It's about time: Circadian rhythm and metabolism

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General discussion and future perspectives

Throughout evolution, humans have lived in synchrony with the natural light-dark cycle. Our bodies were used to going to sleep a few hours after dark, and waking up just before dawn. However, in modern society the unambiguous availability of artificial light as well as the need to have 24/7 access to goods and services have desynchronized our biological clock from the naturally occurring day and night. The consequence of artificial light exposure in the late evening or early night is a backward shift of our biological clock. This forms a problem when we set our alarm clock to forcefully wake up in the morning to go to work, resulting in sleep deprivation and misalignment of our biological clock and behavior. Sleep is an essential process for our health and well-being. Short-term sleep deprivation mainly has psychological consequences such as increased stress, mood disorders, impaired cognition and memory, and behavior problems [1]. On the long-term, sleep deprivation also has major physical consequences, such as increased risk of obesity, osteoporosis, dyslipidemia, and cardiovascular disease (CVD) [2-5]. In line with this, exposure to light at night, which impairs sleep quality and quantity, is associated with obesity and dyslipidemia [6]. Thus, adhering to your biological clock by maintaining a consistent sleep schedule with a sufficient sleep duration is essential to maintain metabolic health.

For many people, maintaining a consistent sleep schedule is simply not feasible. In order to meet the demands of globalization and our 24/7 economy, a significant part of the population has to work outside of regular office hours. Working nights or in rotating shifts disrupt the biological clock, and like sleep deprivation, are associated with increased risk of among others cardiometabolic diseases and metabolic bone disorders [7-9]. Nowadays, 20% of the global working population is involved in some form of shift work [10-12], which could thus have major health consequences. Given the high prevalence of shift work, the development of novel strategies to reduce disease risk in this population is of utmost scientific priority.

This thesis has provided novel insights into the mechanisms through which circadian disruption leads to (cardio)metabolic diseases. Furthermore, two main approaches have been investigated to prevent diseases associated with circadian disturbances, namely (1) by limiting disruption of the circadian timing system, and (2) by directly targeting the affected tissues. The promise and future of these therapeutic strategies will be discussed in this final chapter.

Strategies to limit disruption of the circadian system

Main factors that can disrupt the biological clock in humans are sleep deprivation, exposure to light at night and circadian misalignment (i.e. misalignment between the internal circadian clock and external environment or behavior) [13], and a combination of these factors could contribute to the adverse health effects observed in shift workers. To investigate causality and underlying mechanisms in the relationship between circadian disturbance and cardiometabolic diseases, various animal models have been developed. Shift work can be experimentally mimicked by manipulating timing of food intake, physical activity, sleep and light exposure [14]. In this thesis, shift work was modelled by modulating timing of light exposure, by exposing mice to repeated shifts in light-dark cycle. Through this approach, we are the first to demonstrate a causal relationship between mistimed light exposure and CVD (**Chapter 2**), as well as metabolic bone disorders (**Chapter 3**).

Although exposure to light-dark shifts results in a well-controlled homogeneous induction of 'shift work', a disadvantage of this approach is that its unifactorial, while the relationship between shift work and cardiometabolic diseases is likely multifactorial [15]. Also, the heterogeneity of shift work complicates the translatability of findings from one animal model to

all human shift workers [14]. Nevertheless, animal models are required to gain mechanistical insights necessary to develop strategies that limit circadian disruption in shift workers. The latter can be achieved by either enhancing rhythm adaptation in shift workers, or by reducing circadian misalignment (e.g. through reducing exposure to light at night or via melatonin supplementation), which is further elaborated in the following sections.

Timed feeding, exercise and temperature

The strongest and most well-studied zeitgeber is light, which synchronizes the circadian system according to the day-night cycle. However, other external zeitgebers have also been identified, such as feeding, physical activity and temperature. These zeitgebers entrain circadian rhythms, and can therefore be utilized in a situation of rhythm disruption.

The importance of feeding as a zeitgeber is demonstrated by studies comparing the effect of *ad libitum* versus time-restricted feeding (TRF) on the circadian timing system. Since mice are nocturnal animals, restricting their food access to the day completely reverses the phase of circadian oscillators in various peripheral tissues [16]. This occurs most rapidly in the liver, but is also observed in other tissues such as kidney, heart and pancreas. The effects of TRF are present under both constant dark and normal light-dark conditions [16], indicating that timed feeding can override light as a zeitgeber of peripheral circadian rhythm. Interestingly, while *ad libitum* feeding of a high-fat diet (HFD) disrupts the circadian timing system and increases body weight in mice [17], restricting HFD access to a period of 4 hours in the active phase largely restores peripheral circadian rhythm and prevents body weight gain [18]. Although the improved metabolic profile observed in these mice could have been the result of a decrease in caloric intake, as the timed HFD-fed mice had a very short eating window, more recent studies showed that food restriction to a period of 8-10 hours in the active phase also results in improved metabolic health without a difference in caloric intake [19, 20]. This has been confirmed in humans, in whom an isocaloric weight loss diet is more effective when caloric intake is high in the morning as compared to the evening [21]. Collectively, these studies indicate that TRF could be a promising strategy to reduce metabolic disorders associated with rhythm disruption. Therefore, in Chapter 6, we investigated whether TRF could also be used to accelerate adaptation of the circadian system in a shift work setting. We subjected mice to weekly alternating light-dark cycles to mimic shift work, in combination with *ad libitum* feeding or TRF in the dark phase, and observed an accelerated adaptation of core body temperature and activity rhythms in mice that were fed exclusively in the dark. This work indicates that timed feeding is indeed a promising strategy to enhance rhythm adaptation in shift workers. Future research is needed to investigate whether this enhanced adaptation also prevents diseases associated with shift work.

Like timed feeding, scheduled exercise has been shown to shift behavioral rhythms in mice, both in constant dark and normal light-dark conditions [22-24]. This is mediated through a direct effect of physical activity on the molecular clock in skeletal muscle. Modulation of this skeletal muscle clock through scheduled wheel running accelerates adaptation in mice exposed to 8 hour shifts in light-dark cycles [25]. This accelerated adaptation following timed exercise is also observed in humans during a night shift [26], suggesting that timed exercise could be a useful therapeutic tool to reduce rhythm disruption in shift workers. However, although many studies focused on the role of timed exercise as a zeitgeber for the biological clock, it is not yet known whether modulating the timing of exercise could ameliorate metabolic disease. Nevertheless, mice with a disrupted skeletal muscle clock develop insulin resistance and obesity [27], and obesity dysregulates the muscle clock in humans [28], indicating that

circadian rhythm in skeletal muscle is tightly linked to whole body metabolism. Recent data from our group indeed suggests that timed exercise could benefit cardiometabolic health, as late but not early endurance exercise training reduces atherosclerosis development in mice (Schönke et al., unpublished). However, whether this also works in a setting of circadian disruption, and whether this finding translates to humans, remains to be investigated.
Pulses and oscillations of temperature modulate rhythmic expression of clock genes in peripheral tissues [29-31], demonstrating the role of temperature as a zeitgeber. It has been reported, however, that temperature is a relatively weak zeitgeber [29], which may be the reason why the role of temperature in circadian regulation has not yet been extensively studied. Interestingly, a recent study suggests that temperature may be more important for circadian rhythm in humans than previously thought [32]. In this study, sleep was investigated in three preindustrial human societies, to evaluate circadian sleeping patterns without the influence of artificial light. Timing of sleep adhered more closely to a reduction in ambient temperature than to the onset of darkness, suggesting that the daily temperature oscillations may be a strong regulator of sleep/wake rhythm. These oscillations in ambient temperature are largely absent in modern societies that use electricity to artificially regulate environmental temperature, which could negatively impact sleep quality and therefore circadian rhythm.
Thus, in addition to light, feeding, exercise and temperature are all potent zeitgebers. We have shown that timed feeding can improve adaptation to shifting light-dark cycles (**Chapter 6**). Others have demonstrated a similar effect for timed exercise [25]. Whether temperature oscillations also expedite adaptation remains to be investigated. Studies in both rodent models and humans are necessary to evaluate the relative importance of different zeitgebers for peripheral circadian clocks, in order to reveal which zeitgeber should preferentially be modulated to achieve a desired (therapeutic) effect. However, as disease risk in shift workers is likely multifactorial, a multifactorial approach may be needed to optimally prevent cardiometabolic diseases associated with shift work. For example, shift work can disrupt the timing of both food intake and physical activity, and a combined intervention of diet and exercise could be more effective than diet or exercise alone. Novel preclinical as well as clinical studies are thus required to study the benefit of such multifactorial approaches. Another factor that complicates implementation of strategies to enhance rhythm adaptation is the heterogeneity of shift work. For shift workers that work in permanent evening or night shifts, quick adaptation of the circadian clock to a new rhythm is desired. However, this may not be the case for people that work in rotating shifts, defined by working schedules with hours that change every 2-3 days. A challenge for the future will lie in designing the optimal therapeutic strategy for each type of shift work, or even a personalized therapeutic strategy for each individual shift worker.

Reducing exposure to light at night

The beginning of this chapter highlighted some of the detrimental effects of light at night on cardiometabolic health. These effects can be largely prevented through simple changes in behavior. For example, a large prospective study recently demonstrated that sleeping in a bedroom with the television or lights on results in an additional weight gain of 1 kg/year [33]. In this context, raising public awareness of the consequences of light at night is very important for disease prevention.
Another deleterious form of light exposure at night is the use of portable light-emitting devices immediately before bedtime, such as mobile phones, tablets and e-readers. The use of these electrical devices is associated with a later sleep onset and increased sleep deficiency [34, 35]. Furthermore, a study in participants randomized to reading either a printed book, or a

light-emitting e-reader before going to sleep demonstrates a circadian phase delay of 1.5 hours in participants using a light-emitting e-reader, that coincides with a reduced morning alertness [36]. Of the various colors of light that are emitted by portable light-emitting devices, blue light appears most detrimental for sleep quality. Humans exposed to blue light with a relatively low wavelength (460 nm) in the evening show an increased alertness and impaired homeostatic sleep regulation at night as compared to those exposed to warmer light with a relatively high wavelength ($\geq$ 550 nm) [37, 38]. These findings led to the development of blue light filters on electronic devices such as smartphones, which have been shown to significantly improve sleep quality [39]. This approach has also been tested in a shift work setting, by providing shift workers with orange-tinted glasses that filter out short-wavelength light. Shift workers that wear these glasses during their night shift show an improved performance during their shift, and an increased sleep quality the day after [40]. Thus, filtering out short-wavelength light during a night shift could be a promising strategy to reduce circadian misalignment and sleep deprivation in shift workers, and thereby prevent shift work associated diseases.

Melatonin treatment

An important hormone regulating the sleep/wake cycle and circadian rhythm in humans is melatonin. The pineal gland produces melatonin in a circadian fashion, with a peak that occurs around 2 hours before the onset of sleep [41]. Melatonin regulates sleep timing by acting on the suprachiasmatic nucleus (SCN) to promote fatigue and sleepiness. Accordingly, administration of melatonin in the afternoon when endogenous levels are low induces sleepiness in human subjects [42], demonstrating the therapeutic potential of melatonin to induce sleep onset and improve sleep quality. In shift workers, melatonin levels are decreased during the night [43], to similar levels as in patients suffering from insomnia [44]. Treatment with melatonin in the hour before going to sleep promotes adaptation to shifts in light-dark cycle [45], and decreases sleep onset latency in shift workers [46], indicating that melatonin could be used to reduce circadian disruption. However, melatonin and its associated receptors also play an important role in glucose homeostasis. Loss of function of the melatonin MT2 receptor as well as low nocturnal melatonin levels are associated with increased risk of type 2 diabetes [47, 48], and acute melatonin administration impairs glucose tolerance in the morning and evening in humans [49]. These results suggest that both low and high melatonin levels in the night contribute to an increased risk of type 2 diabetes. Therefore, when administering melatonin as a strategy to reduce circadian disruption, dosage and timing of treatment should be carefully considered.

Of note, most laboratory mouse strains such as the C57BL/6 strain do not produce melatonin, as the activity of enzymes that synthesize melatonin is compromised in these mice [50]. Since we mostly used C57BL/6 mice for the research described in this thesis, we thus revealed melatonin-independent effects of induced shift work on the development of CVD (**Chapter 2**) and metabolic bone disorders (**Chapter 3**). Futures studies in different mouse strains are necessary to evaluate a potential role of melatonin rhythm in the development of shift work associated diseases.

Novel strategies to directly target metabolic diseases

In addition to limiting disruption of the circadian timing system to prevent associated diseases, novel therapeutic strategies should focus on directly targeting the negatively affected tissues. The sections below describe the potential of such novel strategies that have been investigated in this thesis.

Reducing cardiovascular disease by restoring vascular function

Disruption of circadian rhythm through shift work or sleep deprivation is associated with increased risk of CVD in humans [2, 7]. In addition, deficiency in the circadian clock gene *Bmal1* increases plasma lipids and cholesterol in mice [51], and disruption of the *Clock* gene increases plasma cholesterol, inflammation and atherosclerosis in *ApoE*$^{-/-}$ and *Ldlr*$^{-/-}$ mouse models of atherosclerosis [52], demonstrating a causal relationship between circadian disruption and CVD risk. In **Chapter 2**, we now showed that mimicking shift work through alternating light-dark cycles also increases atherosclerosis development in mice, by using the APOE*3-Leiden.CETP humanized mouse model of atherosclerosis. We did not observe any effect on plasma cholesterol or circulating immune cells, but identified vessel wall dysfunction, i.e. increased oxidative stress, inflammation and chemoattractant properties, as a likely cause of the increased atherosclerosis. All of these processes have been targeted before in the context of atherosclerosis. Various antioxidants have been shown to reduce atherosclerosis in mouse models [53-58]. Although the benefit of antioxidants in reducing atherosclerosis in humans is still being questioned, trials have demonstrated a reduction in cardiovascular death and myocardial infarction following natural antioxidant therapy [59, 60]. The potential of anti-inflammatory therapy is illustrated by the recent CANTOS trial. This trial investigated whether targeting IL-1β, an interleukin which was upregulated in the vessel wall of mice exposed to light-dark shifts in our study, could reduce CVD in human subjects [61]. Indeed, IL-1β inhibition strongly reduced primary CVD endpoints (i.e. myocardial infarction, stroke and cardiovascular death), but was associated with a higher incidence of fatal infection by impairing host defence. Adverse effects due to an impaired host defence is also a drawback of therapies targeting cell-adhesion molecules, that otherwise seem rather promising in reducing CVD risk [62]. A way to overcome this obstacle and reduce adverse effects without losing therapeutic efficacy is through chronotherapy, which will be further discussed below (in the section 'chronotherapeutic strategies'). When side adverse effects can be limited, pharmacotherapies that reduce inflammation and prevent vascular dysfunction could be used to prevent CVD, and may be particularly effective in people that suffer from chronic rhythm disruption by e.g. shift work.

Activating brown adipose tissue to prevent cardiometabolic diseases

Not only an impaired vascular health, but also an impaired brown adipose tissue (BAT) activity has been implicated in the association between circadian disturbances and cardiometabolic diseases [63]. Therefore, activation of BAT could be a promising strategy to improve cardiometabolic health. Activation of BAT through cold exposure has already been shown to increase energy expenditure and reduce adiposity in humans [64]. Moreover, cold exposure reduces plasma cholesterol levels in human subjects [65], and subjects with detectable cold-activated BAT show lower plasma cholesterol levels as compared to subjects without detectable BAT [66]. These results support the notion that activation of BAT could be employed to improve metabolic health and protect from CVD. Indeed, activation of BAT by β3-adrenergic receptor stimulation protects from atherosclerosis development in APOE*3-Leiden.CETP mice [67]. A high dose of the synthetic β3-adrenergic receptor agonist mirabegron has been shown to also activate BAT in humans [68]. However, mirabegron cross-reacts with other β-adrenergic receptors such as the β1-adrenergic receptor, which results in tachycardia and questions the safety of this approach to activate BAT [68]. Therefore, novel approaches to increase BAT activity are highly warranted.

In **Chapter 7**, we investigated the therapeutic potential of activating the G protein-coupled receptor 120 (GPR120), a fatty acid sensing receptor that is highly expressed by brown adipocytes.

We found that stimulation of GPR120 promotes BAT activity and reduces body weight and fat mass in mice, suggesting that GPR120 agonism is a promising strategy to increase lipid combustion and reduce obesity. It would be interesting to study whether stimulation of the GPR120 could also prevent the development of CVD through activation of BAT. The role of omega-3 fatty acids, natural ligands of GPR120, has already been investigated with respect to CVD development. Early trials show beneficial effects of capsules with omega-3 fatty acids on CVD outcomes [69, 70]. However, follow-up trials could not consistently reproduce this finding [71-75] possibly due to short treatment periods or low dosages of omage-3 fatty acids [76]. In our study, we used a synthetic agonist with a high potency and selectivity for GPR120 [77], a strategy that is likely more effective as compared to treatment with omega-3 fatty acids. When proven to be safe for humans, synthetic GPR120 agonist could be very effective in improving metabolic health and reducing CVD risk.

In addition to positive regulators of BAT activity such as omega-3 fatty acids, negative regulators of BAT activity exist. An example of such a negative regulator is the sex hormone testosterone, which is predominantly present in males, and has been reported to reduce thermogenic gene expression in cultured brown adipocytes [78]. In **Chapter 8**, we further investigated the role of testosterone in BAT activity. We demonstrate that depletion of testosterone by castration of male mice strongly increases BAT activity, in line with the fact that females with low levels of testosterone show a higher BAT activity as compared to males [79, 80]. These results suggest that differences in testosterone production are at least in part responsible for evident sex differences in human BAT activity. Thus, future (clinical) studies should determine whether promising BAT-activating therapies are similarly effective in both males and females, and whether therapeutic dose and treatment duration, besides timing of administration (see below), should be adjusted in a sex-dependent manner.

Chronotherapeutic strategies

Aside from developing novel therapeutic strategies to prevent health effects of circadian disruption, we can also use our knowledge on circadian rhythm to coordinate the biological clock with existing medical treatment, a concept named ‘chronotherapy’. The use of chronotherapy can improve therapeutic efficacy while reducing adverse effects, as explained in the sections below.

Timing of medication to increase therapeutic efficacy

When a drug is ingested, various organs play a role in its absorption, distribution and excretion. These organs, such as the intestine, liver and kidneys, all show a physiological circadian rhythm. As a result, the bioavailability of drugs is dependent on circadian timing [81]. In addition to bioavailability, the effectiveness of a drug is also dependent on circadian timing, due to a presence of circadian rhythm in the target tissue(s). For example, the immune system shows a strong circadian regulation, and vaccination has proven to be more effective in the morning versus the afternoon [82]. Thus, time of drug administration is an important factor to consider. Not only physiological processes, but also pathological processes demonstrate a day-night rhythm. Already in 1963, the observation was made that the risk of a myocardial infarction is higher during the waking state than during sleep [83], which has been confirmed by many subsequent studies [84]. The presence of a rhythm in CVD incidence is not surprising, as many cardiovascular processes (e.g. heart rate, blood pressure, vascular endothelial function) demonstrate a 24 hour rhythm. Thus, application of chronotherapy for CVD logically followed these observations. Preliminary clinical studies have been performed with aspirin, demonstrating a

strong time-dependent effect of aspirin on platelet activity [85]. However, whether chronotherapy with e.g. aspirin could be useful in further reducing CVD risk in humans on the long-term remains to be investigated. Preclinical studies have already shown benefit of chronotherapy in mouse models of CVD. The CCL2-CCR2 axis has been demonstrated to regulate rhythmic leukocyte recruitment in the context of atherosclerosis [86]. Pharmacological blocking of the CCL2-CCR2 axis is effective in reducing atherosclerosis development in *Apoe*$^{-/-}$ mice, but only when treatment is applied in the inactive phase. Of note, increased CCL2 expression may be an underlying reason for the aggravated atherosclerosis development in mice exposed to shifting light-dark cycles (**Chapter 2**). If these experimental data can be translated to humans, chrono-pharmacological targeting of the CCL2-CCR2 axis could be a promising strategy to reduce CVD in a shift work setting.

Aside from cardiovascular processes, various processes involved in bone remodeling demonstrate robust circadian rhythms [87]. We have demonstrated that circadian disruption by shifting light-dark cycles affects bone remodeling and bone structure in mice (**Chapter 3**), emphasizing the importance of circadian rhythm for bone health. This notion is substantiated by studies applying chronotherapy in osteoporosis, that show a time-of-day dependent treatment efficacy [88, 89]. Optimal dosing time increased the effect on bone turnover markers and further improved bone mineral density. This will likely reduce the long-term risk of osteoporosis-related fractures, although this remains to be confirmed.

We (**Chapter 4**) and others also demonstrated circadian rhythms in BAT activity in both mice and humans [90, 91]. In **Chapter 7**, we used this knowledge on rhythmic BAT activity to design a novel therapeutic strategy to optimally active BAT. In mice, BAT activity is highest at the onset of the dark phase. Therefore, we injected mice with a novel BAT-activating compound 2 hours before initiation of the dark phase, to coordinate circulating levels of the compound with a peak in physiological BAT activity rhythm. This timed treatment was highly effective in further increasing BAT activity, and thereby improving metabolic health. However, as we did not compare treatment efficacy between different dosing times, we cannot comment as yet on the potential of BAT-activating chronotherapy. Future studies should thus investigate whether timed therapy could be an effective strategy to optimally increase BAT activity in both mice and humans, and prevent the development of metabolic disease.

Although chronotherapy in general is a very intuitive and promising strategy to improve treatment efficacy, it is not yet broadly applied in clinical practice. A recent evaluation of all registered clinical trials reported that less than 0.2% of currently ongoing trials involve a form of circadian intervention [81]. Of those trials that involve a form of chronotherapy, most are focused on neurological disorders. A mere 4.6% and 4.5% of trials involving chronotherapy are focused on metabolic disease and heart diseases, respectively. Even less studied are diseases of muscle, bone and cartilage, representing only 1% of chronotherapeutic trials. These numbers emphasize the need for additional clinical studies that investigate circadian inventions to prevent and/or treat cardiometabolic and bone diseases.

Timing of medication to reduce adverse treatment effects

By increasing therapeutic efficacy, chronotherapy could reduce treatment dosage and duration. This limits drug exposure, thereby reducing the incidence of adverse drug reactions that nowadays cause around 200,000 deaths annually throughout the European Union alone [92]. Of note, diminishing adverse drug reactions through chronotherapy could be particularly valuable for CVD prevention strategies, wherein adverse effects often limit the use of otherwise promising novel drugs.

Another example of chronotherapy to limit adverse effects is the therapeutic use of glucocorticoids. Physiological circulating glucocorticoid levels demonstrate robust circadian oscillations that are regulated by the SCN [93, 94]. These glucocorticoid oscillations are important in mediating peripheral circadian rhythm. We observed that disruption of glucocorticoid rhythm in mice impairs BAT activity and metabolic health (**Chapter 4**), and promotes the development of osteoporosis (**Chapter 5**), stressing the importance of glucocorticoid rhythm for essential physiological processes. In humans, glucocorticoids are widely used as treatment for a variety of inflammatory diseases, due to their immunosuppressive properties. In fact, it has been estimated that around 10 million people worldwide are receiving long-term glucocorticoid therapy [95]. Long-term glucocorticoid therapy is associated with an increased risk of metabolic diseases and the bone disease osteoporosis [96, 97]. It was long thought that this risk was solely attributable to supraphysiological glucocorticoid levels resulting from glucocorticoid treatment. However, our findings from **Chapters 4 and 5** demonstrate that a disturbed glucocorticoid rhythm could also contribute substantially. Oral glucocorticoid therapy is currently dosed to mimic physiological glucocorticoid rhythm in humans, by giving a high dose in the morning and one or two lower doses in the afternoon and/or evening. Primarily due to a short half-life of glucocorticoids in the circulation, this treatment does not mimic the endogenous glucocorticoid rhythm adequately [98]. Therefore, other strategies to administer glucocorticoids, such as circadian infusion or delayed-release formulations, are being developed [99, 100]. It would be very interesting to see whether these chronotherapy-based strategies are able to mimic the endogenous glucocorticoid rhythm more closely and therefore limit adverse effects associated with glucocorticoid therapy.

Concluding remarks

The general population increasingly suffers from circadian disruption. For most people, this is the result of unhealthy habits, e.g. staying up late, using bright light-emitting devices shortly before bedtime, and late-night snacking. Raising awareness on the adverse health effects associated with these causes of circadian disruption is paramount. However, for some people circadian disruption is an occupational hazard. The atypical working schedules of shift workers disturbs the tightly regulated biological clock, thereby increasing the risk of cardiometabolic disorders. Therefore, there is an urgent need to identify novel strategies that limit health risks associated with inevitable circadian disruption as occurs in shift work, as summarized in **Figure 1**.

On the one hand, strategies can focus on improving circadian rhythm in a shift work setting, which can be achieved in various ways. Disturbing effects of light during the night shift can be reduced by wearing glasses that filter out short-wavelength light. Negative effects of light exposure on the melatonin system and the sleep/wake cycle can be prevented through oral administration of melatonin. In addition, correct timing of zeitgeber exposure (e.g. feeding, exercise and temperature) could enhance rhythm adaptation in a shift work setting, thereby reducing circadian disruption. This thesis focused on feeding as a zeitgeber and demonstrated that timed feeding is a very effective strategy in promoting rhythm adaptation. However, future studies are needed to compare the effectiveness of different strategies in improving rhythm of shift workers and shift work associated disease. Considering the multifactorial disease risk in shift workers and the variability in human shift work behavior, there may not be a 'one size fits all' approach. A personalized strategy that takes individual behavior and chronotype into account may provide more benefit to shift workers. Such an approach requires extensive

data collection, and smartphone applications that keep track of daily eating, sleeping and activity patterns have already been developed for this purpose [101]. Integration of these data with artificial intelligence to design the perfect therapeutic strategy for each individual is an important area for future research.

On the other hand, strategies can focus on directly targeting shift work associated diseases. As we demonstrated that experimental shift work aggravates atherosclerosis by increasing vascular inflammation and oxidative stress, future studies should aim at restoring vascular function to prevent CVD risk in shift workers. Aside from promoting vascular function, cardiometabolic health in shift workers may be improved by promoting brown fat activity. We have developed a novel approach to activate brown fat by using a synthetic GPR120 agonist, which was extremely effective in improving the metabolic profile of mice (i.e. mice treated with the agonist lost more than 70% of their fat mass within 3 weeks). It will be very interesting to see whether this approach is also able to effectively (and safely) improve cardiometabolic health in humans.

Of note, as many (patho)physiological processes involved in the development of shift work-associated diseases demonstrate a circadian rhythm, selecting the optimal therapeutic window could be essential. This is substantiated by our results, showing that administration of glucocorticoid therapy at the wrong time negatively affects both cardiometabolic health and metabolic bone health. However, an optimal dosing time can tremendously increase the efficacy of a drug, while reducing adverse effects. Thus, implementation of chronotherapy is crucial, and should be further investigated, both for novel and existing therapies.

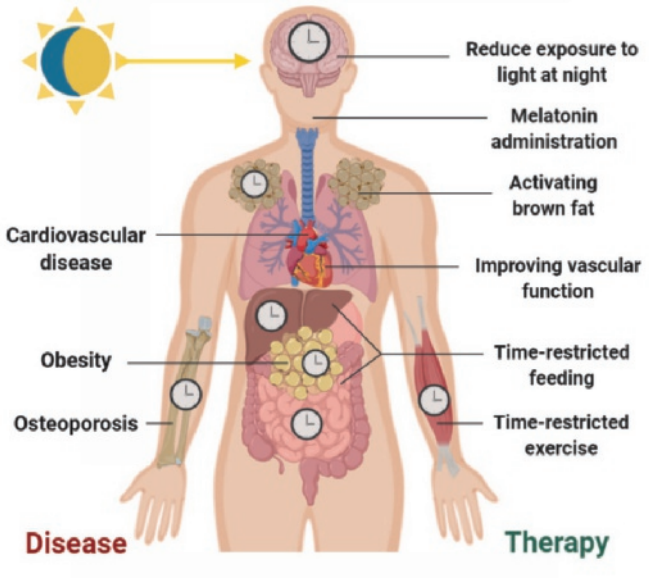

Figure 1. Novel strategies to prevent diseases associated with circadian disruption. Chronic circadian disruption, as occurs in shift work, is associated with amongst others cardiovascular disease, obesity and osteoporosis in humans. These health risks can be limited by improving circadian rhythm (e.g. by reducing exposure to light at light, administering melatonin, and correct timing of zeitgeber exposure, such as feeding and exercise), or by directly targeting the disease (e.g. by activating brown fat or improving vascular function).

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