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Greased lighting : implications of circadian lipid metabolism for cardiometabolic health

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Chapter 1

General Introduction and outline

GENERAL INTRODUCTION AND OUTLINE

Cardiovascular disease (CVD) is the leading cause of mortality worldwide [1]. The main contributor to CVD is the formation of atherosclerotic plaques in the vessel wall, a process largely driven by hypercholesterolemia [2]. Indeed, cholesterol-lowering drugs such as statins have substantially lowered CVD related mortality [3]. In spite of this success, the incidence of CVD has continued to rise, prompting the search for additional modifiable risk factors. Perturbation of our biological clock has recently been recognized as a potential modifiable risk factor for metabolic diseases [4]. Epidemiological studies demonstrated an association between disturbed day-night rhythms, such as due to shift work [5], short sleep [6] and light at night [7-9], and several metabolic disturbances, such as obesity, dyslipidemia, insulin resistance and coronary heart disease. The overall aim of the projects described in this thesis is to investigate the mechanisms by which the biological clock contributes to lipid metabolism and how derangement of the biological clock may contribute to metabolic disorders. I will therefore provide an overview of the basic aspects of lipid metabolism and mammalian chronobiology, and the interplay between these two fields.

Within the topic of lipid metabolism, I will focus on lipoprotein metabolism and the role of brown adipose tissue (BAT) in lipoprotein metabolism. I will discuss the pathophysiology of atherosclerosis with a focus on dyslipidemia and obesity as risk factors. Within the topic of chronobiology, I will briefly address the basic organization of the mammalian biological clock, as this thesis describes studies performed in mice and humans. In this thesis, I will also explore the role of the hypothalamus-pituitary-adrenal (HPA)-axis in circadian rhythms. Therefore, the HPA-axis will be addressed in more detail. Next, since in humans short sleep duration is a common behavioral disruption of physiological day-night rhythms, I will briefly introduce human sleep physiology with a focus on short sleep duration and energy metabolism. Finally, I will combine these two fields and discuss circadian rhythmicity in lipid metabolism and give an overview of the evidence for a causal role of disturbed rhythms in metabolic disease.

Metabolism

Lipoprotein physiology

Lipids are hydrophobic molecules which encompass different molecular substances. For this thesis, I will focus on triglycerides (TG) and cholesterol, since these lipids are most involved in metabolic health and development of CVD. TGs are the most abundant lipids in human diet and their function for metabolism is to deliver energy in the form of fatty acids (FAs). TG consists of three FA chains esterified to a glycerol backbone. Cholesterol is an abundant dietary lipid in a typical Western type diet, with an entirely different structure and function. Cholesterol serves to build membranes, and serves as a precursor molecule for synthesis of bile acids and steroid hormones. Due to their hydrophobic nature, both TGs and cholesteryl esters, the storage form of cholesterol, have to be properly packaged into lipoprotein particles to be transported in the circulation. Lipoproteins are large spherical

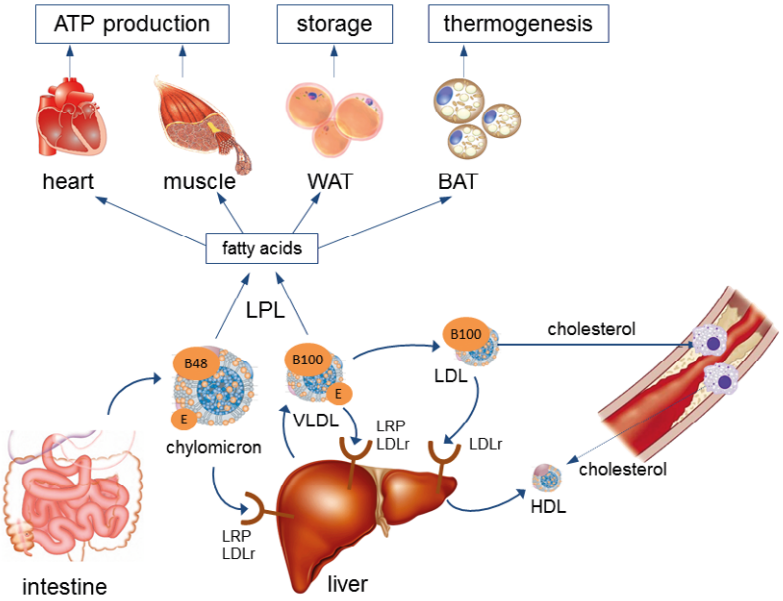

Figure 1. Overview of lipoprotein metabolism. *ATP = adenosine triphosphate, BAT = brown adipose tissue, LDLr = LDL receptor, LPL = lipoprotein lipase, LRP = LDL-receptor related protein, TG = triglyceride, WAT = white adipose tissue. See text for explanation.*

macromolecular complexes, with a lipid rich core consisting of TGs and cholesteryl esters and an outer layer composed of proteins, free cholesterol and phospholipids. They are classified based on their diameter and density: very low density lipoproteins (VLDL), low density lipoproteins (LDL), intermediate density lipoproteins (IDL), high density lipoproteins (HDL), and chylomicrons (CM) (chyle refers to lymph).

Since TGs either derive either from the diet or from de novo synthesis in the liver, the latter predominating during fasting, there are two lipoprotein synthesis routes: the endogenous and exogenous pathway [10]. After feeding, dietary TGs within the intestinal lumen are hydrolyzed into FAs and 2-monoacylglycerol, both of which are taken up by enterocytes and re-esterified into TGs. Likewise, cholesteryl esters are hydrolyzed an cholesterol is taken up by enterocytes and re-esterified. In the enterocytes, apolipoprotein B (apoB48 in humans, apoB100 in rodents) is added onto droplets consisting of TGs and cholesteryl esters by the enzyme microsomal triglyceride transfer protein (MTP), eventually producing chylomicrons during exit of enterocytes by coating with a shell composed of phospholipids, cholesterol and additional apolipoproteins. These particles are secreted into the lymphatic fluid, and eventually reaches metabolically active cells via the circulation. In case of fasting, this role is taken over by the liver, which continuously produces VLDL particles according to intracellular mechanisms similar to chylomicrons, although they are built from apoB100 in both humans and rodents [11-13]. In the circulation, these large TG-rich VLDLs or CM are trapped onto the endothelium by proteoglycans in metabolically

active tissues where TGs are hydrolyzed by lipoprotein lipase (LPL) [14]. The liberated FA can then be taken up by parenchymal cells via FA transporters such as CD36 and FA acid transport protein (FATP). Over time, VLDL and CM are delipidated; they decrease in size and increase in relative density, turning into remnant particles, namely VLDL remnants, or IDL, and CM remnants. In the blood stream, they acquire additional apolipoproteins, importantly ApoE. ApoE can bind to both the LDL-receptor (LDLr) and LDLr-related protein (LRP) on hepatocytes, mediating their uptake [15]. Further delipidation of IDL results in LDL, which have lost all apolipoproteins except for ApoB100 that induces uptake by the LDLr only. HDL is a separately synthesized particle within the liver and intestine uniquely containing ApoA1 and/or ApoA2. HDL is secreted as a cholesterol-poor discoid particle, which has the capacity to take up cholesterol from various peripheral tissues, including macrophages. Also, in humans, HDL can take up cholesteryl esters from LDL particles, in exchange for TG via cholesteryl ester transfer protein (CETP). While ApoB-containing lipoproteins are associated with increased risk for CVD, ApoA-containing HDL is associated with a decreased risk for CVD [10] (Figure 1).

Dyslipidemia and the development of atherosclerosis

The major part of CVD is attributable to atherosclerotic disease [16]. Atherosclerosis is a chronic and active pathological process within the vessel wall, consisting of an inflammatory and a lipid-driven component. The lipid-component is driven by elevated circulating concentrations of LDL particles. LDL particles can enter the vessel wall, where they are prone to modification by *e.g.* oxidation and aggregation. Macrophages take up these modified LDL particles via SRA and CD36, resulting in 'foam cells': large cholesterol-rich macrophages. Eventually, foam cells die and attract more immune cells, precipitating a pro-inflammatory cascade. On the other hand, HDL is described as anti-atherogenic, not only because of its anti-oxidative and anti-inflammatory properties, but mainly by its ability to extract cholesterol from macrophages via ABCA1 and ABCG1 for transport to the liver for excretion into the feces, a process that is termed reverse cholesterol transport [17]. Atherosclerotic plaques occlude the vascular lumen, diminishing laminar blood flow, without prominent clinical symptoms. However, upon plaque rupture, rapid platelet aggregation results in formation of thrombi that can completely occlude the lumen of a blood vessel, resulting in hypoxia and death of tissues downstream of the occlusion. Occlusion of the carotid artery results in a stroke, while in the coronary artery this results in a myocardial infarction.

White adipose tissue and its role in obesity

The prevalence of overweight and obesity has reached epidemic numbers worldwide. According to the World Health Organization, in 2014 1.9 billion people were overweight and 600 million were obese [18]. Obesity is a major risk factor for dyslipidemia and cardiovascular disease, but also for development of a myriad of other diseases, such as type 2 diabetes (T2D) and cancer [19, 20]. Although obesity is classified most commonly according to the body mass index (BMI, in kg/m^2), this poorly reflects the underlying

pathophysiology, since it ignores body composition. The body composition plays an important role in the predisposition to metabolic disease, where the accumulation of white adipose tissue (WAT) is pivotal. Upon a positive energy balance, i.e. a surplus of energy intake over energy expenditure, excess energy is stored in WAT in the form of TGs. When a critical level of TGs in WAT is reached, the white adipocytes produce pro-inflammatory mediators, initiating a pro-inflammatory state within the WAT which perpetuates itself. This low-grade inflammatory status induces insulin resistance and leads to steatosis of other organs including skeletal muscles and the liver by 'lipid overflow', causing insulin resistance in those tissues. Prolonged insulin resistance may result in the development of T2D where insulin sensitizers and eventually insulin is administered to control pathological consequences to high blood glucose levels. Insulin resistance of the liver results in a higher VLDL particle output, which increases circulating TG levels, thus aggravating dyslipidemia [21]. Therefore, promoting weight loss and preventing obesity are key strategies to prevent CVD.

Brown adipose tissue: a target to promote metabolic health

Besides the white fat depots which have an important role in obesity, both humans and rodents also possess brown fat depots. In contrast to WAT, BAT burns energy to produce heat. Cold is the most important physiological stimulus to activate BAT. Cold signals from the skin are processed by the anterior hypothalamus, which increases the sympathetic outflow towards the densely innervated BAT. Upon release of noradrenalin, the β3-adrenergic receptor on brown adipocytes is activated, leading to a downstream signaling cascade, ultimately resulting in increased hydrolysis of intracellular TG stores and increased FA oxidation by mitochondria. The mitochondria-dense brown adipocytes uniquely express uncoupling protein 1 (UCP1), which allows dissipation of the proton gradient generated by the electron transport chain into heat instead of ATP production. To replenish intracellular lipid stores, brown adipocytes increase the uptake of TG-derived FA as well as glucose from the circulation (Figure 2) [22].

In rodents, increasing BAT activity, either by physiological or pharmacological stimuli, has been proven to prevent and diminish diet-induced obesity and to lower dyslipidemia and atherosclerosis development [24-26]. In recent years, efforts have been made to translate these results to humans. In healthy young volunteers, a 3 week mild cold exposure intervention increased BAT volume (as detected by [^{18}F]fluorodeoxyglucose uptake by PET-CT) and decreased fat mass by approx. 800 g [27]. Additionally, BAT activation by cold acclimation improved insulin sensitivity in individuals with T2D [28]. South Asians, an ethnic group with a predisposition for dyslipidemia, obesity and CVD, have lower BAT detectability accompanied by lower resting energy expenditure [29]. Therefore, the activation of BAT in humans is a viable therapeutic strategy to combat cardiometabolic diseases including adiposity, dyslipidemia and CVD. Current treatment strategies aim to recruit more BAT volume and/or to stimulate BAT activity to increase energy expenditure [30].

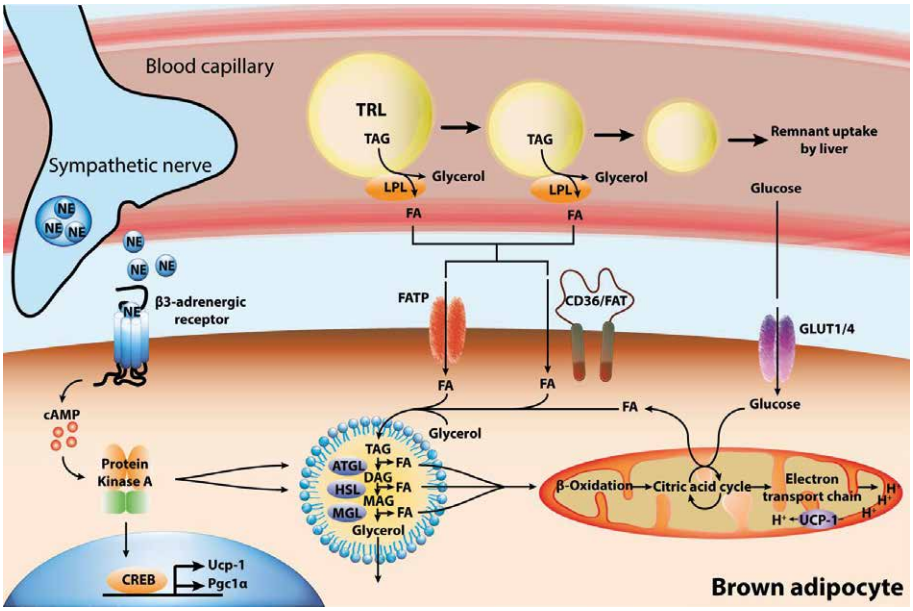

Figure 2. Brown adipocyte physiology. *DG = diglyceride, FA = fatty acid, FATP = fatty acid transport protein, LPL = lipoprotein lipase, MG = monoglyceride, TG= triglyceride, TRL = triglyceride-rich lipoprotein, UCP-1 = uncoupling protein-1 . See text for explanation. From Hoeke et al. [23]*

Chronobiology

Organization of the mammalian biological clock

The earth turns around its axis, resulting in the daily rhythm of light and dark, while the rotation of the earth around the sun results in a yearly rhythm of different day length. These 'circadian' (from Latin *circa*= about; *dies*=day) and seasonal rhythms have resulted in the evolution of biological clock systems throughout living organisms on earth (see box for more circadian terminology). Biological clocks are ubiquitous; they are present in bacteria, fungi, plants and animals. Presumably, the DNA damaging effects of ultraviolet radiation during the day has been one of the first triggers for a compartmentalization of cellular processes. DNA has to be unfolded for replication which renders it more susceptible to breaks. Darkness is therefore a more favorable period for DNA replication and repair. Furthermore, during the light period, energy from sunlight is available for photosynthesis, giving rise to a natural circadian rhythm in energy availability. Seasonal rhythms are represented by changing light exposure duration and are highly associated with changes in temperature. Rather than reacting to these changing environmental circumstances, the biological clock system evolved to anticipate to these recurrent events [31].

The light-dark cycle is the most important cue for the mammalian biological clock. The biological clock system is hierarchy organized, with a central clock, located in the suprachiasmatic nuclei (SCN) of the hypothalamus, which governs peripheral clocks throughout the body. Light is perceived by the eye and information about the intensity and wavelength of light is transferred directly to the SCN via the retino-hypothalamic tract [32]. The SCN, consisting of approx. 20,000 individual neurons, has the unique capacity to produce a rhythmic electrical firing rate, which is sustained *ex vivo*. The individual neurons synchronize each other through neuropeptide coupling, synaptic signaling and gap junctions [33]. The output of the SCN consists of this rhythmic electrical signal as well as an endocrine output, most importantly vasoactive intestinal polypeptide and arginine vasopressin [34, 35]. The SCN is the only part of the biological clock which allows entrainment of our biological clocks to light. However, light is not the only environmental cue conferring information on 24h rhythms and indeed the SCN can be synchronized by other "Zeitgebers" (German for 'time givers'). These Zeitgebers include food intake and physical activity [36] (Figure 3).

BOX: RELEVANT CIRCADIAN TERMINOLOGY

Amplitude: the distance between the peak or trough and the mean value of an oscillatory function
Chronobiology: the scientific field that studies biological rhythms

Chronotherapy: treatment based on knowledge of chronobiology. May be used for different aspects, such as timing of drug administration, using of light as treatment or treatments aimed to improve sleep-wake rhythms.

Circadian: taking place or functioning in cycles of approximately 24 hours. Conventionally, circadian rhythms are required to be endogenously generated and therefore must be determined under constant conditions (such as in mice under constant darkness).

Daily: taking placing every 24 hours

Diurnal: for this thesis same as daily. Can also refer to species which have their habitual activity period during the light phase (contrary to *nocturnal*)

Free-running period: the period of an oscillation (rhythm) in the freerun state

Jet lag: adverse health effects associated with the disruption of circadian rhythms, typically caused by time zone travel

Peak: highest value within a cycle

Period: the amount of time between the completion of one cycle

Trough: lowest value within a cycle

Zeitgebers: synchronizing environmental signals

Zeitgeber time: convention to display time where onset of light is defined as ZT0. Measured in hours

The SCN orchestrates 24h rhythms in physiological processes and behavior to align them to the environment. The SCN synchronizes peripheral tissues both directly via hormonal and neuronal output originating from the SCN and indirectly, via regulating brain areas and dictating rhythmicity in hormonal output that is generated outside the SCN. The SCN has many direct neuronal connections to other brain regions, *e.g.* nuclei that control food intake behavior (e.g. the arcuate nucleus), body temperature (the pre-optic area) and hormonal output (e.g. pineal gland and paraventricular nucleus (PVN)) [37]. In addition, direct neuronal connections exist with peripheral tissues, including metabolic organs such as liver, adipose tissue and muscle. Likely, this holds true for additional metabolic organs which have not been investigated up to now [38-40].

Peripheral tissues exhibit rhythmicity in their tissue-specific function. While not every cell of the mammalian body has been studied, for now it seems safe to state that all tissues display a degree of rhythmicity. Estimates of the extent of rhythmicity in peripheral tissues are often derived from microarray studies, which report that 5-10% of the transcriptome displays a circadian rhythm [41, 42]. Data derived from transcriptome analysis are probably an underestimation of the cellular circadian processes, since besides transcription, also histone modification, translation, post-translational modification and protein degradation display a circadian rhythm, [43, 44]. All mammalian cells that have been studied so far contain a molecular clock, driven by a transcriptional-translational feedback loop. In the past decade, the most important proteins of the molecular clock have been elucidated. Upon dimerization of core clock proteins BMAL1 and CLOCK (or within the same tissues their analogues BMAL2 and NPAS2), they form an active transcription factor. They bind to the promotor element E-Box, which promotes the transcription of downstream genes. Among these genes are both clock gene repressors and the 'clock controlled genes' (ccgs), which exert their tissue-specific functions. The clock gene repressor genes encode for proteins, namely PERIOD (PER1, PER2), CRYPTOCHROME (CRY1, CRY2) and NR1D1 (better known as REV-ERBα), which translocate back to the nucleus and inhibit the BMAL1-CLOCK dimer, thereby forming the negative arm of the transcription-translation feedback loop. This molecular clock system is equal amongst different cell types, also within the SCN (Figure 4) [45, 46].

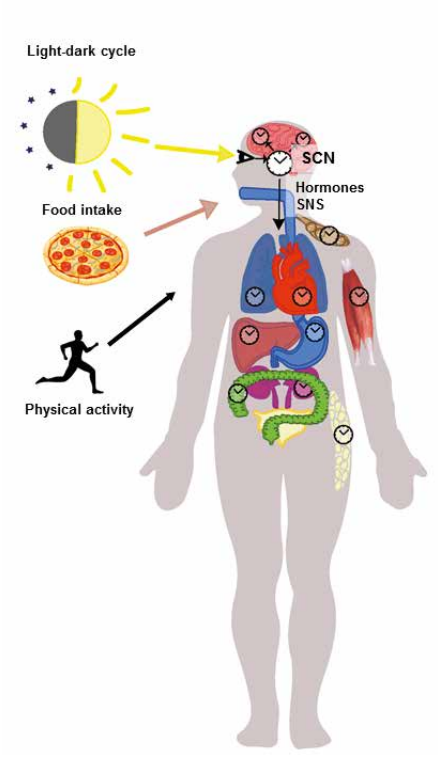

Figure 3. Organization of mammalian circadian clock system. *SCN = suprachiasmatic nuclei; SNS = sympathetic nervous system. See text for explanation.*

HPA-axis physiology and its role in chronobiology

The SCN function and the molecular clock in peripheral tissues are well-described. Nevertheless, the exact signals that transfer the timing signal of the SCN to peripheral tissues are still largely unknown. There is some evidence for glucocorticoids to confer timing signal to peripheral tissues [47, 48]. Glucocorticoids are able to synchronize the circadian rhythm in clock gene expression *in vitro* [49, 50]. Also, complete absence of endogenous

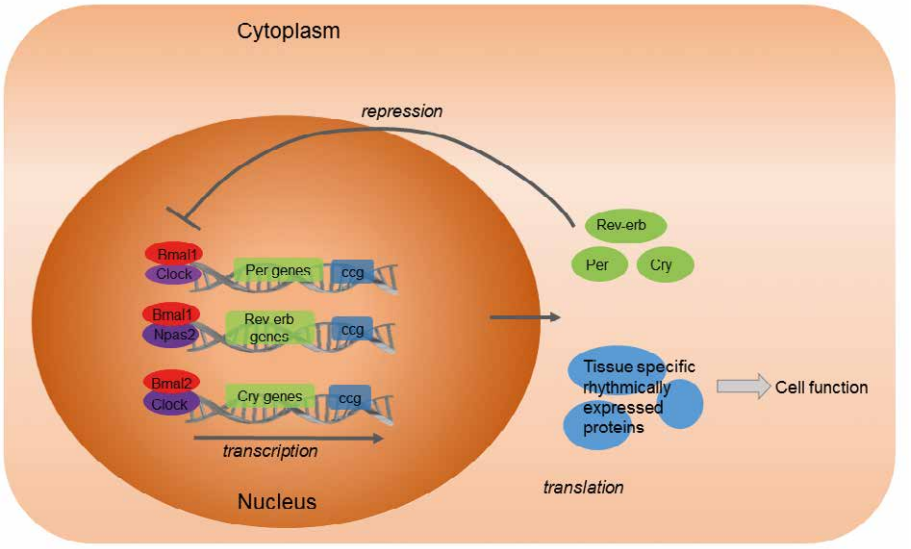

Figure 4. Cell autonomous clock. *Every mammalian cell contains a core clock machinery which controls circadian gene expression via transcriptional translational feedback loop. Ccg = clock controlled gene. See text for explanation.*

glucocorticoids by adrenalectomy shifts the circadian rhythm in clock genes in peripheral tissues [51]. In humans subjected to a constant food, sleep and activity protocol, an oral dose of glucocorticoids also shifted clock gene expression pattern in peripheral blood mononuclear cells [52].

Glucocorticoid secretion is regulated via the HPA-axis, which is the endocrine system that is best known for its response to stress. Upon a physiological or psychological stressor, the PVN secretes corticotrophin-releasing hormone (CRH). CRH stimulates the pituitary to secrete adrenocorticotropic hormone (ACTH) into the circulation, which stimulates the adrenals to secrete glucocorticoid hormones (cortisol in humans, corticosterone in rodents). ACTH and glucocorticoids exert a negative feedback on the hypothalamus and on the pituitary which dampen the response. Interestingly, in absence of stress, plasma glucocorticoid concentrations are highly rhythmic. In both humans and rodents, glucocorticoids reach a peak concentration before waking up ('arousal') and decrease throughout the wakeful period reaching the trough before sleep [53].

Glucocorticoids are steroid hormones which travel to the cell nucleus where they bind to their receptors: the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). Since the MR has a higher binding affinity than the GR, the MR is bound throughout the 24h period, while the GR is only bound during the peak concentrations, such as during stress or the circadian peak [54]. MR and GR are present throughout the body, including the brain and metabolic organs such as liver, adipose tissue and muscle. In metabolism, glucocorticoids act as catabolic hormones, i.e. to increase available energy. GR agonism

increases glucose release, gluconeogenesis, and lipolysis while inhibiting glucose uptake by muscle and adipose tissue. The circadian peak of glucocorticoids thereby increases energy availability before waking up.

Human sleep physiology and sleep insufficiency

Humans have a well-defined circadian sleep-wake rhythm. Sleep takes place during the night, while during the day we are awake and active. It is important to note that sleep physiology is not solely an output of the biological clock, but two processes: sleep timing and sleep pressure. Therefore, sleep insufficiency is not synonymous to a disturbance of the biological clock, but also affects the sleep pressure physiology. Sleep timing is the process determined by the biological clock. The SCN determines a circadian rhythm in wakefulness which is aligned with the light-dark cycle. Furthermore, sleep timing ensures that sleep-wake cycles are synchronized to circadian rhythms in other bodily functions [55]. This is relevant to energy metabolism, as sleep coincides with fasting and a nearly absent physical activity levels. Sleep pressure is a direct function of wakefulness. Upon wakening, sleep pressure builds up and upon sleep, sleep pressure decreases. The regulation of onset of sleep and maintaining sleep is a delicate interplay of brain areas promoting wakefulness, suppressing wakefulness and promoting sleep. For the regulation of sleep timing, it is clear that melatonin plays an important role. How sleep pressure is regulated is not completely understood [56].

Sleep insufficiency is highly prevalent, with 70.1 million adults reporting less than 6 hours sleep per night [57]. Epidemiological studies strongly associate habitual short sleep duration (<5 hours per night) with many metabolic disorders [6, 58-60]. Interestingly, while most studies report a linear relationship between sleep duration and metabolic outcome parameters, others also report an association between long sleep duration (usually >9-10 hours per night) and metabolic disorders, resulting in a U-shaped association [61, 62]. A recent study demonstrated that short sleep duration was a risk factor for BMI, but not perceived sleep loss [63]. The notion that optimal sleep duration exists, is supported by studies on sleep duration in absence of modern lifestyle demands such as artificial light and setting an alarm clock. Individuals living in pre-industrialized communities have a reported sleep duration of approximately 7 hours per night [64]. Likewise, young adults camping one week outdoors, banned from electrical lights, alarm clocks and watches, display similar sleep-wake cycles [65]. In both cases, sleep duration was not equal to the total hours of darkness, but rather was kept constant by staying awake by making campfires. Together these data suggest that the optimal sleep duration is about 7 hours sleep per night.

Interplay between lipid metabolism and the biological clock

Rhythms in lipid metabolism

It has long been investigated whether human plasma lipids display circadian variation. Under standardized meal circumstances, it is clear that TG levels are highly rhythmic with a reported variation of 30-60%. TG levels also display a clear postprandial excursion [66-68].

The postprandial TG excursion shows variable peak levels throughout the day, with a higher excursion in the morning than in the afternoon [66, 69]. Circadian rhythm in TG uptake by the intestine may be a contributor, since enterocytes express clock genes [70]. Mice with a mutation of the *Clock* gene have increased lipid absorption and doubled plasma TG and cholesterol levels [71]. Cholesterol levels seem more stable throughout the day; some studies show some degree of rhythmicity [67, 72] while others do not [66, 73]. Furthermore, extensive lipidome analysis demonstrated that circulating plasma lipids are rhythmic independent of food intake in humans [74, 75] and rodents [76]. Therefore, the production and/or uptake of lipids by metabolic organs must be rhythmic. In rodents, genes involved in both TG and cholesterol production and uptake in the liver were shown to be rhythmic [76, 77]. In humans, cholesterol synthesis was estimated by determination of cholesterol precursors in plasma, and appeared to display a diurnal variation [73]. It is currently not yet known, whether the lipid uptake by metabolically active tissues displays a circadian rhythm.

Circadian disruption as a causal risk factor for metabolic disorders

In the past decade, animal studies have provided ample evidence for a causative link between disturbance of the biological clock and cardiometabolic disorders. Specific electrical ablation of the SCN induces obesity and insulin resistance in rodents [78]. Also, several genetic knock-out models of clock genes have been generated, which resulted in adverse metabolic phenotypes, including dyslipidemia and obesity [79-82]. The adverse metabolic phenotype observed in whole-body knock-out of clock genes may have a similar etiology as the SCN ablation, since the genetic deficiencies also affect the SCN [83]. Interestingly, tissue-specific disturbances of rhythm also induce metabolic disturbances. Mice with an adipocyte-specific knock-out of *Bmal1* have higher body weight than control animals due to increased food intake. Interestingly, this was attributed to an increased FA release by WAT during daytime, which increases orexigenic signals in the hypothalamus [84]. Furthermore, tissue-specific disruption of clock genes can enhance atherosclerosis formation. *Ldlr-/-* mice that received bone marrow from *Rev-erbα-/-* mice exhibit a pro-inflammatory phenotype due to M1 polarization of macrophages [85]. *Apoe-/-* mice that received bone marrow from *Clock* mutant mice have impaired cholesterol efflux from macrophages [71].

Unfortunately, these genetic models for disturbed circadian rhythms are not readily translatable to humans. Alternatively, light exposure changes can modulate the biological clock output without inducing genetic defects. In mice, constant light exposure induces a fast increase in weight gain, independent of physical activity and food intake. Instead, constant light exposure lowers total energy expenditure [86-88]. Mechanistically, constant light dampens the circadian electrical output of the SCN [86] and it was previously shown that constant light dampens circadian glucocorticoid levels [89, 90]. In humans, an experimental study showed that 2 hours of blue light exposure in the evening changes energy expenditure the following morning. This indicates that light exposure, presumably via the SCN, can causally influence metabolism in humans [91].

OUTLINE OF THIS THESIS

The overall aim of this thesis is to investigate the role of circadian rhythm in metabolism, specifically involving lipid metabolism and its implications for metabolic disease.

Part I describes animal models of circadian rhythms with particular focus on the role of BAT. Since light exposure has been associated to increased body weight and BMI in humans, we set out to investigate how light exposure affects metabolism. In **chapter 2** we investigated the effect of prolonged light exposure on body composition and evaluated underlying mechanisms to show that prolonged light dampens BAT activity. In **chapter 3**, we included both shortened and prolonged light exposure as modulators of the biological clock, and investigated diurnal rhythms in BAT activity and their consequences for plasma lipid metabolism. Next, we set out to further elucidate the mechanism by which the biological clock regulates BAT rhythmicity. We focused on the role of the HPA-axis by evaluating the effect of a dampened glucocorticoid rhythm on BAT rhythmicity in **chapter 4**. Since light exposure can alter metabolism, at least via BAT activity, we hypothesized that rotating light schedules may also underlie the association between shift work and atherosclerotic disease. In **chapter 5** we, therefore, investigated the effect of light schedules on atherosclerosis development.

Part 2 focuses on human models for circadian rhythms. A common human disturbance of physiological day-night rhythms is shortened sleep duration. Previously, it was found that one night of short sleep increases insulin resistance. In **chapter 6**, we aimed to better understand the underlying mechanism for this finding by performing metabolomic analysis on plasma. Another human condition associated with deterioration of circadian rhythms is aging. We made use of this fact to better understand whether circadian rhythms in cholesterol levels are affected by the biological clock and may relate to human health. In **chapter 7** we, therefore, determined diurnal plasma cholesterol concentrations in a cohort of individuals with a familial predisposition for longevity and their spouses.

The results and implications of these studies are discussed in **chapter 8**.

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