKII-dependent necroptosis	20 mg/kg i.p.	120 mg	257
In a murine model of diabetic cardiomyopathy melatonin improved heart function involving the Syk-mitochondrial complex I-SERCA pathway	20 mg/kg i.p.	120 mg	258
In a rat model of doxorubicin-induced cardiotoxicity, melatonin counteracted cardiac damage	40 mg/kg i.p.	480 mg	259
Melatonin protected against streptozotocin-induced diabetic cardiomyopathy through the mTOR signaling pathway in rats	50 mg/kg i.p.	600 mg	260
In a murine model of septic cardiomyopathy melatonin protected against cardiac dysfunction by regulating apoptosis and autophagy via activation of SIRT1	30 mg/kg i.p.	180 mg	261
In a murine model of septic cardiomyopathy melatonin suppressed cardiac injury by regulating mitochondrial activity and cytoskeletal organization	20 mg/kg i.p.	120 mg	262
Melatonin alleviated cardiac fibrosis via inhibiting NLRP3 inflammasome and TGF-β1/Smads signaling in murine diabetic cardiomyopathy	10 mg/kg intragastrically	60 mg	263
In a murine model of doxorubicin-induced cardiomyopathy melatonin attenuated cardiotoxicity, and decreased oxidative stress and apoptosis	10 mg/kg p.o.	60 mg	264
In a rat model of doxorubicin-induced cardiotoxicity melatonin was more effective than a thymoquinone to reduce cardiotoxic effects	10 mg/kg i.p.	120 mg	265
In a murine model of septic cardiomyopathy melatonin attenuated ER stress and mitochondrial damage via BAP31 upregulation and MAPK-ERK pathway	10 and 20 mg/kg i.p.	60, 120 mg	122
The cardioprotective effect of melatonin against doxorubicin-induced cardiotoxicity in rats are through preserving mitochondrial function and dynamics	10 mg/kg p.o.	120 mg	266
In a rat model of doxorubicin-induced cardiotoxicity melatonin was more effective than adrenomedullin to reduce cardiotoxic effects	10 mg/kg i.p.	120 mg	267
Melatonin attenuated rat diabetic cardiomyopathy and reduces myocardial vulnerability to I/R injury by improving mitochondrial quality control via SIRT6	10 mg/kg p.o.	120 mg	268
Synergistic cardioprotective effects of melatonin and deferoxamine through the improvement of ferritinophagy in doxorubicin-induced acute cardiotoxicity	20 mg/kg i.p.	240 mg	269
In a rat model of diabetic cardiomyopathy, melatonin alleviated hyperglycemia-induced cardiomyocyte apoptosis via regulation of long non-coding RNA H19/miR-29c/MAPK axis	10 mg/kg p.o.	120 mg	270
In a murine model of doxorubicin-induced cardiomyopathy melatonin restored autophagic flux via SIRT3/TFEB signaling pathway	20 mg/kg p.o.	120 mg	271
In a rat model of doxorubicin-induced cardiomyopathy melatonin affected cardiac metabolic reprogramming	10 mg/kg p.o.	120 mg	272
Melatonin ameliorated arsenic-induced cardiotoxicity through the regulation of the SIRT1/Nrf2 pathway in rats	10–30 mg/kg p.o.	120–360 mg	273
In a murine model of diabetic cardiomyopathy, melatonin-activated Parkin translocation and rescues impaired mitophagic activity	20 mg/kg i.p.	120 mg	274

Akt Ak strain transforming, *BAPI* AMP- BRCA1 associated protein-1, *CamKII* Ca2+/calmodulin-dependent protein kinase II, *c-mtNOS* constitutive mitochondrial nitric oxide synthase, *ER* endoplasmic reticulum, *ERK* extracellular signal-regulated kinase, *HDAC* histone deacetylase, *imt* Inducible mitochondrial nitric oxide synthase, *iNOS*; inducible nitric oxide synthase, *Mst1* macrophage-stimulating 1, *mTOR* mammalian target of rapamycin, *UCP2* mitochondrial uncoupling protein 2, *MAPK* mitogen-activated protein kinase, *MLKL* mixed lineage kinase domain-like pseudokinase, *nNOS* neuronal nitric oxide synthase, *NLRP3* NLR family pyrin domain containing 3, *Nrf2* Nuclear factor erythroid 2-related factor 2, *PGC-1α* peroxisome proliferator-activated receptor coactivator 1 α, *PI3K* phosphoinositide 3-kinase, *RIP3* receptor-interacting protein 3, *SERCA* sarcoplasmic/endoplasmic reticulum Ca2+-ATPase, *SIRT* Sirtuin, *TFEB* transcription factor EB, *TGF-β1* transforming growth factor beta 1.

In all cases the human equivalent dose (HED) for a 75 kg adult was calculated by normalizing body surface area from animal doses[188–190]. Body surface area has been advocated as a factor to use when converting a dose for translation from animals to humans because it correlates well with several biological parameters such as O_2 consumption, caloric expenditure, basal metabolism, blood volume, circulating plasma proteins, and renal function across several mammalian species. Allometric calculations based on animal studies predict that cytoprotective melatonin doses for humans should be in the 100 mg/day range, doses which are rarely used clinically with some exceptions e.g. in dose escalation studies with doses up to 100 mg, melatonin did not show any toxicity in humans[191,192]. It is important to note that melatonin is extremely atoxic. The lethal dose-50 for melatonin i.p. injection was determined for rats (1168 mg/kg) and mice (1131 mg/kg), but the lethal dose for melatonin oral administration (assessed up to 3200 mg/kg in rats) and melatonin s.c injection (assessed up to 1600 mg/kg in rats and mice) could not be determined[193]. In humans, melatonin has an excellent safety profile and is generally well tolerated[194,195]. Therefore melatonin's potential and usefulness in CVDs need to be explored and further investigated, which calls for multicenter double-blind trials.

Concluding remarks

CVDs, cancer, respiratory diseases, diabetes, and neurological diseases account for >80% of non-communicable disease deaths, which are strongly linked to disability, reliance, and long-term care needs[196]. In this review, we covered two main etiopathogenic processes that lead to CVDs: inflammation and daily rhythm disturbance, the latter produced by living in a 24/7 Society that spoils the sleep/wake cycle. As a result of sleep/wake cycle dysregulation, a plethora of pathophysiological alterations that accelerate ageing occur. Melatonin treatment emerges as a feasible non-toxic chronobiotic/cytoprotective strategy in this scenario.

Because of the HED of melatonin determined from preclinical studies, melatonin dosages need to be reviewed. Indeed, given the number of scientific/medical papers that have recommended its use, melatonin's failure to garner attention as a potential treatment for healthy aging is discouraging. The pharmaceutical business is not motivated to promote the use of melatonin because it is not patentable. Nonetheless, it would be wise for the pharmaceutical business to research the possibility of a profitable and medically effective combination of melatonin with specific medications. Due to its low cost, minimal toxicity, and ability to be taken orally, melatonin would be particularly advantageous.

Received: 21 February 2024; Accepted: 16 July 2024;
Published online: 09 September 2024

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Acknowledgements

This research did not receive a specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contributions

Writing—original draft preparation, D.P.C.; writing—review and editing, D.E.V. Both authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare no competing interests.

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