

Chronodisruption, Metabolic Homeostasis, and the Regulation of Inflammation in Adipose Tissues

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Molecular circadian clocks align daily behavioral and metabolic rhythms with the external day-night cycle. Priming energy metabolism for recurring changes on a 24-hour basis, these clocks are deeply interlinked with metabolic homeostasis and health. Circadian rhythm disruptions, as occurring in shift work or sleep disorders, are often accompanied by metabolic disturbances – from the promotion of overweight and type-2 diabetes to the development of the metabolic syndrome. An important indicator of the adverse outcomes of overweight seems to be a systemic low-grade inflammation which is initially observed in adipose tissues and is promoted by circadian misalignment. Interestingly, the genetic disruption of circadian clocks in rodents leads to metabolic dysregulations very comparable to what is observed in shift workers and with the development of tissue specific clock gene knockout mice, the importance of single-tissue clocks for the metabolic regulation was further deciphered. In this review, we summarize the current knowledge on the role of mistimed behavior in metabolic health and outline behavioral interventions aiming at reducing the metabolic ramifications of chronodisruption.

CIRCADIAN INFLUENCE ON ADIPOSE METABOLISM, OVERWEIGHT, AND METAINFLAMMATION

Today's society is facing an overweight pandemic with an obesity rate of more than 30 percent in adults and a similarly high obesity rate of 16 percent in children in the United States [1]. In the last decades a profound progression towards a sedentary lifestyle in combination with over-nutrition increased the prevalence of obesity and its

complications [2]. Metabolic health and body weight regulation are deeply interlinked with the endogenous circadian clock system that orchestrates 24-hour rhythms of behavior and metabolic regulation [3]. Perturbation of this regulatory system might amplify metabolic dysregulation and the adverse effects of typical modern-day and lifestyle factors such as shift work, long active hours, and reduced overall sleep, which all have the capability to disrupt daily behavioral routines. In combination with a constant food availability, this challenged metabolic

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†Abbreviations: TGs, triglycerides; BMAL1, Brain and Muscle Arnt-like protein-1; CLOCK, Circadian locomotor Output Cycles Kaput; WAT, White Adipose Tissue; BAT, Brown Adipose Tissue; HFD, High Fat Diet; IL-6, Interleukin 6; TNF- α , Tumor Necrosis Factor α .

Keywords: circadian clocks, chronodisruption, adipose tissue, metabolism, inflammation

Author Contributions: IK and HO conceptualized the paper. IK and HO wrote and revised the manuscript.

regulation is further stressed, and weight gain and impaired glucose tolerance might arise [4]. In this review we evaluate the interaction of modern 24-hour lifestyles, circadian clocks, and their role in metabolic regulation and health.

THE FAT CENTURY

Today's permanent and ubiquitous availability of food changed human nutrition to a state where – in stark contrast to all times up to now – the worldwide probability of being overweight has become higher than that of suffering from undernutrition [5]. Particularly during the last few decades overweight and obesity have steadily increased throughout the population and in line with this weight increase, the risk was elevated for sequelae like type-2 diabetes, cardiovascular disorders, and the metabolic syndrome [6]. These metabolic alterations are often associated with a modern lifestyle; shift workers in particular have an elevated risk of developing at least one metabolic disorder in their lifespan with risk ratios being directly proportional to the time period spent in shift work [7,8]. In shift work at least three factors are combined that already each on its own can impact health and metabolism: changed sleep patterns, nocturnal light exposure, and mistimed food intake. Roenneberg and Merrow developed a comprehensive model for the interaction of these factors in regard to the circadian misalignment and the development of obesity and other health issues [9]. Humans are diurnal species sleeping mainly during the dark hours of the night. Habitual sleep lasting around seven hours per day on average associates with the lowest death risk in comparison to shorter or longer sleep hours where the mortality rate is higher, probably due to an increased systemic inflammation or an altered overall health status [10]. Outcomes of short sleep times worsen if sleep duration is further decreased or mistimed [11]. In school children, for example, not only the lack of sleep, but also a shift to later sleeping hours is associated with increased weight gain during the summer holidays [12]. Nocturnal wake episodes or night shift work often involve increased light exposure during the dark, which further disrupts molecular circadian rhythms. In mice, already low levels of light at night shift behavioral and metabolic rhythms and lead to increased weight gain [13,14]. Shortened sleep is also associated with reduced levels of the satiety-inducing hormone leptin and elevated levels of the hunger-promoting peptide ghrelin [15]. With regard to glucose metabolism, postprandial insulin release from the pancreas is decreased close to the rest phase compared to the beginning of the activity phase in humans [16]. Thus, late meals towards the end of the active phase, *i.e.* the evening in humans, the morning in nocturnal rodents, lead to prolonged high serum glucose

concentrations that are more likely to be stored as triglycerides (TGs) in adipose tissues [16,17].

Natural sleep and meal rhythms are often severely disrupted by shift work. Mimicking such conditions in the laboratory, in subjects following a 3-day behavioral and environmental aligned (day work) or misaligned (night work) schedule circadian misalignment resulted in a pre-diabetic state of impaired glucose tolerance and decreased insulin sensitivity [18]. Along with metabolic changes and disrupted circadian rhythms, the immune system is affected in shift workers resulting in altered numbers of circulating lymphocytes and natural killer cells and increased inflammatory cytokines like interleukin 6 (IL-6) and tumor necrosis factor α (TNF- α) that contribute to a pro-inflammatory phenotype [19-23]. Nocturnal light exposure also directly leads to a drop of the epiphyseal hormone melatonin [24]. Low melatonin levels – or light pulses at night – are associated with an increased breast cancer risk in night shift working nurses [24]. With regard to energy metabolism, melatonin supplementation was able to improve body weight regulation and metabolism in a rat model for obesity-related type-2 diabetes [25]. Oral supplementation in humans with melatonin deficiency increases volume and activity of brown adipose tissue (described later) and improves total cholesterol and triglyceride levels without affecting body weight [26]. Melatonin can reset the circadian sleep cycle and has beneficial short-term effects on shift workers [27]. Its stabilizing effect on sleep-wake regulation is further improved when combined with bright light exposure in the morning [28,29]. Interestingly, so far, no long-term effects of melatonin supplementation have been investigated.

SNACK THE WEIGHT UP – MISTIMED FOOD INTAKE AND ITS METABOLIC CONSEQUENCES

Being active in the normal rest phase – *e.g.* during shiftwork – is often accompanied by mistimed food intake which not only increases the total caloric intake, thereby promoting weight gain, but also has adverse effects on energy metabolism (see Figure 1). This was first observed in mice that were fed exclusively during their inactive phase (*i.e.* the day), gaining more weight than their active phase-fed littermates already after nine days [30]. The inactive phase fed group shifted circadian rhythms in metabolic active organs. Moreover, overall caloric intake was elevated with the majority of food being consumed shortly after it became available [30,31]. Lifestyle influences on everyday eating behavior and bodyweight homeostasis in humans have been observed. In a small-scale study by the Panda group, non-shift workers ate at almost random intervals over a time window of 14-15 hours of the

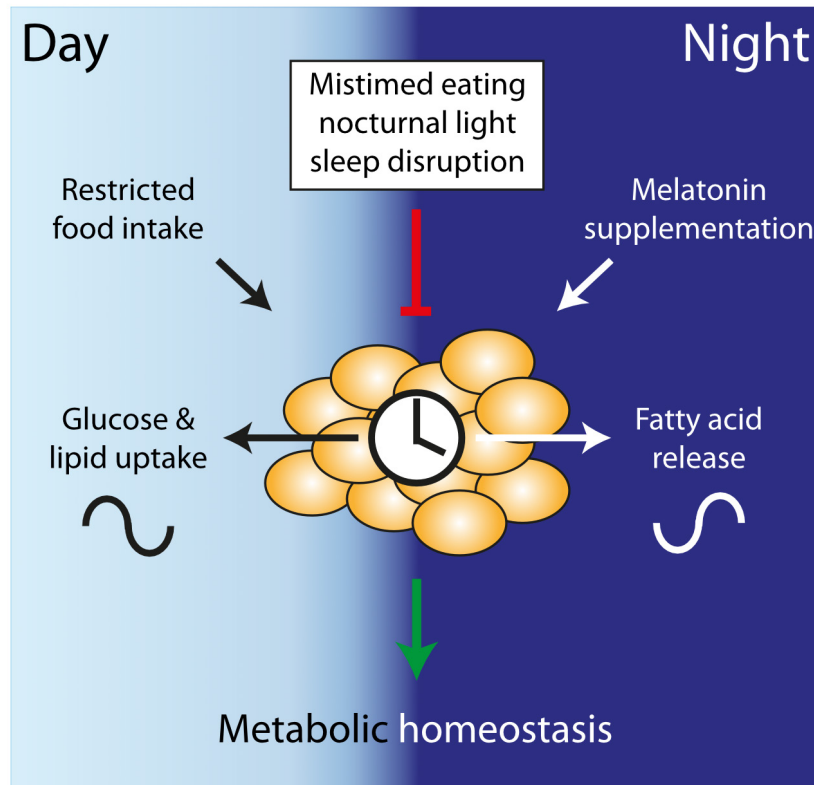


Figure 1. Diurnal adipose tissue homeostasis. During active/ daytime hours food is consumed and white adipose tissue stores Glucose and lipids. During the dark and fasting night fatty acids are released, supported by melatonin. Undisturbed cycles stabilize the metabolic homeostasis (green). Mistimed eating, nocturnal light and sleep disruption inhibit this timed processes (red).

day. If overweight participants from this group reduced their daily eating timespan to 10 hours, without any other restrictions, they not only lost weight but also improved their sleep rhythm [32]. Food is a strong *Zeitgeber* for peripheral organs and the timing of food intake can disrupt or amplify the coordinated clock system [33]. The importance of meal timing is also seen in mice with access to a high-fat diet (HFD). If provided *ad libitum*, HFD weakens rhythmic behavior with mice eating more during their inactive phase, promoting hyperphagy and obesity [34]. However, when HFD is only provided during the nighttime (*i.e.* the active phase in mice), mice stay slim and healthy [35,36]. Interestingly, especially the late phase of the activity phase may be crucial for regulating energy homeostasis. Mice that are offered an HFD snack at the end of their active phase show increased adiposity and hypoleptinemia compared to those receiving an early active phase snack [31]. In a repeated jet lag experiment (6 hours of phase advance every week) mice with *ad-libitum* food access become overweight within 10 days. In this setting obesity is prevented by restricting the food availability to the dark phase [37].

ADIPOSE TISSUE CIRCADIAN CLOCKS IN THE REGULATION OF ENERGY METABOLISM

Adverse outcomes of misaligned behavior have attributed to disruptions of the endogenous circadian clock apparatus. Circadian clocks have evolved to anticipate predictable changes in environmental conditions, priming the organism for optimal metabolic function and nutrient utilization [38]. On the molecular level, circadian time-keeping is based on 24-hour oscillations of interlocked transcriptional-translational feedback loops that regulate cell type-specific programs of clock-controlled genes by binding to circadian enhancer elements [39,40]. At the center of this molecular clock apparatus are the transcription factors BMAL1 (*Brain and Muscle Arnt-like protein-1*) and CLOCK (*Circadian locomotor Output Cycles Kaput*). Circadian clock oscillations are self-sustained but remain sensitive to resetting or entraining signals and can thereby adapt to changing environmental conditions and time zones [41]. Strong entrainment signals are light exposure and food intake that during specific phases reset

central and peripheral clock, respectively, thus aligning the endogenous rhythm with external time. Like most tissues, adipose tissues harbor circadian clocks and local metabolic processes like carbohydrate and lipid metabolism are rhythmically regulated in adipocytes [42].

White adipose tissue (WAT) presents the major fat mass in the mammalian body, but in the last decades brown adipose tissue (BAT) depots regained scientific and public interest. In contrast to white adipocytes that isolate and store energy in form of TGs, the primary task of BAT is active mitochondrial heat production characterized by uncoupling protein 1 (UCP1) expression [43]. BAT harbors intrinsic clocks and in rodents UCP1 expression follows a diurnal pattern [44-46]. Interestingly, mice lacking UCP1 remain lean at room temperature but gain weight at thermoneutrality [47,48], while genetic BAT ablation directly leads to the development of obesity [49]. Circadian BAT activity and energy expenditure can directly be modulated by *Bmal1* deletion in the murine ventromedial hypothalamus circumventing SCN signaling, leaving the local BAT clock intact but leading to a reduced body weight in mice [50]. In humans, BAT occurrence was originally believed to be restricted to infants. Recent studies, however, have shown that significant amounts of BAT can be detected in adults. BAT activity is stimulated by acute cold exposure, but correlates negatively with age and BMI [51-56]. Human BAT utilizes glucose for heat production with a thermogenic, but still glucose responsive circadian rhythm [57]. High BAT abundance is correlated with low glycemia, but with regard to potential therapeutic benefits of BAT in obesity and type-2 diabetes, it has to be considered that even with upregulated glucose uptake due to cold exposure, BAT mediated energy turnover remains minor compared to that of skeletal muscle [57-59].

Molecular Clock Disruption and Metabolic Outcomes

Dysfunctional circadian clocks and rhythms are associated with impaired metabolic homeostasis (see Table 1). A mutation of the clock core component, *Clock*, in mice causes blunted rhythmic behavior and elevated food intake during the inactive light phase and increased weight gain compared to age-matched wildtype animals [60]. Already on a (low-fat) normal-chow diet provided *ad libitum*, these mutant mice show symptoms of the metabolic syndrome with elevated adiposity and blood triglyceride, cholesterol, and 24-hour glucose levels [60]. Mice with a deletion of the CLOCK partner protein BMAL1 show a diverse adipose phenotype. On one hand, they fail to establish sufficient amounts of adipose depots and display no ectopic fat formation in liver or muscle tissue but instead they suffer from elevated levels of circulating fatty acids with high blood triglycerides, free fatty acids, and

cholesterol [61]. The inability of fat storage in *Bmal1*^{-/-} mice persists if they are fed HFD [61]. Tissue-specific deletion of *Bmal1* has been widely used to delete molecular clock function in metabolic tissues. Abolished clock function in liver interferes with the stabilization of blood glucose levels, leading to hypoglycemic states during the diurnal fasting periods [62]. While the liver clock drives a rhythmic hepatic glucose release, pancreatic beta-cell clock ablation leads to a loss of insulin release after glucose stimuli and a hypoinsulinemic state in mice and *Bmal1* deletion in muscles leads to weight gain [62-64]. Adipose tissue cells are programmed to take up energy during the active/feeding and release it during the rest/fasting phase [42]. If adipose clocks are genetically disabled in mice, plasma concentrations of triglycerides and free fatty acids during the day are elevated, feeding rhythms attenuated and animals gain excessive amounts of adipose tissue [65].

METABOLIC INFLAMMATION

Adipose tissue is not only involved in metabolic, but also in inflammatory responses through secretion of pro- and anti-inflammatory cytokines [66]. Under obesogenic conditions, immune cells are recruited to adipose stores which can further amplify inflammatory responses [67]. Increased body weight is not inevitably accompanied by further metabolic disorders such as cardiovascular diseases or type-2 diabetes, but a so-called “healthy overweight state” can transform into the pathogenic obese phenotype [6,68]. A hallmark for this shift is the adipose immune state and systemic low-grade inflammation is a key attribute of the metabolic syndrome [69]. During inflammation processes neutrophils are the first innate immune cells recruited to the tissue and later cleared by tissue resident macrophages [70]. In mice, the number of adipose tissue infiltrating neutrophils increases already after a short exposure to HFD [71,72]. Neutrophil intrinsic clocks drive cellular aging and regulate tissue infiltration and defense behavior [73]. In the obese state the number of pro-inflammatory adipose tissue resident immune cells rises rapidly, increasing and the risk for developing systemic inflammation [74]. Neutrophils contribute to impaired insulin sensitivity in adipose tissue with the release of the proteolytic enzyme elastase that continues to affect the tissue, even after the number of neutrophils is again reduced [72]. Further innate immune cells resident in WAT are macrophages which contribute to systemic inflammation with rhythmic secretion of TNF- α and IL-6 [75]. This secretion rhythm is depended on a functional intrinsic clock of the macrophages [75]. In shift workers, which have an elevated risk to develop the metabolic syndrome, leukocyte counts are elevated [76]. In rats, chronic advanced but not delayed shifts of

Table 1. Adipose phenotypes of clock disruption in mice.

Mouse/Mutation/Experiment	Phenotype	Reference
Light exposure at night	Metabolic and behavioral phase shifts, weight gain	[13,78]
Mistimed food intake	Tissue clock phase shifts, weight gain	[30,31]
High-fat diet <i>ad libitum</i>	Blunted behavioral and tissue clock rhythms, weight gain, hyperphagy	[34]
Chronic jet lag (6h advance/week)	Weight gain	[37]
Constant light exposure	Weight gain	[101]
SCN lesion	Loss of behavioral and molecular rhythms, obesity, hyperphagy	[102]
<i>Clock</i> mutation	Blunted behavioral rhythms, weight gain, hyperphagy	[60]
<i>Bmal1</i> KO	Lean, hyperlipidemia	[61]
<i>Per1/2</i> double KO	Leptin resistance	[103]
<i>Per2</i> KO	Altered lipid metabolism, hypotriglyceridemia, weight gain under HFD	[104,105]
<i>Per3</i> KO	Increased fat mass	[106]
<i>Cry1</i> KO	Resistant to diet induced obesity through HFD (not <i>Cry2</i> KO)	[107]
<i>Cry1/2</i> double KO	Loss of behavioral rhythms, hyperinsulinemia, weight gain	[108-110]
<i>Reverba/β</i> double KO	Loss of behavioral rhythms, hyperlipidemia, hyperglycemia, hepatic steatosis	[111-113]
Liver <i>Bmal1</i> KO	Hypoglycemia during fasting period	[62]
Pancreas <i>Bmal1</i> KO	Hypoinsulinemia	[63]
Adipose tissue <i>Bmal1</i> KO	Weight gain	[65]
Muscle <i>Bmal1</i> KO	Weight gain	[64]

the light-dark cycle lead to an increase of inflammation [77]. Further, dim light at night amplifies TNF- α expression in WAT in mice under a high-fat diet [78]. In adipose tissue, this inflammation directly leads to alterations in triglyceride and glucose homeostasis [79,80]. Treatment with hormones involved in circadian system organization can affect adipose inflammation suggesting that adipose clocks may be involved in determining adipocyte inflammatory state. A supplementation with glucocorticoids can dampen adipose tissue inflammation and human microarray studies revealed that dexamethasone treatment alters immune and inflammatory response pathways in adipocytes [81,82]. Additionally, glucocorticoids inhibit further immune cell recruitment to adipose tissue in obesity [83]. The effect of melatonin supplementation varies from pro- to anti-inflammatory properties, depending on the concentration, setting, and the measured parameter [84]. In young Zucker diabetic fatty rats melatonin treatment improves their phenotypic low-grade inflammation and reduces plasma IL-6 and TNF- α levels by about 10 percent [85]. In leptin deficient *ob/ob* mice, melatonin supplementation improves adipose tissue inflammation [86]. Melatonin partly counteracts the 24-h endocrine rhythm disruption caused by an HFD [87], inhibits the development of metabolic syndrome under different obe-

sogenic light regimes [88] (see Table 1), and normalizes biochemical parameters in mild diet induced inflammation in rats [89]. In humans, melatonin treatment in metabolic syndrome improves blood pressure values and lipid profiles [90] and reduces TNF- α concentrations [91]. In obese women, melatonin treatment decreases inflammatory parameters [92] while, in contrast, in healthy women glucose tolerance is impaired under melatonin treatment [93]. Another player in metabolic adipose tissue inflammation is the gut microbiome, which displays a diurnal rhythm in species composition, is highly influenced by clock genes [94-96], and *vice versa*, can also modulate the circadian clock [97]. Further dysregulations of the microbiome are associated with pro-inflammatory metabolic states like type-2 diabetes and obesity [98]. Germ-free mice display alterations in the rhythmic transcriptome and metabolites. Interestingly, in these mice rhythmic cytokine signaling in WAT is ablated [99] promoting low-grade WAT inflammation [100].

CONCLUSION

The demands of modern societies promote a health impairing lifestyle with disrupted sleep and eating patterns. Natural circadian regulation is weakened, and

averse metabolic outcomes may be the consequence. With increasing knowledge on rhythm interfering factors and their mechanisms of action, the next goal of chronobiology and medicine will be the implementation of this knowledge into daily life and clinical practice. It may be naïve to strive for an abolishment of shiftwork in a globalized economy, but – if shiftwork cannot be avoided – chronotherapeutic interventions aiming at stabilizing the circadian clock network, such as scheduled meal timing, timed light exposure/avoidance, or melatonin supplementation, may become useful tools to reduce adverse health effects and rebalance energy metabolism under chronodisruptive conditions and generally improve metabolic and glucose homeostasis and fitness.

Acknowledgments: This work was funded by grants of the German Research Foundation (DFG; GRK-1957 & OS353-7/1) and a Lichtenberg Fellowship of the Volkswagen Foundation (HO).

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