It's about time: Circadian rhythm and metabolism

Schilperoort, M.

Summary

Noncommunicable diseases are the leading causes of mortality worldwide, accounting for more than 70% of all deaths. An important risk factor for the development of noncommunicable diseases is disruption of the internal biological clock. The biological clock adjusts physiological processes to day-night cycles by generating circadian rhythms throughout the body. Maintaining consistent circadian rhythms is essential for long-term health, and chronic circadian disturbances have been strongly associated with cardiometabolic diseases and metabolic bone disturbances in humans. This thesis provides insight into the role of circadian rhythm in the development of (cardio)metabolic diseases, and elucidates novel approaches to prevent diseases associated with circadian disturbances. **Chapter 1** gives a general introduction on the regulation of circadian rhythm, and its role in health and disease.

In **Chapter 2**, we investigated causality and underlying mechanisms in the association between chronic circadian disruption, as occurs in shift work, and cardiometabolic disorders. To this end, we subjected APOE*3-Leiden.CETP mice, a well-established mouse model of human-like atherosclerosis development, to weekly shifts in light-dark cycle to mimic human shift work. We found that shifting light-dark cycles promotes atherosclerosis development, thereby revealing a causal relationship between mistimed light exposure and cardiovascular disease. We did not observe differences in plasma cholesterol and the activation status of immune cells, but identified increased vascular inflammation and oxidative stress as a likely cause for the increased atherosclerosis. Thus, preventing vascular dysfunction in shift workers could be a promising strategy to reduce cardiovascular disease. This could potentially be achieved by timed-restricted feeding, which can override light as a zeitgeber of peripheral circadian rhythm, thereby limiting effects of circadian disruption on tissues such as the vasculature.

In **Chapter 3**, we used the same experimental model for human shift work to further investigate the association between circadian disruption and the metabolic bone disease osteoporosis. We identified strong rhythms in the expression of circadian clock genes within the bones of mice, which was clearly disrupted by shifting light-dark cycles. Repeated shifts in light-dark cycle decreased plasma levels of markers for bone formation and resorption, indicative of a reduced bone turnover. Consistent with these observations, we found abnormalities in trabecular bone structure and an increased cortical bone mineralization. Altogether, the bones of mice exposed to shifting light-dark cycles show an osteoporotic phenotype that may explain the increased incidence of bone fractures in shift workers.

After establishing causality in the relationship between circadian disruption and cardiometabolic disorders, we intended to further investigate underlying mechanisms in a tissue that was previously identified as a potential mediator of this relationship, namely brown adipose tissue (BAT). It is well-known that glucocorticoid hormone entrains rhythm in peripheral organs to modulate their activity. However, whether glucocorticoids also orchestrate the circadian rhythm in BAT activity and thereby contribute to (cardio)metabolic health remained to be elucidated. In **Chapter 4**, we demonstrated that corticosterone, the primary murine glucocorticoid, dictates BAT activity rhythm in mice. This effect was independent of glucocorticoid receptor expression in adipocytes. Rather, our results suggest that corticosterone modulates sympathetic innervation of BAT by acting on the central nervous system. In dyslipidemic APOE*3-Leiden.CETP mice, experimental flattening of the corticosterone and thereby BAT activity rhythm resulted in adiposity. Collectively, these data show that disruption of glucocorticoid rhythm contributes to the metabolic symptoms associated with circadian disturbances.

In **Chapter 5**, we investigated whether a disturbed glucocorticoid rhythm could also underlie

the association between circadian disruption and osteoporosis. Experimental flattening of the corticosterone rhythm in mice tilted the balance in bone remodeling towards bone resorption, thereby reducing bone volume and density. The observed alterations in bone structure negatively affected the mechanical properties of bone, as reflected by a decreased bone strength and stiffness. Together, these results indicate that a disturbed glucocorticoid rhythm can indeed increase the risk of osteoporotic fractures. This is not only relevant for shift workers, but also for patients that have a blunted endogenous glucocorticoid rhythm due to exogenous glucocorticoid administration. In these patients, well-designed chronotherapy with glucocorticoids may reduce osteoporotic adverse effects.

It is unlikely that the number of individuals that suffers from circadian disruption will decrease in the near future. Therefore, it is essential to identify novel strategies to prevent diseases associated with circadian disruption. In **Chapter 6**, we investigated an approach to limit disruption of the circadian timing system, by subjecting mice to time-restricted feeding. In accordance with mice being nocturnal animals, restricting food access to the light phase impaired adaptation of 24-hour patterns of core body temperature and physical activity to a new rhythm, while restricting food access to the dark phase markedly enhanced adaptation. Time-restricted feeding could therefore be a promising strategy to limit disruption of the circadian timing system and reduce the associated disease risk. Studying this in the context of disease development, including atherosclerosis and osteoporosis, would be an important next step.

We continued to develop novel strategies to prevent diseases associated with circadian disruption by directly targeting one of the affected tissues. BAT importantly contributes to total energy expenditure and (cardio)metabolic health, and BAT rhythm and activity are negatively affected by circadian disturbances. For these reasons, we investigated a novel approach to promote BAT activity in **Chapter 7**, by stimulating the fatty acid-sensing G protein-coupled receptor 120 (GPR120) that is highly expressed by brown adipocytes. Selective GPR120 agonism in mice acutely increased fat oxidation and lipid uptake by BAT. This resulted in a strong reduction in fat mass, already within one week of treatment. Mechanistically, GPR120 signaling promoted calcium-dependent mitochondrial depolarization and fragmentation, which increased the oxidative activity of brown adipocytes. We therefore concluded that activation of brown fat by GPR120 agonism is a promising strategy to increase lipid combustion and improve (cardio)metabolic health. It remains to be investigated, however, if GPR120 agonism is able to prevent diseases caused by circadian disruption and whether appropriate timing of pharmacotherapy is critical.

Considering that males show lower BAT activity as compared to females, in **Chapter 8** we investigated whether the male sex hormone (or androgen) testosterone could reduce BAT activity. Depletion of testosterone by castration in male mice promoted lipid uptake by BAT while decreasing its lipid content, demonstrating increased BAT activity. Castration had the same effect in brown adipocyte-specific androgen receptor-deficient mice as compared to wildtype controls, demonstrating an indirect effect of testosterone on BAT. Noradrenalin levels were increased in BAT of castrated mice, suggesting that testosterone reduces BAT activity by diminishing sympathetic outflow to BAT. These results demonstrate that differences in testosterone are likely responsible for sex differences in brown fat, and that novel (chronotherapeutic) strategies to activate BAT may not be equally effective in men and women.

To conclude, in **Chapter 9** the results of this thesis were placed in the context of the current scientific literature, and the potency of novel strategies to prevent diseases associated with circadian disruption was discussed. Collectively, the results described in this thesis emphasize

the importance of circadian rhythm for (cardio)metabolic health, and have improved our knowledge on the mechanisms that underlie disease risk in the ever-increasing population of individuals who suffer from circadian disturbances.