It's about time: Circadian rhythm and metabolism

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General introduction and outline

In 2017, the Nobel prize in Physiology or Medicine was awarded to Jeffrey Hall, Michael Rosbash and Michael Young for their discovery of the molecular mechanism controlling circadian rhythm. The laureates started their research in the 1980s and provided a foundation in circadian biology, which is now recognized to have great implications for human health. The role of circadian rhythm in health and disease is further explored in this thesis, and by the end of it you will have realized that when talking about metabolic diseases – It's all about time.

The origin of the biological clock

Day and night exist because of the earth's rotation on its axis around the sun, which occurs once every 24 hours. Moreover, the axis of this rotation is tilted at an angle of 23.5 degrees, which generates the seasons. The Northern Hemisphere above the equator is angled towards the sun during its summer, while at the same time, the Southern Hemisphere is further away from the sun and has its winter. Summer is characterized by a longer day length and higher temperatures, while winter is known for a shorter day length and lower temperature. Thus, the rotation of the earth results in an environment with profound variations in light and temperature depending on time, which requires adaptation of the organisms living in such an environment. This resulted in the origin and evolution of the biological clock, that adjusts physiological processes to day-night cycles. The biological clock dictates circadian rhythm, a term that originates from the Latin words circa (around) and dies (day). As the name implies, circadian rhythm refers to all physiological processes that have a period of roughly 24 hours. Circadian rhythm is controlled by a cell-autonomous molecular machinery that produces robust rhythms in gene expression. The molecular clocks in different tissues require coordination to regulate coherent physiological rhythms that are adapted to the day-night cycle. This coordination is mediated through external or environmental cues called 'zeitgebers', which literally translates to 'time givers'. The strongest zeitgeber is light, which signals to the suprachiasmatic nucleus (SCN) of the hypothalamus, a small brain region that is also referred to as the 'master clock' of the body. The SCN transfers these signals to peripheral molecular clocks mainly via the autonomous nervous system and through daily variation in hormones levels, of which glucocorticoids are particularly important. These processes coordinate peripheral tissue rhythms with the external environment. In the sections below, I will elaborate further on the various components that together comprise or regulate the biological clock.

Internal regulation of circadian rhythm

The cell-autonomous core molecular clock is maintained through a transcriptional/translational feedback loop consisting of two activator proteins (CLOCK and BMAL1) and two repressor proteins (PER and CRY) [1]. The activator proteins CLOCK and BMAL1 form a heterodimer, and initiate transcription of the repressor genes *Per* and *Cry*. PER and CRY proteins subsequently heterodimerize and translocate to the nucleus to inhibit the CLOCK and BMAL1 complex, thereby inhibiting their own transcription. The activity of CLOCK and BMAL1 remains low until PER and CRY proteins are degraded through ubiquitin-mediated pathways, which restarts the cycle. Together, CLOCK, BMAL1, PER and CRY form the core molecular clock, as illustrated in Figure 1. Of note, regulation of the entire molecular clock is more complex, and additional proteins (i.e. REV-ERB and ROR) exist that finetune and strengthen cell-intrinsic rhythm [2]. This results in robust cycling of genes and proteins comprising the molecular clock (collectively named 'clock genes' and 'clock proteins'), with a period of about 24 hours. Clock proteins

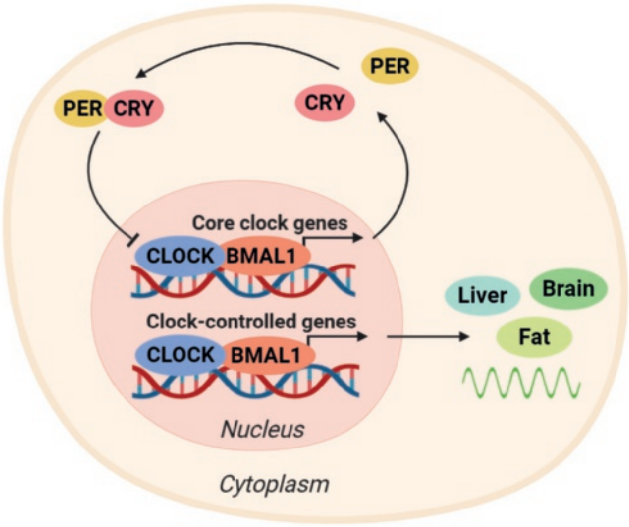

Figure 1. The core molecular clock machinery. Cell-intrinsic rhythm is regulated by CLOCK and BMAL1 which heterodimerize to drive transcription of *Cry* and *Per*. CRY:PER heterodimers subsequently inhibit transcription of CLOCK and BMAL1, resulting in self-sustaining oscillations of core clock genes. CLOCK:BMAL1 heterodimers additionally regulate transcription of tissue-specific genes, in for example the liver, brain and adipose tissue ('fat'), thereby inducing both central and peripheral circadian rhythm.

not only regulate the expression of clock genes, but can also initiate transcription of tissue-specific target genes (Figure 1). As a result, many important tissue-specific genes and proteins demonstrate a circadian rhythm [3, 4]. In mice, around 40% of protein-coding genes show a circadian rhythm in transcription [5]. Even more striking, in the primate *Papio anubis* (baboon), a species closely related to humans, more than 60% of the transcriptome shows 24 hour rhythms in gene expression [6].

Cellular molecular clocks are autonomous, but require synchronization by the SCN in order to coordinate physiological processes. This synchronization can occur in various ways, one of which is through regulation of glucocorticoid hormone. The release of glucocorticoids is regulated by the hypothalamic-pituitary-adrenal (HPA) axis. The HPA response starts with release of corticotropin releasing hormone (CRH) by the hypothalamus. CRH stimulates the secretion of adrenocorticotropic hormone (ACTH) by the anterior pituitary, which in turn acts on the adrenal cortex to promote glucocorticoid production and release. The HPA axis is importantly involved in the stress response, characterized by a very high production of glucocorticoids necessary for a fight-or-flight response. Additionally, the HPA axis is tightly controlled by the SCN [7-9], resulting in a day-night rhythm in circulating glucocorticoid levels. The daily peak levels of glucocorticoids are around 5- to 10-fold higher as compared to the trough values [10], although still considerably lower than the levels reached during a stress response. By binding to the glucocorticoid receptor (GR), glucocorticoids can synchronize rhythms in peripheral cellular clocks *in vitro* and *in vivo* [11]. The promotor of the clock gene *Per1* contains a hypersensitive glucocorticoid-response element (GRE), that induces *Per1* transcription in response to GR activation [12]. PER1 subsequently triggers expression of other clock genes, thereby inducing and maintaining peripheral circadian rhythm [13]. Disruption of circadian glucocorticoid rhythm by chronic synthetic glucocorticoid administration or adrenalectomy blunts rhythmic clock gene expression in peripheral tissues [14, 15]. Furthermore, in the absence of SCN input, GR activation can restore 60% of the circadian transcriptome in the liver [16]. These studies demonstrate the importance of glucocorticoids in transferring signals from the SCN, thereby regulating circadian rhythm (Figure 2).

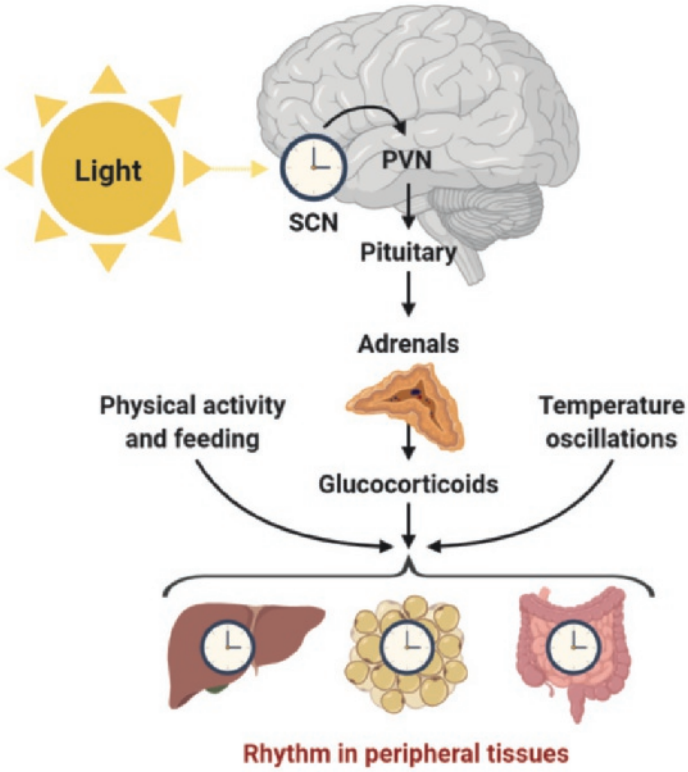

Figure 2. Regulation of rhythm in peripheral tissues by the suprachiasmatic nucleus and zeitgebers. Light signals are received by the suprachiasmatic nucleus (SCN), which promotes rhythmic glucocorticoid release from the adrenals via signaling through the paraventricular nucleus (PVN) of the hypothalamus and the pituitary. Glucocorticoids subsequently induce rhythm in peripheral tissues, such as the liver, adipose tissue and the gut. Additional 'zeitgebers' such as physical activity, feeding and temperature are able to modulate peripheral circadian rhythm independent of the SCN.

External cues that affect circadian rhythm

Aside from the SCN that acts as an internal synchronizer, various external stimuli or zeitgebers can affect peripheral circadian rhythm, either directly and/or by providing feedback to the SCN. The most apparent and well-studied zeitgeber is light. Signals of light are perceived by intrinsically photosensitive retinal ganglion cells (ipRGCs) in the retina of the eyes [17]. These neurons are particularly sensitive for blue light of the visible light spectrum [18], and transfer these light signals to the SCN via the retinohypothalamic tract. The SCN responds by modulating its electrical activity, which subsequently affects central and peripheral circadian rhythm. Through this mechanism, circadian rhythms adapt to the light-dark cycle with respect to changes in phase, intensity or duration of the light cycle (i.e. photoperiod). Rhythms adapted to the light-dark cycle are called diurnal rhythms, while formally only rhythms that persist under constant environmental conditions are circadian. For the sake of simplicity, I will not discriminate between circadian and diurnal rhythms within this thesis. The importance of light as a zeitgeber is illustrated by studies in rodents showing that continuous light dampens rhythm in neuronal activity in the SCN, as well as rhythms in behavioral activity [19, 20]. In contrast to light, which only affects rhythm through the SCN, other zeitgebers such as temperature, physical activity and feeding have been shown to also directly affect peripheral circadian clocks (Figure 2). Pulses or oscillations in temperature can modulate rhythmic expression of clock genes in peripheral mouse tissues such as liver, pituitary and lung, without affecting the SCN [21-23]. However, temperature is considered to be a relatively weak zeitgeber as compared

to light [24]. Physical activity through exercise has similar effects on circadian rhythm in humans as compared to light [25]. Although exercise can affect rhythm of clock genes in the SCN of mice [26], direct effects on the molecular clock in skeletal muscle have also been reported [27]. Like physical activity, feeding can act as a strong zeitgeber, independent of the SCN. In fact, restricting food intake to the inactive phase in mice has been shown to uncouple circadian rhythm in peripheral tissues from the SCN [28, 29]. While the SCN clock follows the light-dark cycle, the molecular clock in the liver strictly aligns to the feeding time [28]. Thus, collectively these studies show that not only our brain, but also our environment and behavior are potent regulators of the biological clock. In our modern society, technological advances such as artificial light impact our 'natural' environment, and potentially disrupt the tightly regulated biological clock.

The role of circadian rhythm in health and disease

The World Health Organization (WHO) states that noncommunicable diseases (i.e. chronic, non-transmittable diseases), such as diabetes, cancer and cardiovascular diseases are currently the largest threat to global health, responsible for over 70% of all deaths worldwide [30]. Physiological processes involved in the etiology of these diseases, such as lipid and glucose homeostasis [31], cell proliferation [32] and the immune response [33] are all controlled by the biological clock. Thus, it should come as no surprise that disturbances of the biological clock are associated with increased risk of disease in humans. This thesis will focus specifically on cardiometabolic diseases and metabolic bone diseases, that have been strongly associated with situations of chronic circadian disruption, as further elucidated in the sections below.

Cardiometabolic diseases

Cardiometabolic disease is a collective term that refers to both metabolic and cardiovascular diseases. Metabolic disease is characterized by a combination of obesity with high blood pressure, increased plasma glucose and/or dyslipidemia, and is a major risk factor for the development of cardiovascular diseases (CVD). As indicated by the name, CVD is a class of diseases affecting the heart and/or blood vessels. The main cause of CVD is atherosclerosis, which is defined by the build-up of atherogenic lipoprotein-derived cholesterol and immune cells in the vessel wall to form so-called atherosclerotic plaques. The most clinically dangerous plaques, by rupturing or eroding, can trigger occlusive luminal thrombosis and thereby cause a heart attack or stroke, depending on the specific artery that is occluded by the thrombus. Of note, almost 18 million people die from CVD each year, making it the number one cause of death globally [34].

Epidemiological studies have associated shift work, a situation of chronic circadian disruption, with increased risk of various cardiometabolic diseases [35-38]. However, association does not imply causality, and a more unhealthy lifestyle (e.g. increased smoking and altered eating habits) has been reported in shift workers which could be a confounding factor [39]. Therefore, follow-up studies using animal models have been performed to further investigate the relationship between circadian rhythm and metabolic health [40]. In these studies, rodents were exposed to repeated shifts in light exposure to mimic human shift work. The results indeed demonstrated a direct relationship between circadian disruption and certain metabolic aberrations, such as a an impaired glucose homeostasis and increased body weight. However, as yet the exact role of the circadian system in regulating lipid metabolism remains largely unknown. Further studies, like those described in this thesis, are required to investigate this suspected relationship, and to study long-term consequences of circadian dysfunction for the development of atherosclerosis.

Brown adipose tissue as a therapeutic target for cardiometabolic diseases

One way through which circadian disruption could affect lipid metabolism is by modulating the activity of brown adipose tissue (BAT), which is also known as 'brown fat'. In comparison to white adipose tissue (WAT) or 'white fat', which makes up around 20-30% of the total body weight, BAT is a relatively small fat depot that only makes up 0.1% of the total body weight [41]. However, unlike WAT, which primarily functions to store energy, BAT has the capacity to burn tremendous amounts of energy, thereby significantly contributing to the body's total energy expenditure. It is estimated that as little as 50 g of BAT can burn up to 20% of the basic caloric need when fully stimulated [41], a feature that is enabled by the presence of uncoupling protein-1 (UCP-1) in the inner membrane of the mitochondria in brown adipocytes [42, 43]. Here, UCP-1 increases the permeability of the inner mitochondrial membrane, thereby disrupting the proton gradient generated by the electron transport chain that is required for ATP synthesis. This results in the release of energy in the form of heat, a process named thermogenesis, which is required to maintain core body temperature in a cold environment. The idea that thermogenesis by BAT importantly contributes to whole-body energy expenditure is supported by data showing that obese individuals exhibit a reduced BAT activity as compared to individuals with a healthy body weight, as determined through radiolabeled glucose uptake by BAT [44]. Interestingly, sex differences in BAT activity have also been reported, with a higher BAT volume and activity in female as compared to male human subjects [45, 46]. Nevertheless, activation of BAT through its natural stimulus, cold exposure, has been shown to reduce fat mass and improve metabolic health in adult human subjects of both sexes [47, 48]. In a human-like murine model of atherosclerosis, pharmacological activation of BAT improved lipid metabolism and reduced atherosclerosis development [49]. Thus, activation of BAT is a promising target to combat cardiometabolic disorders.

Interestingly, recent studies have demonstrated a strong circadian rhythm in BAT activity in both mice and humans (i.e. a high activity at the start of the active phase and a low activity at the start of the inactive phase) [50, 51]. Disruption of circadian rhythm through prolonged light exposure reduced BAT activity in mice, thereby impairing metabolic health [52]. These data suggest that a reduced BAT activity (rhythm) could be an important mediator in the association between circadian disturbances and cardiometabolic diseases. However, the mechanism(s) that underlie rhythmic BAT activity are not fully understood, and require further investigation. Also, before activation of BAT could be implemented in a clinical setting to reduce shift work associated cardiometabolic diseases, other issues need to be addressed. Current pharmacological approaches to activate BAT (with e.g. mirabegron) produce unwanted side effects [53], and research should focus on elucidating novel targets that can be used to safely activate BAT in humans. Furthermore, it should be determined whether such a BAT-activating therapy is effective in both males and females, or whether sex differences exist in the treatment response. Lastly, as BAT displays a rhythm in activity, the response of BAT to (pharmaco)therapy could also differ depending on the time of day. Strategies to activate BAT should therefore be coordinated with the biological clock, a concept named 'chronotherapy'.

Metabolic bone diseases

By now it has become quite clear that the biological clock plays an important role in the function of classical metabolic organs, such as the liver, muscle and adipose tissue. Interestingly, evidence emerges that circadian rhythm may also regulate the metabolism of bone tissue. Bone metabolism (or bone remodeling) is a continuous process of bone resorption and bone formation,

required to maintain bone strength throughout life. This process needs to be tightly regulated, as disturbances in the balance between bone resorption and bone formation can result in metabolic bone diseases such as osteoporosis. In osteoporosis, bone resorption exceeds bone formation, resulting in a reduced bone mass and increased fracture risk. There are many well-known risk factors for osteoporosis, such as declining estrogen levels in postmenopausal women, decreased calcium intake and vitamin D deficiency [54]. In addition, shift work has been associated with a low bone mineral density and increased fracture risk [55, 56], indicating that a disturbed circadian rhythm could be a novel risk factor for osteoporosis.

The potential role of circadian rhythm in bone metabolism is further supported by the presence of daily fluctuations in circulating bone turnover markers in humans [57]. However, it is not yet known to what extent rhythmicity exists in bone, and which factors are important to mediate a healthy bone rhythm. An important zeitgeber for bone could be glucocorticoid rhythm. Synthetic glucocorticoids are widely used to treat inflammatory diseases, but have substantial adverse effects on bone that result in glucocorticoid-induced osteoporosis. The fracture risk in glucocorticoid-induced osteoporosis is dose-dependent, indicating that an overexposure to glucocorticoids is deleterious for bone health. However, whether a disrupted rhythm in glucocorticoids has (additional) effects on bone health remained unknown, and was further explored in this thesis.

Interventions to limit adverse effects of circadian rhythm disturbances

The sections above illustrate how detrimental circadian disruption can be for (cardio)metabolic health. In our current society, many people suffer from circadian disruption by misaligning their biological and social time. An example of this is social jetlag, a phenomenon defined by a different sleeping pattern in the weekends than during the work week [58]. It is important to raise awareness and educate the general public on the health implications of not adhering to your internal biological clock. Nevertheless, in our current 24 hour society we cannot fully prevent the occurrence of circadian disturbances. Our economy relies on shift work as well as intercontinental traveling, both of which pose significant public health concerns. Therefore, research should focus on improving circadian rhythm in e.g. shift workers, to prevent the associated metabolic disorders.

The effect of physical activity and feeding on peripheral circadian clocks has been discussed above (see 'External cues that affect circadian rhythm'). As the timing of physical activity and feeding both directly affect peripheral circadian rhythm, they could form a basis for future therapeutic strategies to prevent health risks in shift workers. Interestingly, a recent study showed that restricting food access to the active phase prevents metabolic disease in mice lacking a circadian clock [59], emphasizing the important role of timing of food intake in circadian energy metabolism. This has been confirmed in humans, in whom meal timing has been shown to affect clock gene expression in adipose tissue and plasma glucose rhythms [60]. In humans, the effect of meal timing on overall metabolic health has also been investigated. Observational studies demonstrated that skipping breakfast and late-night eating is associated with increased risk of obesity and CVD [61-64]. These findings suggests that consuming most of the daily energy intake in the beginning of the day is beneficial for metabolic health. Indeed, hypocaloric intervention studies have shown that among obese subjects who consume the same amount of calories per day, eating the majority of these calories for breakfast results in significantly increased weight loss as compared to eating the majority of these calories for dinner [61]. Thus, meal time-based strategies could be employed to prevent obesity and associated cardiometabolic diseases. As shift workers show profound changes in their eating

habits (e.g. changing meal patterns, skipping meals, consuming more food at unconventional times, etc.) [65], it would be of high interest to investigate whether meal-time based strategies can also be effective in preventing cardiometabolic disease in a shift work setting.

Outline of this thesis

The aim of this thesis was to further explore the role of circadian rhythm in the development of metabolic diseases. It is well known that chronic circadian disruption, as occurs in shift work, is associated with cardiometabolic disorders. In **Chapter 2**, we investigated whether this association is of a causal nature, by exposing mice to shifting light-dark cycles to mimic human shift work. We hypothesized that shifting light-dark cycles increases atherosclerosis development in a well-established mouse model for human-like lipid metabolism and atherogenesis, thereby demonstrating a causal relationship between mistimed light exposure and CVD. Aside from cardiometabolic disorders, shift work has been associated with a low bone mineral density and increased fracture risk, suggesting that a disturbed circadian rhythm could be a novel risk factor for metabolic bone diseases such as osteoporosis. In **Chapter 3**, we used the same mouse model for human shift work to further investigate the association between circadian rhythm and osteoporosis, to address the hypothesis that circadian disruption is detrimental for bone health. After establishing whether circadian disruption is causally related to cardiometabolic disorders and metabolic bone diseases, we proceeded with investigating underlying mechanisms. In **Chapter 4**, we studied whether rhythm in glucocorticoid hormone could be important for cardiometabolic health. As an impaired BAT activity rhythm was shown to be an important mediator in the association between circadian disturbances and cardiometabolic diseases, we specifically focused on this tissue. We investigated whether a flattened glucocorticoid rhythm could result in a disrupted BAT activity rhythm, and thereby negatively impact cardiometabolic health. In **Chapter 5**, we continued on investigating the importance of physiological rhythm in glucocorticoids, but now concentrating on bone metabolism. We hypothesized that circadian fluctuations in glucocorticoids are essential to maintain bone health, which could partly explain the increased risk of osteoporosis in patients treated with synthetic glucocorticoids. The next chapters of the thesis focused on novel strategies to limit the occurrence of disease associated with circadian disruption. An established strategy to improve circadian rhythm is time-restricted feeding. In **Chapter 6**, we investigated whether time-restricted feeding could also be effective in reducing metabolic disturbances following circadian disruption by shifting light-dark cycles. In **Chapter 7**, instead of improving circadian rhythm to reduce cardiometabolic risk, we aimed to directly target metabolic disorders by exploring novel BAT-activating strategies. We report on the therapeutic potential of the G protein-coupled receptor 120 (GPR120), a fatty acid sensing receptor which is highly expressed in BAT. Hereafter, we aimed to find additional targets to activate BAT and improve metabolic health. Considering that males with high levels of testosterone show reduced BAT activity as compared to females, in **Chapter 8** we investigated whether the sex hormone testosterone could affect BAT activity. Finally, the results from these studies and their therapeutic implications are discussed in **Chapter 9**.

References

1. Partch, C.L., C.B. Green, and J.S. Takahashi. Molecular architecture of the mammalian circadian clock. Trends Cell Biol. 2014;24(2):90-9.
2. Preitner, N., F. Damiola, L. Lopez-Molina, J. Zakany, et al. The orphan nuclear receptor REV-ERBalpha controls circadian transcription within the positive limb of the mammalian circadian oscillator. Cell. 2002;110(2):251-60.
3. Miller, B.H., E.L. McDearmon, S. Panda, K.R. Hayes, et al. Circadian and CLOCK-controlled regulation of the mouse transcriptome and cell proliferation. Proc Natl Acad Sci U S A. 2007;104(9):3342-7.
4. Panda, S., M.P. Antoch, B.H. Miller, A.I. Su, et al. Coordinated transcription of key pathways in the mouse by the circadian clock. Cell. 2002;109(3):307-20.
5. Zhang, R., N.F. Lahens, H.I. Ballance, M.E. Hughes, et al. A circadian gene expression atlas in mammals: implications for biology and medicine. Proc Natl Acad Sci U S A. 2014;111(45):16219-24.
6. Mure, L.S., H.D. Le, G. Benegiamo, M.W. Chang, et al. Diurnal transcriptome atlas of a primate across major neural and peripheral tissues. Science. 2018;359(6381).
7. Buijs, R.M., J. Wortel, J.J. Van Heerikhuize, M.G. Feenstra, et al. Anatomical and functional demonstration of a multisynaptic suprachiasmatic nucleus adrenal (cortex) pathway. Eur J Neurosci. 1999;11(5):1535-44.
8. Buijs, R.M., J. Wortel, J.J. Van Heerikhuize, and A. Kalsbeek. Novel environment induced inhibition of corticosterone secretion: physiological evidence for a suprachiasmatic nucleus mediated neuronal hypothalamo-adrenal cortex pathway. Brain Res. 1997;758(1-2):229-36.
9. Kalsbeek, A., R.M. Buijs, J.J. van Heerikhuize, M. Arts, et al. Vasopressin-containing neurons of the suprachiasmatic nuclei inhibit corticosterone release. Brain Res. 1992;580(1-2):62-7.
10. Chung, S., G.H. Son, and K. Kim. Circadian rhythm of adrenal glucocorticoid: its regulation and clinical implications. Biochim Biophys Acta. 2011;1812(5):581-91.
11. Balsalobre, A., S.A. Brown, L. Marcacci, F. Tronche, et al. Resetting of circadian time in peripheral tissues by glucocorticoid signaling. Science. 2000;289(5488):2344-7.
12. Yamamoto, T., Y. Nakahata, M. Tanaka, M. Yoshida, et al. Acute physical stress elevates mouse period1 mRNA expression in mouse peripheral tissues via a glucocorticoid-responsive element. J Biol Chem. 2005;280(51):42036-43.
13. Reddy, T.E., J. Gertz, G.E. Crawford, M.J. Garabedian, et al. The hypersensitive glucocorticoid response specifically regulates period 1 and expression of circadian genes. Mol Cell Biol. 2012;32(18):3756-67.
14. Koyanagi, S., S. Okazawa, Y. Kuramoto, K. Ushijima, et al. Chronic treatment with prednisolone represses the circadian oscillation of clock gene expression in mouse peripheral tissues. Mol Endocrinol. 2006;20(3):573-83.
15. Sotak, M., J. Bryndova, P. Ergang, K. Vagnerova, et al. Peripheral circadian clocks are diversely affected by adrenalectomy. Chronobiol Int. 2016;33(5):520-9.
16. Reddy, A.B., E.S. Maywood, N.A. Karp, V.M. King, et al. Glucocorticoid signaling synchronizes the liver circadian transcriptome. Hepatology. 2007;45(6):1478-88.
17. Berson, D.M., F.A. Dunn, and M. Takao. Phototransduction by retinal ganglion cells that set the circadian clock. Science. 2002;295(5557):1070-3.
18. Qiu, X., T. Kumbalasiri, S.M. Carlson, K.Y. Wong, et al. Induction of photosensitivity by

heterologous expression of melanopsin. Nature. 2005;433(7027):745-9.
19. Coomans, C.P., S.A. van den Berg, T. Houben, J.B. van Klinken, et al. Detrimental effects of constant light exposure and high-fat diet on circadian energy metabolism and insulin sensitivity. FASEB J. 2013;27(4):1721-32.
20. Ohta, H., S. Yamazaki, and D.G. McMahon. Constant light desynchronizes mammalian clock neurons. Nat Neurosci. 2005;8(3):267-9.
21. Brown, S.A., G. Zumbrunn, F. Fleury-Olela, N. Preitner, et al. Rhythms of mammalian body temperature can sustain peripheral circadian clocks. Curr Biol. 2002;12(18):1574-83.
22. Buhr, E.D., S.H. Yoo, and J.S. Takahashi. Temperature as a universal resetting cue for mammalian circadian oscillators. Science. 2010;330(6002):379-85.
23. Kornmann, B., O. Schaad, H. Bujard, J.S. Takahashi, et al. System-driven and oscillator-dependent circadian transcription in mice with a conditionally active liver clock. PLoS Biol. 2007;5(2):e34.
24. Refinetti, R. Entrainment of circadian rhythm by ambient temperature cycles in mice. J Biol Rhythms. 2010;25(4):247-56.
25. Yamanaka, Y., S. Hashimoto, S. Masubuchi, A. Natsubori, et al. Differential regulation of circadian melatonin rhythm and sleep-wake cycle by bright lights and nonphotic time cues in humans. Am J Physiol Regul Integr Comp Physiol. 2014;307(5):R546-57.
26. Mendoza, J.Y., H. Dardente, C. Escobar, P. Pevet, et al. Dark pulse resetting of the suprachiasmatic clock in Syrian hamsters: behavioral phase-shifts and clock gene expression. Neuroscience. 2004;127(2):529-37.
27. Wolff, G. and K.A. Esser. Scheduled exercise phase shifts the circadian clock in skeletal muscle. Med Sci Sports Exerc. 2012;44(9):1663-70.
28. Damiola, F., N. Le Minh, N. Preitner, B. Kornmann, et al. Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. Genes Dev. 2000;14(23):2950-61.
29. Hara, R., K. Wan, H. Wakamatsu, R. Aida, et al. Restricted feeding entrains liver clock without participation of the suprachiasmatic nucleus. Genes Cells. 2001;6(3):269-78.
30. World Health Organization (WHO). Ten threats to global health in 2019. [cited 2019 July 10]; Available from: https://www.who.int/emergencies/ten-threats-to-global-health-in-2019.
31. Poggiogalle, E., H. Jamshed, and C.M. Peterson. Circadian regulation of glucose, lipid, and energy metabolism in humans. Metabolism. 2018;84:11-27.
32. Shostak, A. Circadian Clock, Cell Division, and Cancer: From Molecules to Organism. Int J Mol Sci. 2017;18(4):873.
33. Scheiermann, C., Y. Kunisaki, and P.S. Frenette. Circadian control of the immune system. Nat Rev Immunol. 2013;13(3):190-8.
34. Benjamin, E.J., S.S. Virani, C.W. Callaway, A.M. Chamberlain, et al. Heart Disease and Stroke Statistics-2018 Update: A Report From the American Heart Association. Circulation. 2018;137(12):e67-e492.
35. Karlsson, B., A. Knutsson, and B. Lindahl. Is there an association between shift work and having a metabolic syndrome? Results from a population based study of 27,485 people. Occup Environ Med. 2001;58(11):747-52.
36. Tenkanen, L., T. Sjoblom, and M. Harma. Joint effect of shift work and adverse life-style factors on the risk of coronary heart disease. Scand J Work Environ Health. 1998;24(5):351-7.
37. Haupt, C.M., D. Alte, M. Dorr, D.M. Robinson, et al. The relation of exposure to shift work

with atherosclerosis and myocardial infarction in a general population. Atherosclerosis. 2008;201(1):205-11.
38. Tuchsen, F., H. Hannerz, and H. Burr. A 12 year prospective study of circulatory disease among Danish shift workers. Occup Environ Med. 2006;63(7):451-5.
39. Shan, Z., Y. Li, G. Zong, Y. Guo, et al. Rotating night shift work and adherence to unhealthy lifestyle in predicting risk of type 2 diabetes: results from two large US cohorts of female nurses. BMJ. 2018;363:k4641.
40. Opperhuizen, A.L., L.W. van Kerkhof, K.I. Proper, W. Rodenburg, et al. Rodent models to study the metabolic effects of shiftwork in humans. Front Pharmacol. 2015;6:50.
41. Elattar, S. and A. Satyanarayana. Can Brown Fat Win the Battle Against White Fat? J Cell Physiol. 2015;230(10):2311-7.
42. Cannon, B. and J. Nedergaard. Brown adipose tissue: function and physiological significance. Physiol Rev. 2004;84(1):277-359.
43. Schilperoort, M., G. Hoeke, S. Kooijman, and P.C. Rensen. Relevance of lipid metabolism for brown fat visualization and quantification. Curr Opin Lipidol. 2016;27(3):242-8.
44. van Marken Lichtenbelt, W.D., J.W. Vanhommerig, N.M. Smulders, J.M. Drossaerts, et al. Cold-activated brown adipose tissue in healthy men. N Engl J Med. 2009;360(15):1500-8.
45. Cypess, A.M., S. Lehman, G. Williams, I. Tal, et al. Identification and importance of brown adipose tissue in adult humans. N Engl J Med. 2009;360(15):1509-17.
46. Pfannenberg, C., M.K. Werner, S. Ripkens, I. Stef, et al. Impact of age on the relationships of brown adipose tissue with sex and adiposity in humans. Diabetes. 2010;59(7):1789-93.
47. van der Lans, A.A., J. Hoeks, B. Brans, G.H. Vijgen, et al. Cold acclimation recruits human brown fat and increases nonshivering thermogenesis. J Clin Invest. 2013;123(8):3395-403.
48. Yoneshiro, T., S. Aita, M. Matsushita, T. Kayahara, et al. Recruited brown adipose tissue as an antiobesity agent in humans. J Clin Invest. 2013;123(8):3404-8.
49. Berbee, J.F., M.R. Boon, P.P. Khedoe, A. Bartelt, et al. Brown fat activation reduces hypercholesterolaemia and protects from atherosclerosis development. Nat Commun. 2015;6:6356.
50. Lee, P., R. Bova, L. Schofield, W. Bryant, et al. Brown Adipose Tissue Exhibits a Glucose-Responsive Thermogenic Biorhythm in Humans. Cell Metab. 2016;23(4):602-9.
51. van den Berg, R., S. Kooijman, R. Noordam, A. Ramkisoensing, et al. A Diurnal Rhythm in Brown Adipose Tissue Causes Rapid Clearance and Combustion of Plasma Lipids at Wakening. Cell Rep. 2018;22(13):3521-3533.
52. Kooijman, S., R. van den Berg, A. Ramkisoensing, M.R. Boon, et al. Prolonged daily light exposure increases body fat mass through attenuation of brown adipose tissue activity. Proc Natl Acad Sci U S A. 2015;112(21):6748-53.
53. Cypess, A.M., L.S. Weiner, C. Roberts-Toler, E. Franquet Elia, et al. Activation of human brown adipose tissue by a beta3-adrenergic receptor agonist. Cell Metab. 2015;21(1):33-8.
54. Kling, J.M., B.L. Clarke, and N.P. Sandhu. Osteoporosis prevention, screening, and treatment: a review. J Womens Health (Larchmt). 2014;23(7):563-72.
55. Feskanich, D., S.E. Hankinson, and E.S. Schernhammer. Nightshift work and fracture risk: the Nurses' Health Study. Osteoporos Int. 2009;20(4):537-42.
56. Quevedo, I. and A.M. Zuniga. Low bone mineral density in rotating-shift workers. J Clin Densitom. 2010;13(4):467-9.
57. Redmond, J., A.J. Fulford, L. Jarjou, B. Zhou, et al. Diurnal Rhythms of Bone Turnover Markers in Three Ethnic Groups. J Clin Endocrinol Metab. 2016;101(8):3222-30.
58. Wittmann, M., J. Dinich, M. Merrow, and T. Roenneberg. Social jetlag: misalignment of

biological and social time. Chronobiol Int. 2006;23(1-2):497-509.
59. Chaix, A., T. Lin, H.D. Le, M.W. Chang, et al. Time-Restricted Feeding Prevents Obesity and Metabolic Syndrome in Mice Lacking a Circadian Clock. Cell Metab. 2019;29(2):303-319.e4.
60. Wehrens, S.M.T., S. Christou, C. Isherwood, B. Middleton, et al. Meal Timing Regulates the Human Circadian System. Curr Biol. 2017;27(12):1768-1775.e3.
61. Cahill, L.E., S.E. Chiuve, R.A. Mekary, M.K. Jensen, et al. Prospective study of breakfast eating and incident coronary heart disease in a cohort of male US health professionals. Circulation. 2013;128(4):337-43.
62. Kahleova, H., J.I. Lloren, A. Mashchak, M. Hill, et al. Meal Frequency and Timing Are Associated with Changes in Body Mass Index in Adventist Health Study 2. J Nutr. 2017;147(9):1722-1728.
63. Kutsuma, A., K. Nakajima, and K. Suwa. Potential Association between Breakfast Skipping and Concomitant Late-Night-Dinner Eating with Metabolic Syndrome and Proteinuria in the Japanese Population. Scientifica (Cairo). 2014;2014:253581.
64. Wang, J.B., R.E. Patterson, A. Ang, J.A. Emond, et al. Timing of energy intake during the day is associated with the risk of obesity in adults. J Hum Nutr Diet. 2014;27 Suppl 2:255-62.
65. Souza, R.V., R.A. Sarmento, J.C. de Almeida, and R. Canuto. The effect of shift work on eating habits: a systematic review. Scand J Work Environ Health. 2019;45(1):7-21.