Greased lighting : implications of circadian lipid metabolism for cardiometabolic health

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ADDENDUM

SUMMARY

Cardiovascular diseases (CVD) are the leading cause of death worldwide, and disturbances in day-night rhythms have recently been implicated as a novel risk factor for CVD. **Chapter 1** provides an overview of both lipid metabolism and the biological clock system and their role in the pathogenesis of CVD is outlined. We introduce the various research question that were addressed by a combination of studies in animal models and humans.

Part I of this thesis focuses on the effects of modulating circadian rhythms on energy metabolism using animal models. Disruption of circadian rhythmicity in humans, *e.g.* through exposure to light at night, is associated with obesity and related disorders, including type 2 diabetes and CVD. The underlying mechanism is unclear. Therefore, we investigated the effect on the duration of light exposure on the metabolic phenotype in **Chapter 2**. Mice that were exposed to day lengths of 16 h and 24 h light, compared to regular 12 h light, showed increased adiposity without food intake or locomotor activity being affected. Mechanistically, we demonstrated that prolonged day length decreases sympathetic input into brown adipose tissue (BAT) and reduces β3-adrenergic intracellular signaling in BAT. As a consequence, prolonging day length decreased the uptake of FAs from TG-rich lipoproteins as well as glucose from plasma selectively by BAT. We concluded that impaired BAT activity is an important mediator in the association between disturbed circadian rhythm and adiposity. Therefore, we anticipate that activation of BAT may overcome the adverse metabolic consequences of disturbed circadian rhythmicity.

Since BAT activity is subject to seasonal regulation, we hypothesized that BAT is also subjected to a circadian, i.e. 24 h, regulation by the central biological clock. In **Chapter 3** we characterized the 24 h rhythm of BAT activity and its implications for lipid metabolism. We observed a high amplitude 24 h rhythm in the TG-derived FA uptake by BAT. The highest uptake was observed at the onset of the active period which was explained by high *Lipoprotein lipase* (*Lpl)* expression and low *Angioprotein-like 4 (Angptl4)* expression, resulting in high LPL protein levels in BAT. Since BAT takes up TG-derived FA to produce heat, we examined fasting and postprandial lipid levels at various time points, and observed that circadian rhythmicity in BAT activity determined daily fluctuations in plasma lipid concentrations as well as lipid clearance. Strikingly, in mice as well as humans we found postprandial lipid excursions to be nearly absent at the onset of the active period and high before sleep, consistent with the diurnal BAT activity pattern. We hypothesize that diurnal rhythm in BAT activity explains previous findings that restriction of food intake to the early wakeful period improves metabolic health.

From the previous chapters, we concluded that the biological clock regulates BAT activity rhythm. However, the mechanisms underlying the regulation of BAT rhythmicity were still unclear. We hypothesized that the circadian rhythm of plasma glucocorticoids, referring to cortisol in humans and corticosterone in rodents, plays a crucial role. In **Chapter 4** we thus addressed this hypothesis by implanting mice with subcutaneous pellets containing a low concentration of corticosterone, which markedly dampened the endogenous corticosterone rhythm. After one week of corticosterone pellet treatment,

the ability of BAT to take up TG-derived FAs from the circulation was determined at AM (onset of the light period) and PM (onset of the dark period). In control mice receiving vehicle, FA uptake by BAT displayed a large AM-PM fluctuation that was in accordance with data shown in Chapter 3. Interestingly, this AM-PM fluctuation was abolished in mice with dampened corticosterone rhythms by preventing the rise in FA uptake PM. Examination of cellular pathways in BAT revealed that dampened rhythm in FA uptake was accompanied by dampened rhythms of the expression of *Lpl* and phosphorylation of CREB, a downstream β3-receptor signaling target. In contrast, known glucocorticoid-responsive element-controlled genes retained rhythmic expression patterns, suggesting that glucocorticoids did not act on brown adipocytes directly. We concluded that short-term dampening of corticosterone rhythm flattens the rhythmicity of BAT activity with respect to the uptake of TG-derived FAs in mice. Our data imply that the biological clock regulates rhythmicity of BAT activity via circadian glucocorticoid levels.

Shift work is a risk factor for atherosclerotic disease, however which aspect(s) of shift work causally induce(s) atherosclerosis remains to be elucidated. In **Chapter 5** we investigated the contribution of mistimed light exposure to atherosclerosis development by performing studies in female APOE*3-Leiden.CETP mice fed a Western-type diet, which is a well-established model for human-like lipoprotein metabolism and atherosclerosis. In two separate experiments, mice were either subjected to constant light (LL) or to three different rotating light-dark schedules, compared to control 12h light-dark (LD) schedule, during 15 weeks of Western-type diet feeding. While LL disturbed the circadian physical activity rhythm to some extent, atherosclerotic lesion development was not affected. In contrast, a weekly 6 h advance light schedule increased plaque severity. Moreover, weekly reversal of LD schedule, which is possibly most effective to disturb circadian rhythmicity, increased plaque severity as well as plaque size. Interestingly, increased atherosclerotic development could not be attributed to higher plasma cholesterol levels, which may suggest that other factors including regulation of immune function by the biological clock may play a role. In conclusion, mistimed light exposure aggravates atherosclerotic development in mice, which may at least partly explain the association between shift work and CVD in humans. Future studies should focus on whether this is mediated via cholesterol metabolism or inflammatory pathways.

Part II of this thesis focuses on the effects of altered day night rhythm and longevity on human lipid metabolism. In **Chapter 6** we investigated the effects of shortened sleep on metabolism. We previously showed that acute sleep curtailment induces insulin resistance, in healthy individuals as well as in patients with type 1 diabetes. Therefore, disturbances in sleep might play a causal role in the pathogenesis of insulin resistance, independent of endogenous insulin production. However, the underlying mechanisms remained unclear. This study aimed to explore the metabolic pathways affected by sleep loss using targeted metabolomics in human fasting plasma samples. Healthy individuals and patients with type 1 diabetes were studied after a single night of short sleep (4 h) versus normal sleep (8 h) in a cross-over design. Remarkably, one night of short sleep specifically increased the plasma levels of acylcarnitines, essential intermediates in mitochondrial FA oxidation. Specifically,

short sleep increased plasma levels of tetradecenoyl-L-carnitine (C14:1), octadecanoyl-L-carnitine (C18:1), and octadecadienyl-L-carnitine (C18:2). Since increased plasma acylcarnitine levels could be a sign of disturbed FA oxidation, it is possible that sleep curtailment acutely induces inefficient mitochondrial function. Inefficient mitochondrial function has been associated to insulin resistance. Also, acylcarnitines have been shown to have pro-inflammatory properties *in vitro* that could disturb insulin signaling. Our observations provide a basis for further research into the role of acylcarnitines as a potential mechanistic pathway by which sleep deprivation – even short term – causes adverse metabolic effects, such as insulin resistance.

In **Chapter 7** we investigated whether longevity may determine circadian rhythms in cholesterol concentrations. The function of the biological clock function deteriorates with increasing age, and aging is associated with dampening of circadian rhythms. Therefore, we hypothesized that individuals with a familial predisposition for longevity have a higher amplitude circadian rhythm in serum cholesterol concentrations. We investigated circadian rhythmicity of serum cholesterol concentrations in offspring of nonagenarian siblings and their partners. Offspring from nonagenarian siblings and their partners as controls were studied in a controlled in-hospital setting over a 24 h period, receiving three isocaloric meals. Serum total cholesterol, HDL-cholesterol and non-HDL-cholesterol were determined every 30 min over a 24 h period. The serum total cholesterol concentrations were higher during day than during night in offspring and in controls. The difference in total cholesterol concentrations between day and night tended to be greater in offspring than in controls, reaching statistical significance in females. Notably, the day-night serum differences in non-HDL-cholesterol were 2-fold greater in offspring than in controls and most explicit in females. We conclude that familial longevity is characterized by a high circadian rhythmicity of non-HDL- cholesterol in healthy elderly offspring from nonagenarian siblings.

Finally, in **Chapter 8**, we discuss the clinical implications and future perspectives of the research described in this thesis. Using animal studies we observed that BAT is strongly regulated by the biological clock, possibly via circadian glucocorticoid rhythms, and attenuated BAT activity through prolonged light exposure increases adiposity. Research focusing on the rhythm in human BAT, and regulation thereof, is necessary to confirm the translational value of our findings. We also observed that mistimed light exposure enhances atherosclerosis development, which may provide a mechanistic link between the known association between shift work and CVD. We anticipate that living according to the natural circadian rhythms presumably contributes to cardiometabolic health. Since disturbances in day-night rhythms are inevitable in modern society, in the future we may advise individuals at risk for development of CVD refrain from shift work and short sleep duration. In addition, data in this thesis may be useful to design strategies to avoid the disadvantageous metabolic effects of shift work.