A metabolically healthy lifestyle

Summary

Virtually all cells, tissues and physiological processes in the human body exhibit an internal rhythm that can be entrained to the environmental rhythm experienced on earth by (sun)light exposure, food intake, and physical activity (so-called time cues or *Zeitgebers*). The biological, circadian rhythm takes approximately 24 hours to complete and is present even in absence of time cues, whereas the day-night rhythm represents the rhythm that has been entrained to the environmental 24h rhythm. The entrainment of the internal rhythm allows the human body to respond efficiently to predictable homeostatic challenges, such as eating and periods of sleep. In turn, the internal rhythm can be adjusted by changes in the environmental rhythm via *Zeitgebers*. As such, when travelling across time zones, humans are able to adapt their internal rhythm to a time zone that is different from their home country. Similarly, humans can adapt to altered day-night rhythms, for example when performing shift work. However, in recent decades, it has become evident that too frequently challenging the internal rhythm by eating and being active at irregular times, such as with night-shift work, has detrimental consequences for metabolic health. Disturbances of the internal rhythm can have a direct, negative effect on metabolic health, but can also indirectly deteriorate metabolic health via a disturbed sleep. Although it is clear that frequently disturbing the internal rhythm results in impaired metabolic health, it is thus far unclear if metabolic health could benefit from lifestyle interventions that are aligned with the internal rhythm. Therefore, in this thesis, we sought to investigate if re-aligning the timing of physical activity and food intake with the natural biological rhythm had potential to improve metabolic health. The emphasis of this thesis was to restore the 24-hour rhythm in feeding and fasting, which becomes disturbed when eating at irregular times. Both observational- as well as clinical studies were performed to address the aim of this thesis.

On a physiological level, circadian rhythms are shaped by an interplay between the central clock (or master pacemaker), which is located in the suprachiasmatic nucleus (SCN) of the posterior hypothalamus and by auxiliary clocks located in peripheral tissues. The most important *Zeitgeber* for the clocks is (sun)light, that is picked up by retinal cells, which subsequently send a signal to the SCN. Ultimately, this process results in the synchronization of all body clocks to sunlight. In addition to light, peripheral clocks can also be attuned by food intake and exercise, and can signal back to the SCN. **Chapter 2** of this thesis provides a theoretical framework on the working mechanisms of the circadian rhythm, as well as an overview of studies examining rhythmicity in human metabolism. Importantly, **chapter 2** also provides an assembly on human intervention studies that have been performed on the effect of timed lifestyle interventions on obesity-related metabolic diseases. From this chapter, it became apparent that it is currently unclear whether an optimal time window to perform physical activity exists to gain most metabolic benefits. Recently, a cross-sectional study showed that people who perform most moderate-to-vigorous physical activity (MVPA) in the afternoon and evening had a higher insulin sensitivity compared to those that evenly distributed their MVPA over the day. It could be hypothesized that performing most activity in the afternoon or evening has different effects on sleep quality compared to activity performed in the morning. Thus, the results from this study may be explained by differences in sleep quality, since sleep is related to metabolic health and regulated by the circadian rhythm. To address this hypothesis, in **chapter 3**, a cross-sectional data analysis was performed to investigate if performing most MVPA in the afternoon and evening was also associated with optimal sleep quality. However, this study did not show associations between the time window in which MVPA was performed and most of the measured sleep characteristics. These results therefore imply that the previously found association between afternoon/evening MVPA and insulin sensitivity was likely not influenced by changes in sleep with afternoon/evening MVPA.

The subsequent chapters of this thesis were aimed at understanding the effects of a longer fasting duration, by advancing the last meal of the day, on metabolic health. These studies were conducted since a previous study showed that most people have an eating time window that spans at least 14 hours and that reducing this eating time window, thereby prolonging fasting time, can result in weight loss. Importantly, the weight loss occurred without any dietary restrictions. Moreover, a subsequent study in males at risk of type 2 diabetes showed that limiting food intake to a more narrow time window, thereby prolonging the overnight fast, results in an increased insulin sensitivity, even without weight loss. Nevertheless, the mechanisms underlying the benefits of limiting food intake to a narrow time window remained unclear. In this thesis, it was hypothesized that when the duration of the daily overnight fast is prolonged, this results in an increased utilization of nutrient stores, such as fat stores and hepatic glycogen, which could improve the uptake of nutrients with the first (breakfast) meal of the subsequent day. Thus, **chapter 4** showed that acutely prolonging the fasting duration from 9.5 hrs to 16 hrs was successful in increasing nocturnal fat oxidation in middle-aged healthy individuals, as well as in middle-aged individuals with a non-alcoholic fatty liver (NAFL). A prolonged fasting duration was, however, not accompanied by alterations in the metabolic response to a standardized breakfast meal or on hepatic glycogen utilization, neither in the healthy nor in the NAFL group. It could be argued that acutely prolonging the overnight fast is too short to improve metabolic health and that repeatedly prolonging the overnight fast would result in more metabolic benefits. Therefore, the effects of a 3-week time restricted eating (TRE) regime were investigated in individuals with type 2 diabetes (**chapter 5**). In contrast to the acute study, prolonging the fasting duration from 10 hrs (control) to 14 hrs (TRE) for a period of 3 weeks did not result in a higher nocturnal fat oxidation. Also levels of hepatic glycogen were not significantly lower after TRE in individuals with type 2 diabetes. Nevertheless, the TRE intervention did result in improvements in glucose homeostasis, which was reflected by a reduction in 24h glucose and fasting glucose levels and more time spent in the normal glucose range. Combined, these

results suggest that prolonging the fasting duration is a promising strategy to improve metabolic health. However, it may be possible that stronger effects are obtained when a more pronounced overnight fasting response is elicited by medication. The type 2 diabetes medication sodium-glucose cotransporter 2 inhibitor (SGLT2i) is promising in this respect, since this medication blocks the resorption of glucose in the proximal renal tubules, which results in a higher urinary glucose excretion. During the night, this medication could lead to a more pronounced fasted state, which subsequently could trigger mechanisms that protect against hypoglycaemia. In **chapter 6**, we showed that two weeks of the SGLT2i dapagliflozin indeed resulted in higher nocturnal fat oxidation, which was accompanied by lower 24-hour glucose levels, higher 24-hour FFA levels, and lower nocturnal levels of beta-hydroxybutyrate in adults with insulin resistance when compared to the placebo. In addition, maximal mitochondrial oxidative capacity was also higher with dapagliflozin. No changes were found in hepatic glycogen and -lipid content.

Finally, since food intake plays a key role in the day-night rhythm of metabolic processes, **chapter 7** of this thesis examined the effect of a 60 hour fast on the day-night rhythmicity in energy metabolism in healthy lean males. In addition, this study visualised the changes in energy metabolism that occur over time when fasted for a longer period of time. This study showed that fasting resulted in changes in rhythmicity of substrate oxidation, whereas the rhythm in energy expenditure remained unaffected, suggesting that the rhythm of energy metabolism is not driven by food intake. Further, the switch to a relatively higher fat oxidation occurred early on during the 60-hour fast and kept gradually increasing over the whole fasting period; nevertheless, carbohydrate oxidation was still present, albeit in minimal quantities.

In sum, this thesis shows that re-aligning the timing of exercise and food intake with the natural day-night rhythm, as well as restoring the rhythm of the fasting-feeding cycle by inducing a more pronounced overnight fast, can improve metabolic health. Importantly, this thesis also emphasizes the need for more human intervention

studies to find the optimal time-of-day to eat and to be active. Furthermore, more mechanistical studies are needed to gain a better understanding of the processes underlying the metabolic health benefits of timed lifestyle interventions.