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### Effects of feeding time and light on energy metabolism in rats

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# 2

## **Rodent models to study the metabolic effects of shift work in humans**

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## ABSTRACT

Our current 24h society requires an increasing number of employees to work nightshifts with millions of people worldwide working during the evening or night. Clear associations have been found between shift work and the risk to develop metabolic health problems, such as obesity. An increasing number of studies suggest that the underlying mechanism includes disruption of the rhythmically organized body physiology. Normally, daily 24h rhythms in physiological processes are controlled by the central clock in the brain in close collaboration with peripheral clocks present throughout the body. Working schedules of shift workers greatly interfere with these normal daily rhythms by exposing the individual to contrasting inputs, i.e., at the one hand (dim) light exposure at night, nightly activity and eating and at the other hand daytime sleep and reduced light exposure. Several different animal models are being used to mimic shift work and study the mechanism responsible for the observed correlation between shift work and metabolic diseases. In this review, we aim to provide an overview of the available animal studies with a focus on the four most relevant models that are being used to mimic human shift work: altered timing of 1) food intake, 2) activity, 3) sleep or 4) light exposure. For all studies, we scored whether and how relevant metabolic parameters, such as body weight, adiposity and plasma glucose were affected by the manipulation. In the discussion, we focus on differences between shift work models and animal species (i.e., rat and mouse). In addition, we comment on the complexity of shift work as an exposure and the subsequent difficulties when using animal models to investigate this condition. In view of the benefit of animal models over human cohorts to study the effects and mechanisms of shift work, we conclude with recommendations to improve future research protocols to study the causality between shift work and metabolic health problems using animal models.

## INTRODUCTION

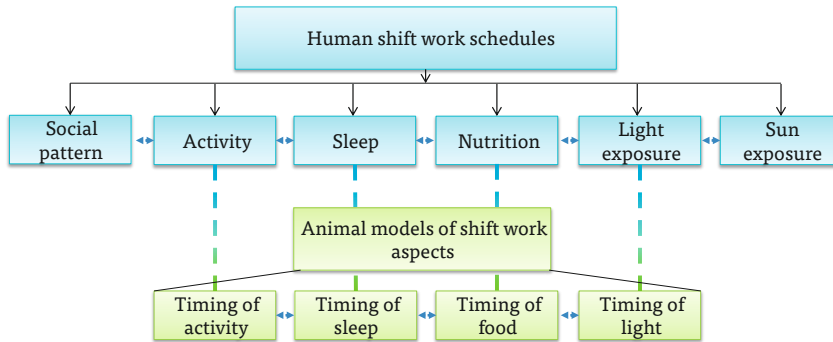
Our current 24h society requires an increasing number of employees to work nightshifts and as a result millions of people worldwide work during the evening or night for a certain period during their life. In the Netherlands, 16% of the working population works regularly or occasionally during the night, whereas 51% of the population sometimes or regularly works during the evening [1]. Epidemiological studies show correlations between shift work and an increased risk of cancer, cardiovascular disease, sleep disturbances, impaired psychosocial health and gastrointestinal problems [2]. Moreover, the last two decades, population-based studies have shown that there is also an association between shift work and development of metabolic problems, including metabolic syndrome [3–15], altered glucose metabolism [16–18], altered lipid metabolism [3, 16, 19] and high blood pressure [5, 16, 20, 21]. Population-based studies are limited in their use for understanding causality and underlying mechanisms to explain the relationship between shift work and disease. Using experimental studies in humans is problematic due to the fact that many metabolic outcomes, such as body weight and composition, are long-term effects. Certainly, acute effects of shift work conditions on metabolic parameters can be studied in humans, which is currently done [22]. Therefore, animal studies have been used to gain more insight in these questions. In the current review, we provide an overview of the different animal models that are available to investigate the mechanism underlying the negative health consequences of shift work.

Daily 24h rhythms are present throughout the body's physiology and can be observed in, for example, sleep, food consumption, body temperature and numerous hormone levels [23]. These rhythms are regulated by the central circadian clock in the suprachiasmatic nucleus (SCN) of the hypothalamus and circadian oscillators in peripheral tissues and organs (the so-called peripheral clocks). The endogenous rhythmicity of the SCN neurons ultimately results from the interaction between a set of rhythmically expressed genes, so-called clock genes, which are expressed in almost every cell of the body. In the SCN, the nearly 24h (i.e., circadian) rhythms produced by these clock-genes are synchronized to the exact 24h rhythms in the outer world by their sensitivity to (sun)light [23]. The synchronizing stimuli for peripheral clocks in

non-SCN tissues are less clear, in addition to nervous and humoral signals from the SCN, behavioural signals such as body temperature, energy metabolism and (feeding) activity also likely play a role [23, 24].

In general, the working schedules of shift workers profoundly interfere with these normal daily rhythms [25, 26]. Shift work leads to a disruption of the circadian rhythms produced by the central and peripheral clocks by confronting them with opposing signals, i.e., light at night and food consumption and activity during the sleep period. Therefore, shift work is a challenge that contains many aspects, which might be related to the negative health effects (Fig. 1): 1) social pattern: shift work affects social life due to working hours that conflict with working hours of social contacts; 2) activity: shift work affects the timing of people's activity and, as a consequence, possibly affects the amount of activity; 3) sleep: shift work affects timing of sleep and possibly duration and quality of sleep; 4) nutrition: shift work affects timing of food intake and possibly meal frequency and composition; 5) light exposure: shift work affects the timing of light exposure, with possibly different intensity and duration of exposure; 6) sun exposure: shift work might affect the duration of sun exposure and as a consequence vitamin D levels. Shift work comprises alterations at different levels of the circadian system that each have their own effect, but are interacting as well (Fig. 1) [25, 26]. For example, the altered timing of activity in shift workers may result in sleep disturbances (duration, quality, and timing), altered nutrition (composition, caloric intake and timing), changed lighting exposure conditions (duration, intensity, and timing), reduced sunlight exposure (possible effect on vitamin D levels) and disturbances in social life. Each of these aspects might, to a greater or lesser extent, contribute to negative health effects. For several aspects, animal models have been developed to examine the metabolic health effects upon manipulation of these aspects of shift work individually or in combination (Fig. 1). To our knowledge, no animal models have so far been developed to study effects of 'social life' and 'sun exposure'.

The aim of the present review is to provide an overview of the available animal studies investigating the mechanism underlying the negative health consequences of shift work and their outcome. In addition, we discuss human relevancy of the available animal models for shift work to gain insight into animal to human translatability and aid future investigations in choosing optimal animal models. Next to animal



**Figure 1. Schematic presentation of shift work aspects.** Shift work can be disentangled into different aspects (blue blocks), for some of these aspects animal models have been developed (green blocks). Each of these aspects might contribute to health risks associated with shift work. However, all aspects strongly interact, making it difficult to separate the effects of each single aspect. In most animal studies, only one of the aspects is manipulated, however, it is important to keep in mind that by manipulation of one aspect, other aspects might be affected as well due to this interaction.

models mimicking circadian disruptive shift work aspects, consequences of circadian disruption have also been studied in animals using genetic manipulation or SCN lesions. These animal models are not within the scope of the present review, since we do not consider them to represent human shift work.

To increase the animal to human translatability the focus of the present review is on animal models investigating the relationship between shift work and metabolic risk factors [27–29] since these factors are easily translatable from humans to animals and vice versa. In addition, these factors can be measured almost non-invasively in humans and often appear before the full-blown disease, allowing for shorter follow-up time and more time for preventive measures or intervention.

Studies were included in the review when they investigated metabolic parameters such as body weight, food intake, activity, glucose metabolism (including plasma glucose, insulin and glucagon levels, glucose tolerance, and glycogen levels), leptin levels, and lipid metabolism (including plasma cholesterol and triglyceride levels).

## MATERIALS AND METHODS

### **Search strategy**

A literature search was performed to obtain an overview of the current scientific literature on studies using animal models for shift work to investigate the relationship between shift work and metabolic function. The search strategy was designed by an information specialist (RIVM) using the MESH-database of Pubmed, to include all MeSH terms and its synonyms and several electronic databases were used (Medline, Embase, BIOSIS Previews and SciSearch). In brief, the search strategy combined keywords related to shift work with keywords related to metabolic risk factors. Examples of key words for shift work: shift work\*, shiftwork\*, night work\*, night shift\*, rotating shift\*, jet lag, working rhythm\*, “irregular working hours”, time restricted, “constant light”, “continuous light”, “light at night”, biological clock\*, body clock\*, chronobiology\*, circadian clock\*. Examples of key words for metabolic risk factors: weight, body weight, weight change, metabolic syndrome, obesity, adiposity, glucose, glucose tolerance, lipid metabolism, energy metabolism, insulin, insulin sensitivity, hypertension, leptin. Only papers published after 1993 were included. For the complete search strategy, see supplementary online data:

<http://www.frontiersin.org/journal/10.3389/fphar.2015.00050/abstract>.

The search resulted in 1550 publications, but only 44 were included as these met the following criteria:

- 1) Using animal models for shift work
- 2) Investigate effects on at least one of the following metabolic risk factors for disease [27, 28]:  
body weight and related measures (BMI, fat percentage) or glucose homeostasis: including plasma glucose levels, glucose tolerance, plasma insulin levels, insulin sensitivity or lipid homeostasis: including plasma levels of triglycerides, cholesterol, free fatty acids, HDL or LDL.

The search included papers in English, Dutch, French, and German. However, only papers in English fitted the above-mentioned criteria. In addition to this search strategy, the present knowledge of the authors and references from included papers (“snowball method”) were used to include additional papers that fitted the above-mentioned criteria (including papers published before 1993) or investigated parameters related to metabolic dysfunction.

### **Categorization of Studies**

Included studies were divided in four different categories as presented in Fig. 1: 1) Models using ‘timing of food intake’, 2) models using ‘timing of activity’, 3) models using ‘timing of sleep’, and 4) models using ‘timing of light’. For each study, the outcome parameters were determined, which included the abovementioned metabolic risk factors for disease as well as circadian parameters (e.g. activity, cortisol) and gene expression. For these parameters, results are described in the text and summarized in the table for overview purposes. In the Tables 1–5, the left column holds the metabolically relevant parameters that were scored for and were most frequently measured in the studies. The *rat* and *mouse* columns represent the number of studies in which an effect of the manipulation (compared to the control condition) was found in this species against the number of studies in which it was measured. In the *total* column, results are divided in direction of effects and presented as the number of studies observing that direction is summarized. This was not done for gene expression. The most right column shows the studies in which the parameter was measured in this category of models. In the tables, studies are counted twice when multiple experiments are performed in one article, for instance when two types of mice were used. Occasionally, the effect of the manipulation was measured on the total level as well as the rhythm of a parameter within the same study. In this case, both effects are included in the *total* column.

With these tables we aim to provide an overview of the metabolic parameters that are influenced per category and type of animals used. Results of effects on gene expression are described in the text and summarized in the table as ‘gene expression’. The present review was aimed at providing a narrative overview of available studies and their findings, due to heterogeneity studies were not assessed for quality.

## **RESULTS**

Animal studies that model human shift work can be divided into four main categories. The first three categories are based on desynchronization of peripheral clocks from the central clock by the unnatural timing of food intake, sleep or activity. The remaining category consists of studies that manipulate the timing of light exposure, including



alterations of duration (i.e., continuous light) and timing of light exposure. Some studies used a shift work model that combines multiple categories and those will be mentioned repeatedly in the different categories if appropriate. Below we discuss the main findings of studies using a shift work model within the categories where the models fit best.

### **3.1 Category 1: Models using 'Timing of food intake'**

Shift work models using shifted and/or restricted timing of food availability are based on the knowledge that food intake is the most important Zeitgeber for peripheral clocks, in the same way as light is for the central clock. Shifting timing of food intake disrupts the orchestrated synchrony between peripheral and brain clocks, which might lead to metabolic problems as peripheral organs such as liver and muscle are essential for energy homeostasis. Shifting the timing of food intake is an interesting approach as metabolic disorders such as obesity are also associated with aberrant dietary habits (i.e., quantity, composition and frequency) and shift workers also have changed dietary habits. Moreover, more recently several studies have suggested that also the timing of food intake is crucial to maintain energy homeostasis [30–34] and shifting the timing of food intake is another characteristic feature of shift workers [35]. All in all making this a relevant model for shift work.

The first evidence for food as a strong entrainment signal for circadian physiology (metabolic and clock gene expression rhythms, hormone secretion rhythms) came from so-called restricted feeding studies. This type of studies usually restricts food availability to a short period (e.g. 2–4h) during the light phase (which is the resting phase of nocturnal rodents) to study entrainment of peripheral clocks. Clearly, these are important studies for chronobiology in general and still are performed frequently to look for and try to understand better the food entrainable oscillator. However, such restricted feeding models are not an adequate reflection of human food intake behaviour during shift work as they restrict the duration of food intake to a (very) short period and therefore were not included in this review.

Food-restriction studies in which food availability is shifted or restricted to a certain phase of the day (i.e., a large part of or the complete light period or dark period) provide a more suitable approach to mimic human feeding behaviour during shift work. One of the first studies with food availability restricted to either the 12h light or 12h dark phase

was done by Damiola *et al.* and they showed a strongly disturbed circadian rhythm according to altered daily body temperature rhythms in mice that could eat only during the light (i.e., resting) phase. Several clock genes in liver adjusted their expression to the timing of food intake [36]. Although alterations in gene expression cannot be translated directly into functional changes, it does indicate that food has strong entraining properties even on a molecular level in mice. More recently, Bray *et al.* performed a short-term experiment and observed metabolic changes within the first 9 days after restricting food intake to the light or dark phase. Whole body energy metabolism was affected within 24h of food-restriction and this was visible in a 5h phase advance of rhythm in energy expenditure, higher resting energy expenditure (RER) and increased caloric intake. Restricting food to the resting phase caused an increase of body weight and blunted plasma glucose and corticosterone rhythm, whereas triglyceride levels were not affected [37]. A short-term experiment by Oyama *et al.* focused on the effects of food timing on inflammatory response but additionally found reduced food intake and body weight in mice fed during the light phase [38]. Jang *et al.* performed the same restriction protocol but studied long-term effects (5–9 weeks). Surprisingly, they observed a protective effect of restricting food to the resting phase with lower body weight and food intake when compared to (chow or high-fat diet) *ad libitum* fed animals. Body weight did not differ from animals pair-fed during the active phase. Also, alterations in the expression of lipogenic, gluconeogenic and fatty acid oxidation-related genes in liver were found in feeding time-restricted animals [39]. Shamsi *et al.* entrained mice to 16L:8D or 8L:16D photoperiods and restricted food availability to light or dark phase. Neither photoperiod nor food timing affected body weight when compared to *ad libitum* feeding. Plasma insulin increased in light phase fed animals despite the photoperiod, whereas plasma glucose tended to be lower and triglycerides significantly decreased when feeding during light was compared to *ad libitum* or dark phase feeding. Rhythms in plasma glucose, insulin and triglyceride secretion shifted by light phase feeding when compared to dark phase feeding and some effects (mostly amplitude) changed over time (i.e., 7 days vs. 35 days). Interestingly, long photoperiod caused light phase fed animals to increase glucose tolerance but decrease insulin tolerance compared to dark phase fed animals and *ad libitum* fed animals respectively. Gene expression of

metabolic and clock genes in liver was altered by feeding during light phase in both amplitude and phase. Corticosterone rhythm was shifted by light phase feeding after 35 days but not after 7 days [40]. Reznick *et al.* took a similar approach with a 3-week study performed with Wistar rats instead of mice. No effect was found on body weight gain or epididymal white adipose tissue, but animals fed during the resting phase decreased their food intake and total activity levels. Rats fed during the light period showed a 12h shift in RER and dampening of activity and energy expenditure diurnal variation. The rhythm of plasma insulin altered with higher 24h levels, corticosterone showed an additional peak and the rhythm of glycogen shifted to an opposite phase in liver but showed increased levels in muscle. Triglyceride levels in liver were reduced whereas muscle content was unaffected in animals fed during the light period. Expression of several other proteins and genes involved in energy metabolism and clock regulation in liver and muscle tissue showed phase changes or altered expression levels. In the same study, this experimental design was used for a group of rats fed a high-fat diet, which aggravated many of the observed effects found in chow day-fed animals with additional disruption of leptin and non-esterified fatty acids (NEFA) levels [41].

A series of studies performed with male Wistar rats used a forced activity protocol as a model for shift work [42–46]. The effects of forced activity will be described below (see category 2), but the non-working ‘control’ groups of these studies are relevant for the timing of food category. When food was restricted to the resting phase, i.e., chow only available from ZT0 to ZT12, rats displayed a dampening of their core body temperature rhythm, an additional peak in the plasma corticosterone rhythm, and a shift in triglyceride secretion, but no differences were observed for the plasma glucose and activity rhythm or total activity. Total food intake remained the same but body weight and peritoneal fat accumulation were increased when compared to *ad libitum* or night fed (food available from ZT12 to ZT24) animals [43]. The observed accumulation of abdominal fat was reproduced by the same group in another study where a decreased glucose tolerance in rats fed during the resting phase was observed, in addition to alterations or dampened rhythms in liver clock and metabolic gene expression [46].

A couple of other groups used comparable food availability approaches in mice on normal chow diet, however, shorter food-restriction

periods were used than 12h during the light period. Yoon *et al.* enforced a 6h advance (ZT6–11) or delay (ZT18–23) in food availability for 9 weeks and observed that body temperature, locomotor activity and triglyceride secretion strongly depend on food timing. Cholesterol and high-density lipoprotein (HDL) levels were moderately increased in both advance and delay groups when compared to *ad libitum* fed animals, and food intake was reduced in the food time advanced group compared to food time delay group and *ad-lib* controls. Fasting glucose levels increased and poor responses to insulin tolerance test intensified over time in daytime fed animals (advance group) [47]. Sherman and colleagues restricted food intake to the light phase, ZT4–8 without caloric-restriction, for 18 weeks and performed this with both high- and low-fat (chow) diets. In both diets, time-restriction was protective for body weight gain, high plasma leptin, insulin, HDL and cholesterol levels. Also, the increased epididymal fat observed in *ad libitum* fed animals was diminished in the food time-restricted groups. In low-fat diet fed animals triglyceride levels were reduced but corticosterone and adiponectin were increased, as compared to the *ad libitum* low and high-fat and restricted high-fat animals. High-fat diet fed animals also showed improved tumour necrosis factor (TNF)-alpha and insulin resistance levels (HOMA-IR) and increased activity levels when food was restricted to the light period, as compared to *ad libitum* fed animals, but were less active than animals on a low-fat diet [48]. Schroder *et al.* focused on the effects on heart rhythm and observed that when food was provided from ZT2–9 only, this negatively affected several aspects of heart rhythm aspects in both wild type and genetically sensitive mice [49].

Most studies mentioned above were performed with normal chow diet, which is low on fat derived content. However, many diet-induced obesity studies use high-fat diet *ad libitum* feeding on which animals will develop obesity, diabetes type 2 and metabolic syndrome [50]. For circadian studies it is important to know that feeding rodents a high-fat diet induces a dampening of the amplitude of daily activity and feeding rhythms, and also metabolic markers, hypothalamic neuropeptides and peripherally expressed factors involved in lipid metabolism are affected [51]. This suggests an interaction between energy metabolism and circadian rhythm control. Some groups, however, combined the restricted feeding paradigm with a high-fat diet. Restriction of food to one phase of the day may re-induce the entrainment lost on high-fat *ad libitum*

feeding. Arble and colleagues made an early attempt and fed mice a high-fat diet solely during the light phase and observed a significant increase in body weight compared to animals fed during the dark phase [52]. Bray *et al.* used four different feeding schedules to study in more detail which phase of the L/D-cycle is most detrimental when ingesting a high-fat diet for 12 weeks. They observed higher body weight gain and adiposity, decreased glucose tolerance next to high insulin, leptin and triglyceride levels in mice consuming their high-fat meal at the end of the active phase instead of at the beginning. Interestingly, when fat was only available in the light phase no metabolic changes were observed with the exception of slightly decreased energy expenditure and oxygen consumption when compared to animals with fat available in the dark phase [53]. Several studies experimented with restriction of a nutrient component to a certain phase of the day for the effects on obesity. For instance, providing the fat component or sugar component of a free-choice high-fat-high-sugar diet only during the light phase affects RER, energy expenditure and body weight [54, 55]. A slightly different approach was taken by Senador *et al.* by offering mice a 10% fructose solution additional to their normal chow and water diet. Fructose was either available for 24h, available for 12h during light phase, available for 12h during dark phase or not available. Increased body weight, higher fasting glucose levels but decreased plasma triglycerides were observed in both groups with 12h fructose availability. Fructose-restriction to light phase additionally caused glucose intolerance and an attenuated amplitude of blood pressure rhythms. *Ad libitum* availability of fructose only caused glucose intolerance when compared to control animals without fructose [56]. Another type of timed food-restriction is done by dividing food intake into 4 or 6 meals equally divided over the L/D-cycle. For instance, Yamajuku *et al.* delivered a high cholesterol diet to rats in a 4-meal schedule (every 6h) without reduction of caloric intake. Those animals developed hypercholesterolemia after 7 days on the protocol and furthermore showed disruption in liver gene expression [57]. These studies are probably highly relevant as shift work models. However, until now it is unclear how shift workers exactly change their dietary habits in timing, composition, frequency and size of meals, making it hard to decide at present what are the best models.

In contrast to studies that restrict food availability to the light phase, Hatori *et al.* showed that restricting a high-fat diet to the natural main

feeding phase (ZT13–21) improved glucose tolerance, insulin sensitivity, adiposity, serum cholesterol levels and leptin levels after fasting or glucose administration, next to prevention of increased liver size and unsaturated fatty acids levels, compared to animals fed *ad libitum* [58].

#### SUMMARY 'TIMING OF FOOD INTAKE' MODELS

Animal models using a restriction of the timing of food intake affect body weight in 64% of the studies, but effects went in different directions with 3 out of 7 studies showing an increase of body weight whereas the four other studies found a decrease in body weight after timed food intake. Restricting food to the light phase resulted in increased body weight compared to animals with food restricted to the dark phase [37, 52] and when compared to *ad libitum* fed [43]. However, others observed reduced body weight after food-restriction to the light phase compared to dark fed animals [38, 47] or *ad libitum* fed animals [47, 48]. Interestingly, in many of these studies food intake was reduced as well. Furthermore, some differences between mouse and rat studies are observed. For example, for body weight, mice studies show a significant effect in 6 out of 8 studies (75%), whereas in rats only in 1 out of the 3 studies (33%) a significant effect was observed. Total food intake and glucose metabolism parameters were measured frequently and were affected in 45% and 67% of the studies, respectively. For a complete overview of all parameters, see Table 1. Together, these results indicate that changing the timing of food intake is effective at influencing several metabolic parameters, although for some parameters results are not consistent. Considering the great variety in types of modulations used, it is not possible to pinpoint this to one aspect of the models used.

### **3.2 Category 2: Models using 'Timing of activity'**

'Work' is a difficult concept to model in animal studies and therefore only a very limited number of studies are available using an actual physical shift work protocol. Obviously, shift work requires shifting of phases of sleep and arousal leading, at least partly, to awakening during the usual sleep period and sleep during the usual active period of the day. The few available physical shift work models used forced activity by housing animals in slowly rotating wheels. These housing conditions force the animals to be active and prevent them to fall asleep, although the animals can lie down and eat. Salgado-Delgado *et al.*

**Table 1. Summary of animal studies in which timing of food intake was manipulated to mimic human shiftwork.** For detailed description of the columns, see Methods section.

+; number of studies with increases, -; number of studies with decreases, ~: number of studies with altered rhythm, o: number of studies with no effect. For *glucose metabolism* a + indicates increased basal levels of glucose, HbA1c or insulin, increased HOMA index or decreased glucose tolerance. *EE* energy expenditure; *RER* respiratory exchange ratio; *BP* blood pressure

	RAT	MOUSE	TOTAL	STUDIES
BODYWEIGHT	1/3	6/8	+ : 3/11 (27.3%) - : 4/11 (36.4%) o : 4/11 (36.4%)	[37-41, 43, 46-48, 52, 53]
FOOD INTAKE TOTAL	1/3	4/8	+ : 0/11 (0%) - : 5/11 (45.5%) o : 6/11 (54.5%)	[37-41, 43, 46-48, 52, 53]
ACTIVITY TOTAL	1/2	2/5	+ : 2/7 (28.6%) - : 1/7 (14.3%) o : 4/7 (57.1%)	[37, 40, 41, 43, 47, 48, 52, 53]
EE TOTAL	1/1	2/2	+ : 0/3 (0%) - : 3/3 (100%) o : 0/3 (0%)	[37, 41, 52, 53]
RER	1/1	2/2	+ : 1/3 (33.3%) - : 1/3 (33.3%) ~ : 1/3 (33.3%) o : 0/3 (0%)	[37, 41, 53]
ADIPOSIITY	2/3	3/4	+ : 3/7 (42.8%) - : 2/7 (28.6%) o : 2/7 (28.6%)	[40, 41, 43, 46, 48, 52, 53]
GLUCOSE METABOLISM	2/3	4/6	+ : 1/9 (11.1%) - : 2/9 (22.2%) ~ : 3/9 (33.3%) o : 3/9 (33.3%)	[37, 39-41, 43, 46-48, 53]
LIPID METABOLISM	2/2	3/5	+ : 1/7 (14.3%) - : 2/7 (28.6%) ~ : 4/7 (57.1%) o : 2/7 (28.6%)	[37, 40, 41, 43, 47, 48, 53]
CORTICOSTERONE	2/2	2/3	+ : 1/5 (20%) - : 0/5 (0%) ~ : 3/5 (60%) o : 1/5 (20%)	[37, 40, 41, 43, 48]
MELATONIN LEPTIN	1/1	0/1	+ : 1/2 (50%) - : 0/2 (0%) ~ : 1/2 (50%) o : 1/2 (50%)	[41, 48, 53]
GHRELIN		1/1	+ : 0/1 (0%) - : 1/1 (100%) ~ : 0/1 (0%) o : 0/1 (0%)	[48]
BP/HEART RATE		2/2	+ : 0/2 (0%) - : 2/2 (100%) ~ : 0/2 (0%) o : 0/2 (0%)	[49]
GENE EXPRESSION	2/2	10/10	12/12 (100%)	[36-41, 46-48]



mimicked human shift work protocols by enforcing the working conditions for 8h per day, during either the active phase or the sleeping phase, 5 days a week for 5 weeks. In addition, they varied the availability of food but always used normal chow diets. A general observation in the groups of animals working during their resting phase was that the animals lost their nocturnal urge to eat and voluntarily consumed their food mostly during their working hours and thereby during their normal resting (i.e., light) phase. Forced activity during the resting phase induced increased body weight and abdominal fat, impaired glucose tolerance, altered plasma triglyceride diurnal variation, dampened daily glucose variation and introduced a secondary peak in the corticosterone rhythm. These effects could be prevented when food availability was restricted to the normal active (i.e., dark) phase [42–46].

An early study by Tsai *et al.* used an extensive design with rats exposed to changes of light schedules (twice a week), forced activity paradigms and combinations of those. They observed higher body weight in the first two months, but not in the third month, in animals that were subjected to a 'shift work' schedule of forced activity (i.e., Tue–Thu work from ZT0–ZT12, Fri–Sun work from ZT12–ZT24, Monday free) combined with changes in light schedule which was in synch (i.e., 12h shift of L/D-cycle twice a week) with the work schedule. Animals exposed to forced activity schedules had lower body weight and lower levels of cholesterol than animals that only underwent shift of light/dark cycle, indicating that forced activity can reverse some effects on metabolism induced by the regular L/D-shifts. Some early effects on body weight and food intake disappeared after two months on the protocol, whereas other effects were only found after a few months on this protocol [59].

A study by Leenaars *et al.* using forced activity in rats did not focus on metabolic parameters, but did observe a decrease in total activity levels in animals that either had to work during their active or during their resting phase compared to freely active animals. Animals working shift work (i.e., work during the normal resting phase) showed reduced body weight gain compared to non-working controls [60].

A study by Hsieh *et al.* used 5-week forced activity in rats and closely studied alterations in activity patterns. The animals in this study are from the same study as [42] but reported more specifically on locomotor activity. A decrease in mean activity levels was observed in shift work animals (i.e., working ZT2–10) during weekdays from week 3 onwards



and during weekend days from week 1 onwards, but not in animals working during their active phase (i.e., working from ZT14–22). Shift work animals showed decreased amplitude of the activity rhythm on weekend days and a differently shaped rhythm of 24h activity on weekdays. Shift work animals showed different activity responses to lights on and off when compared to non-shift working rats [61]. The observed disruption of normal activity patterns was reported as an indication for circadian disruption as similar changes have been observed in SCN lesioned animals.

#### *SUMMARY 'TIMING OF ACTIVITY' MODELS*

Models using an altered timing of activity have only been carried out with rats and therefore no numbers on mouse studies are available. The limited number of studies and contributing research groups with this paradigm resulted in high numbers of affected studies on all parameters. As shown in Table 2, all parameters showed a 100% effectiveness of the different studies, except for body weight, total food and total activity levels intake, which were affected in 5, 2 and 4 out of 6 studies, respectively. These results suggest that 'timing of activity' has metabolic effects, although considering the limited number of studies and research groups these results should be interpreted with some caution.

### **3.3 Category 3: Models using 'Timing of sleep'**

Alterations in timing of activity are directly related to alterations in timing and duration of sleep. However, changes in the timing of sleep are also separately used as a model for shift work. These studies are different from the previously described activity models as their first target is to disturb or shorten sleep and affect the timing of sleep, but not necessarily alter activity levels or food intake. However, undoubtedly changes in sleep behaviour will also affect activity and feeding patterns. Interestingly, (chronic) sleep-restriction is associated with metabolic disorders in both animals and humans [62, 63]. We came across a diversity of methods used to disrupt the normal sleep/wake pattern, including sleep-disruption, sleep-restriction, sleep-fragmentation, sleep-perturbation or sleep-deprivation. Some of those might be other designations of the same intentions, such as sleep-fragmentation and -perturbation. These models either use shifting the timing of the normal sleep phase along the light/dark cycle, perturbing sleep in the normal phase, reducing

**Table 2. Summary of animal studies in which timing of activity was manipulated to mimic human shiftwork.** For detailed description of the columns, see Methods section.

+: number of studies with increases, -: number of studies with decreases, ~: number of studies with altered rhythm, o: number of studies with no effect. For glucose metabolism a + indicates increased basal levels of glucose, HbA1c or insulin, increased HOMA index or decreased glucose tolerance. *EE* energy expenditure; *RER* respiratory exchange ratio; *BP* blood pressure

	RAT	MOUSE	TOTAL	STUDIES
BODYWEIGHT	5/6		+ : 3/6 (50%) - : 2/6 (33.3%) o : 1/6 (16.7%)	[42, 43, 46, 59, 60, 96]
FOOD INTAKE TOTAL	2/6		+ : 1/6 (16.7%) - : 1/6 (16.7%) o : 4/6 (66.7%)	[42-44, 46, 59, 96]
ACTIVITY TOTAL	4/6		+ : 0/6 (0%) - : 4/6 (66.7%) o : 2/6 (33.3%)	[42-44, 46, 60, 61]
EE TOTAL				
RER				
ADIPOSIITY	2/2		+ : 2/2 (100%) - : 0/2 (0%) o : 0/2 (0%)	[43, 46]
GLUCOSE METABOLISM	3/3		+ : 1/3 (33.3%) - : 2/3 (66.7%) ~ : 0/3 (0%) o : 0/3 (0%)	[42, 43, 46]
LIPID METABOLISM	2/2		+ : 0/2 (0%) - : 0/2 (0%) ~ : 2/2 (100%) o : 0/2 (0%)	[42, 43]
CORTICOSTERONE	2/2		+ : 0/2 (0%) - : 0/2 (0%) ~ : 2/2 (100%) o : 0/2 (0%)	[42, 43]
MELATONIN				
LEPTIN				
GHRELIN				
BP/HEART RATE				
GENE EXPRESSION	1/1		1/1 (100%)	[46]

the total number of sleep hours or completely withholding sleep. Most of these sleep studies focused on the effects of sleep-perturbation on sleep parameters (such as percentage rapid eye movement (REM) and non-REM sleep, electroencephalogram (EEG) recordings), behavioural changes or other non-metabolic factors. In this review, only studies using a shift in the timing of sleep to the dark phase and studies using total

sleep-deprivation for a few hours were included, as those were considered most relevant for shift work models.

Methods to perturb sleep are diverse and forced activity, as mentioned before, is one of them. Another method involves gentle handling for a few hours, by touching the animal every time it tries to fall asleep. Short-term effects of sleep-restriction during the first 6h of the normal sleep phase (ZT0–ZT6) were described in two studies. Barclay *et al.* observed moderate alterations in the timing of food intake (in the direction of light phase feeding) and locomotor activity (increased levels during light and decreased levels during dark phase), next to major disruptions in liver transcriptome rhythms enriched for lipid and glucose metabolism pathways after two weeks of sleep-restriction. A decreased response in the pyruvate test, a dampening of the daily rhythms in plasma glycerol, plasma triglyceride, plasma corticosterone and hepatic glycogen levels, and disrupted expression of several clock genes were all rescued by restricting food intake to the dark phase in the sleep-restricted groups [64].

Another study [65] used the same method but subjected the animals to sleep-restriction for only five consecutive days followed by a recovery week during which several parameters were measured. Sleep-restriction led to increased food intake despite increased leptin levels, together these changes are indicative for leptin resistance. Blood metabolites such as glucose and triglycerides were increased, but levels improved again during the recovery week. However, a trend towards higher body weight gain was observed during the recovery week suggesting long-term effects even when the period of sleep-restriction has terminated. Analysis of white adipose tissue transcriptome showed that sleep-restriction affects many genes involved in lipid metabolism, including increased fatty acid synthesis and triglyceride production and storage.

A series of sleep-restriction studies [66–68] have been done using sleep-restriction protocols of different severities: sleep-restriction for 20h each day (sleep ZT0–4; awake ZT4–24) or sleep-disruption (14h sleep-10h awake in four 2-3h episodes). Sleep-disturbance and sleep-restriction both led to decreased body weight when compared to home cage control animals; although food intake was equal and the slight increase in locomotor activity is unlikely to explain the body weight differences. Animals exposed to sleep-disruption or sleep-restriction showed decreased baseline plasma glucose and insulin levels, decreased

glucose tolerance and an attenuated insulin response to the glucose infusion within 8 days. A five-day recovery period attenuated the sleep restriction-induced decrease of plasma leptin, insulin and glucose levels although body weight gain did not recover. Corticosterone levels and food intake were not affected [68]. When the same protocol was performed for 4 weeks, but with a work-weekend-schedule (5 workdays, 2 non-work days) to resemble human shift work conditions, the same authors observed an increase of food intake after the first weekend, possibly to compensate for increased energy expenditure. Body weight gain increased during weekends in sleep-restricted animals and plasma leptin and insulin levels were decreased when measured during working weeks 1 and 4. Rest during weekend days induced recovery of plasma leptin and insulin levels [66].

#### SUMMARY 'TIMING OF SLEEP' MODELS

The number of studies using perturbation of sleep as a model for shift work with focus on metabolic parameters is limited and therefore it is not yet completely clear if this type of manipulation influences metabolic functioning. Glucose metabolism appears to be affected often (in 5 out of 5 studies), whereas body weight (3/5) and food intake (2/4) were not always affected by sleep perturbation. For a complete overview of all parameters, see Table 3.

#### **3.4 Category 4: Models using 'Timing of light exposure'**

In literature, several 'timing of light exposure' models have been reported. These models all use timing of light as a means to disturb the circadian system and, as such, their main influence is on the SCN, in contrast to the previous three categories of models described. One type of these models uses continuous light exposure, i.e., light is present 24h per day. The continuous light models can be further subdivided in models using constant light (similar amounts of light exposure during 24h of the day) and models using dim light at night (together with bright light during the day). In addition to models using continuous light, models using changes in light/dark schedules have been used. These models can be further subdivided into models using alterations in period length (e.g. light/dark periods shorter or longer than 24h) and models using repeated shifts of the light/dark schedule. Studies investigating these types of exposure in relation to metabolic health effects

**Table 3. Summary of animal studies in which timing of sleep was manipulated to mimic human shiftwork.** For detailed description of the columns, see Methods section.

+; number of studies with increases, -; number of studies with decreases, ~: number of studies with altered rhythm, o: number of studies with no effect. For *glucose metabolism* a + indicates increased basal levels of glucose, HbA1c or insulin, increased HOMA index or decreased glucose tolerance. *EE* energy expenditure; *RER* respiratory exchange ratio; *BP* blood pressure

	RAT	MOUSE	TOTAL	STUDIES
BODYWEIGHT	3/3	0/2	+ : 1/5 (20%) - : 3/5 (60%) o : 2/5 (20%)	[64-68]
FOOD INTAKE TOTAL	1/2	1/2	+ : 2/4 (50%) - : 0/4 (0%) o : 2/4 (50%)	[64-67]
ACTIVITY TOTAL	0/1	0/2	+ : 0/3 (0%) - : 0/3 (0%) o : 3/3 (100%)	[64-66]
EE TOTAL	0/1		+ : 0/1 (0%) - : 0/1 (0%) o : 1/1 (100%)	[66]
RER				
ADIPOSIITY				
GLUCOSE METABOLISM	3/3	2/2	+ : 3/5 (60%) - : 2/5 (40%) ~ : 0/5 (0%) o : 0/5 (0%)	[64-68]
LIPID METABOLISM		2/2	+ : 2/2 (100%) - : 1/2 (50%) ~ : 0/2 (0%) o : 0/2 (0%)	[64, 65]
CORTICOSTERONE	1/2	1/2	+ : 2/4 (50%) - : 0/4 (0%) ~ : 0/4 (0%) o : 2/4 (50%)	[64-66, 68]
MELATONIN				
LEPTIN	2/2	2/2	+ : 1/4 (25%) - : 3/4 (75%) ~ : 0/4 (0%) o : 0/4 (0%)	[64-66, 68]
GHRELIN				
BP/HEART RATE				
GENE EXPRESSION		2/2	2/2 (100%)	[64, 65]

are discussed below per type of model. For overview purposes, models using continuous light are presented together in Table 4 and models using changes in light/dark schedules are presented together in Table 5.

### 3.4.1.1 CONTINUOUS LIGHT EXPOSURE

Exposure to constant bright light conditions (LL) has been shown to abolish/diminish the circadian rhythmicity of many laboratory animals. This has, for example, been shown for locomotor activity [69, 70], melatonin [70, 71], food intake [72], and SCN neurons [72]. Hence, constant bright light conditions may be used to severely disrupt circadian rhythms. Since shift workers will be exposed to light during most of the day these models might be relevant for shift work modelling, although the intensity of all-day light may differ from real-life light exposures.

Disruption of circadian rhythms by constant bright light exposure has also been reported to affect metabolic function. Exposure to continuous light (150–180 lux; 4–8 weeks) led to increased body weight gain in two studies in mice [73, 74]. This phenotype was apparent in mice fed normal chow [73, 74] as well as a high-fat diet [73]. In addition, a third study reported increased fat mass after exposure to continuous light [75]. Total food intake was unaltered in these studies, but more food was consumed during the subjective day, indicating changes in the timing of food intake [73, 74]. The effect of continuous light exposure on total activity levels is less clear: unaltered activity levels [74], reduced energy expenditure [73] and a non-significant trend towards a decrease in activity levels [75] have been reported. Apart from changes in body weight and fat mass, continuous light affected other metabolic parameters, such as increased RER, reduced glucose tolerance and altered rhythmicity of insulin sensitivity [73, 74]. However, two studies using relative short-term exposure (6–10 weeks) to continuous bright light in Sprague Dawley rats reported no changes in body weight [70, 76], indicating a possible difference between rats and mice in this model. Interestingly, long-term exposure to bright light for 35 weeks did enhance body weight in Rapp-Dahl rats (model for hypertension). In addition, increased systolic blood pressure was observed [77]. Short-term exposure to continuous bright light did increase glucose levels and alter the rhythmicity of lipids in the study by Dauchy *et al.* [76]. In contrast, in the study by Gale *et al.* changes in glucose metabolism (increased glucose levels and decreased glucose- and arginine- stimulated insulin secretion) were only observed in diabetes-prone HIP rats, but not in wild-type Sprague Dawley rats [70]. Considering these contradicting results, the differences in duration and strains of rats used, as well as the limited number of studies no firm conclusions can be drawn regarding the effects of continuous bright light exposure

on metabolic function in rats. On the other hand, in mice results are more consistent and indicate that disruption of circadian rhythms by continuous bright light exposure increases body weight and alters glucose metabolism. This is associated with an altered timing of food intake, but not with change in total amount of food consumed over 24h (Table 4).

#### 3.4.1.2 DIM LIGHT AT NIGHT

Dim light at night (LDim) also affects circadian rhythmicity, but seems less disruptive for circadian rhythms compared to constant bright light. In respect to human circadian disruptions caused by shift work, models using dim light might be more relevant, since human shift workers will also experience alterations in the level of light exposure during a day, i.e., dim light at work in the office and bright light when commuting. In addition, models using dim light at night are relevant for studying the possible health consequences of light contamination at home, i.e., evening and nocturnal light is present in an increasing amount in our western society.

In contrast to constant bright light exposure, with dim light exposure at night circadian rhythms remain largely intact. This has for example been observed in rhythms of locomotor activity [74] and corticosterone [76]. Metabolic parameters including plasma levels of glucose and fatty acids also remain intact. Interestingly, dim light at night did affect the circadian pattern of food intake with relatively more food being consumed during the rest phase [74]. However, when the brightness of dim light exceeds a certain limit circadian rhythmicity of melatonin and corticosterone will be affected [76].

In three mice studies, exposure to dim light at night (for 2 and 6 weeks) increased body weight [74, 78, 79]. This was observed in male [74, 78] and female mice [79]. In female mice, dim light at night resulted in decreased food intake after 4 weeks [79]. In addition, in dim light exposed animals increases in fat mass and reduced glucose tolerance were observed [74], as well as changes in energy expenditure and RER (reduced whole body expenditure and increased carbohydrate over fat oxidation) [78]. In contrast, body weight was not affected in a rat study using exposure to dim light at night for 6 weeks [76]. In this study, different intensities of dim light at night were used (0.02–0.08  $\mu\text{W}/\text{cm}^2$  dim light). The highest intensity of dim light at night disrupted circadian rhythms of plasma corticosterone, melatonin and glucose, but body weight was not affected during the 6 weeks of exposure.

**Table 4. Summary of animal studies in which continuous light (LL) or dim light (LDim) at night exposure was used to mimic human shiftwork.** For detailed description of the columns, see Methods section.

+: number of studies with increases, -: number of studies with decreases, ~: number of studies with altered rhythm, o: number of studies with no effect. For *glucose metabolism* a + indicates increased basal levels of glucose, HbA1c or insulin, increased HOMA index or decreased glucose tolerance. *EE* energy expenditure; *RER* respiratory exchange ratio; *BP* blood pressure

	RAT	MOUSE	TOTAL	STUDIES
BODYWEIGHT	1/5	5/6	+ : 6/11 (54.5%) - : 0/11 (0%) o : 5/11 (45.4%)	[70, 73, 74, 76-79]
FOOD INTAKE TOTAL	0/2	2/7	+ : 1/9 (11.1%) - : 1/9 (11.1%) o : 7/9 (77.7%)	[73-76, 78, 79]
ACTIVITY TOTAL		0/5	+ : 0/5 (0%) - : 0/5 (0%) o : 5/5 (100%)	[74, 75, 78, 79]
EE TOTAL		2/2	+ : 0/2 (0%) - : 2/2 (100%) o : 0/2 (0%)	[73, 78]
RER		2/2	+ : 2/2 (100%) - : 0/2 (0%) o : 0/2 (0%)	[73, 78]
ADIPOSITY		1/1	+ : 1/1 (100%) - : 0/1 (0%) o : 0/1 (0%)	[75]
GLUCOSE METABOLISM	3/4	3/3	+ : 4/7 (57.1%) - : 0/7 (0%) ~ : 2/7 (28.6%) o : 1/7 (14.3%)	[70, 73, 74, 76]
LIPID METABOLISM	1/2		+ : 0/2 (0%) - : 0/2 (0%) ~ : 1/2 (50%) o : 1/2 (50%)	[76]
CORTICOSTERONE	2/2		+ : 1/2 (50%) - : 0/2 (0%) ~ : 2/2 (100%) o : 0/2 (0%)	[76]
MELATONIN	4/4		+ : 0/4 (0%) - : 2/4 (50%) ~ : 2/4 (50%) o : 0/4 (0%)	[70, 76]
LEPTIN				
GHRELIN				
BP/HEART RATE				
GENE EXPRESSION				



Considering the still limited number of studies using dim light, firm conclusions are not possible yet. However, it appears that similar to continuous bright light exposure dim light at night affects body weight in mice, but not in rats. The currently available studies suggest that glucose metabolism is affected in mice as well as in rats by dim light at night. Interestingly, the study by Fonken *et al.*, reported that the increases in body weight gain and fat mass by dim light at night can be prevented by restricting food access to the dark phase [74]. These results suggest an important role for altered timing of food intake in the effects of continuous light on body weight, although none of these three dim light studies clearly quantified the circadian changes in food intake.

#### SUMMARY 'CONSTANT LIGHT' MODELS

Models using constant light seem to affect body weight in 55% of the studies (Table 4), all increases in body weight. However, there is a clear difference between rat and mice studies, with 1 out of 5 rat studies reporting effects on body weight and 5 out of 6 mice studies. Total food intake is not affected in most studies (only affected in 2 out of 9 studies), whereas glucose metabolism is affected in a majority of studies (6/7, 86%). Interestingly, the clear difference observed between rats and mice in the effects of constant light on body weight is not that pronounced for glucose metabolism. Thus, these results indicate that models using constant light exposure influence glucose metabolism in both species, while body weight is mainly affected in mice. For the other parameters, only a limited number of studies are available making firm conclusions difficult. For a complete overview of all parameters, see Table 4.

#### 3.4.2.1 CHANGES IN LIGHT/DARK SCHEDULES - PERIOD LENGTH

Under normal conditions, one cycle of light and darkness on the planet earth matches exactly 24h. Exposure to altered period lengths (<23h or >25h) usually requires a constant re-entrainment of the circadian system and experiments using such protocols have therefore been used to investigate the effects of circadian disruption. On the other hand, when very short period lengths are used entrainment is not possible, which will result in either free-running rhythms or an abolishment of circadian rhythms. Shorter period lengths have been reported to cause

alterations in several circadian parameters, such as locomotor activity [80, 81], drinking pattern [80] and body temperature [82].

Altered circadian rhythms due to an aberrant period length have been implicated in metabolic disturbances as well. For example, increases in body weight have been observed in mice and rats exposed to short period lengths of 6–23h for 9–10 weeks [80–83]. In addition, changes in glucose homeostasis (increased glucose levels and glucose intolerance), lipid homeostasis (increased cholesterol levels) and expression of liver genes related to glucose metabolism have been reported in one of these models [81]. However, human relevancy of these models is poor since period length remains unaltered during shift work. Of course, partial shifts in light exposure might occur during shift work where light is present during working hours and is avoided during subsequent sleeping hours, but therefore models using shifts in light exposure are more relevant to the human situation than changes in period length.

#### 3.4.2.2 CHANGES IN LIGHT/DARK SCHEDULES - SHIFTS

Shifts in light exposure require re-entrainment of the circadian system causing (temporary) disturbance of circadian rhythms. Repeated phase shifts have been investigated using numerous schedules which differ in shift size (1–12h), frequency (every day-once a week), duration (acute effects-chronic effects), and direction (forwards or backwards), resulting in very heterogeneous study results. For example, a 6h forward shift every 3 days for 10 weeks abolished locomotor and melatonin rhythmicity [70] whereas rhythmicity in locomotor activity and body temperature remained but was disturbed (lengthened period and reduced amplitude) after an 8h forward shift every 2 days for 10 days [84].

Circadian disruption by shifts in light exposure has also been investigated in relation to metabolic function. To our knowledge, four studies using this type of model have been published. A study by Tsai *et al.* in rats, observed an increase in body weight gain during exposure to 12h shifts twice a week [85]. This increase was only observed during the first 2 months of exposure, and body weight gain was unaltered during the third month and a subsequent 10-day recovery period. Interestingly, in this model, food intake was increased and locomotor activity was reduced which could both be linked to the observed increase in body weight gain. However, in contrast to the body weight gain, these changes were present at all time-points of the experiment.

A study by Gale *et al.* did not observe effects on body weight in rats exposed to a 6h shift every 3 days for 10 weeks. Similar to continuous light exposure, effects of this light shift model on glucose metabolism were only observed in diabetes-prone HIP rats but not in wild type rats (increased glucose levels and decreased glucose- and arginine- stimulated insulin secretion) [70]. The model used in a third study, by Bartol-Munier *et al.*, was exposure to 10h shifts twice a week and restriction of food to the dark phase for 5 months. In this study, no effects on body weight were observed whether animals were on normal chow or on a high-fat diet, but changes in glucose metabolism (lower insulin levels) were present in animals fed normal chow and exposed to the shifts [86]. In the most recent study, mice were exposed to a 12h shift once a week for 12 weeks on a normal chow diet and an additional 10 weeks on a high-fat and high-sugar diet to investigate effects on the gut microbiome. In this study, a small but significant increase in body weight was observed in the shifted mice on a normal chow diet. When the diet was changed to a high-fat and high-sugar diet, no additional effects by lighting schedule on body weight were observed [87].

#### SUMMARY 'CHANGES IN LIGHT/DARK SCHEDULES' MODELS

Models using changes in light/dark schedules affect body weight in 56% (5 out of 9) of the studies, with studies using mice finding effects more often (3 out of 4) compared to studies using rats (2 out of 5). The total amount of food intake is affected in half of the studies (50%; 3 out of 6). Glucose metabolism is affected in 83% of studies (5 out of 6) with almost an equal number of studies showing an effect when using mouse or rat. These results suggest that changes in the light/dark cycle affect some of the metabolic parameters (body weight and glucose metabolism). For other parameters, the number of studies is very low making interpretations difficult. For a complete overview of all parameters, see Table 5.

## DISCUSSION

With this review, we aim to provide an overview of the available animal studies investigating the relationship between shift work and metabolic risk factors. Shift work in humans consists of a multi-aspects exposure

**Table 5. Summary of animal studies in which exposure to L/D shifts was used to mimic human shiftwork.** For detailed description of the columns, see Methods section.

+: number of studies with increases, -: number of studies with decreases, ~: number of studies with altered rhythm, o: number of studies with no effect. For *glucose metabolism* a + indicates increased basal levels of glucose, HbA1c or insulin, increased HOMA index or decreased glucose tolerance. *EE* energy expenditure; *RER* respiratory exchange ratio; *BP* blood pressure

	RAT	MOUSE	TOTAL	STUDIES
BODYWEIGHT	2/5	3/4	+ : 4/9 (44.4%) - : 1/9 (11.1%) o : 4/9 (44.4%)	[70, 80-83, 85-87]
FOOD INTAKE TOTAL	2/3	1/3	+ : 2/6 (33.3%) - : 1/6 (16.7%) o : 3/6 (50%)	[80-83, 85, 86]
ACTIVITY TOTAL	2/2		+ : 0/2 (0%) - : 2/2 (100%) o : 0/2 (0%)	[85, 86]
EE TOTAL				
RER				
ADIPOSIITY				
GLUCOSE METABOLISM	2/3	3/3	+ : 4/6 (66.7%) - : 1/6 (16.7%) ~ : 0/6 (0%) o : 1/6 (16.7%)	[70, 80-82, 86]
LIPID METABOLISM	0/1	1/2	+ : 1/3 (33.3%) - : 0/3 (0%) ~ : 0/3 (0%) o : 2/3 (66.7%)	[80, 81, 86]
CORTICOSTERONE				
MELATONIN	2/2		+ : 0/2 (0%) - : 0/2 (0%) ~ : 2/2 (100%) o : 0/2 (0%)	[70]
LEPTIN		1/1	+ : 1/1 (100%) - : 0/1 (0%) ~ : 0/1 (0%) o : 0/1 (0%)	[82]
GHRELIN				
BP/HEART RATE	1/1		+ : 0/1 (0%) - : 0/1 (0%) ~ : 1/1 (100%) o : 0/1 (0%)	[85]
GENE EXPRESSION		1/1	1/1 (100%)	[81]

(Fig. 1). We focused on the four most relevant manipulations that are being used to mimic human shift work conditions in animals: altered timing of food intake, altered timing and/or duration of activity, altered timing and/or duration of sleep, and irregular lighting conditions. The

overview provided in this review shows that these types of models are very useful in modelling one aspect of shift work and investigating the role of these separate aspects. However, the interaction between the different aspects of shift work is an important component of shift work as well, which would be beneficial to model in animals. Unfortunately, the heterogeneity of shift work in humans as an exposure (i.e., number of subsequent shifts, duration of recovery periods, direction of rotation, etc.) and the variability in behavioural coping responses to shift work amongst human individuals (for instance in sleeping and eating strategies) makes modelling shift work in animals a very challenging exercise. To develop an animal model that incorporates the interaction between multiple shift work aspects requires complete knowledge of human shift work behaviour (i.e., light exposure, sleep behaviour and dietary habits). Although in recent years first attempts have been made to achieve the latter, a complete knowledge has not been reached yet. Clearly, an animal model incorporating multiple shift work aspects would have advantages. Firstly, whereas human studies require over 20 years to observe long-term health effects of shift work, such as development of metabolic disease or cancer, in an animal experiment “long-term” health effects can be studied much faster (~1 year). Hence, animal studies could accelerate the unravelling of the underlying mechanisms explaining the relationship between shift work and health. Secondly, animal models provide opportunities to study parameters and processes that would be impossible or extremely invasive to study in humans. In this discussion, we summarize the main findings of this review, differences between models and species and touch upon possible underlying mechanisms.

## MAIN FINDINGS OF THIS REVIEW

### ***All categories of models***

While selecting articles for this review, we came across a very diverse collection of food, activity, sleep and light manipulations, which were all rather different from each other. Although we grouped the studies into four main categories, nearly none of the experimental setups was copied by another research group or was it used in a different species or strain. Furthermore, metabolic parameters were not equally frequent

or extensively measured in the different models, which is another factor making it difficult to compare results between models. Five of our selected metabolic parameters (body weight, total food intake, total activity, glucose metabolism and lipid metabolism) were described in all four categories, but only three of them were described in both mouse and rat studies in each category. First, we will summarize the main results of all studies, followed by a discussion of the main differences between categories of models and species.

Table 6 represents the percentage of studies reporting effects of the manipulation for the listed parameters in exposed groups compared to the control group. Numerous different parameters were described in the included studies but we concentrated on a few that were measured in most studies. Body weight was described in most studies and 62% (26/42) of the studies reported an effect provoked by the manipulated shift work aspect. Total food intake and total activity levels were less often affected, in 39% (14/36) and 39% (9/23) of the studies respectively. Other metabolic parameters including energy expenditure (80% (5/6)) glucose metabolism (83% (25/30)), lipid metabolism (69% (11/16)) and adiposity (80% (8/10)) were affected frequently by shift work. In most studies, circadian parameters were included as well and often showed alterations (mainly in rhythm, including changes in amplitude and phase). For example, the circadian rhythm of corticosterone was altered in 54% (7/13) of the studies.

In summary, effects on metabolism are observed in a substantial number of studies, however, results are not completely consistent. Moreover, changes in metabolism did not always translate in changes in body weight (gain) or adiposity. Indeed, we have to take into account that there might be a publication bias as perhaps mainly parameters that were affected are described and therefore the actual percentages of studies finding an effect might be lower.

### ***Are there differences between categories?***

Body weight is one of the parameters that was measured in all categories of models and was affected in 64% (7/11) of the food studies, in 83% (5/6) of the activity studies, in 60% (3/5) of the sleep studies, in 56% (5/9) L/D-shift studies and in 55% (6/11) of the continuous light studies. Total food intake showed to be affected in about 45% of the food studies (5/11), in 50% of sleep studies (2/4) and L/D-shift studies

(3/6), whereas only 33% (2/6) of the activity-studies and 22% (2/9) of the LL studies demonstrated an effect. Factors involved in glucose and lipid metabolism were affected in all categories of models, although light models showed low percentages for lipid metabolism (33% (1/3) in L/D-shift studies; 50% (1/2) in continuous light studies). The single other parameter which was measured in all five models was total activity levels and this was affected in 43% of food studies (3/7), 67% of activity studies (4/6), 0% of sleep studies (0/3), 100% of L/D studies (2/2) and 0% of LL studies (0/5). These results show that large differences exist between the effects of different categories, however, caution is required when interpreting these results since often only a limited number of studies was available. Another important limitation to draw firm conclusions is the low number of reproducible results for many parameters. On the other hand, remarkable to notice is the 100% score for nearly all parameters measured in the studies that manipulated activity. One possible reason for this might be that 5 out of 8 of these studies came from the same research group and thereby the experimental setup was exactly the same each time, i.e., these authors produced very reproducible results. In conclusion, it is most likely that the variability between the studies (species, type of manipulation, duration of exposure etc.) is important for whether a parameter is affected by the manipulation. This is another representation of the heterogeneity of shift work and increases the complexity to model shift work. When comparing parameters and categories of models for which multiple studies are available differences are not large. Consequently, a category with the largest metabolic consequences cannot be appointed. However, when considering human relevance of the models, the use of models using constant light and alterations in period length is least informative.

### ***Are there differences between rat and mouse studies?***

In the articles included for this review, we observed that rats and mice are used interchangeably for shift work models. Interestingly, however, thus far the observed effects are not identical between species even when exactly the same procedure is carried out [41, 52]. In general, in most categories either mouse (e.g. 0 out of 9 studies in activity-models) or rat studies (e.g. only 3 out of 13 studies in food-models) were underrepresented, thereby making it difficult to compare between the

species. When focusing on parameters reported in at least 8 experiments in both species, neglecting the exact model category, total activity (63% (7/11)), and lipid metabolism (71% (5/7)) were more often affected in rat than in mice studies (17% (2/12), 67% (6/9) respectively). On the other hand, effects were more often observed in mice for body weight (70% (14/20)), total food intake (40% (8/20)), glucose metabolism (86% (12/14)) and total energy expenditure (100% (4/4)) than in rats (55% (12/22), 38% (6/16), 81% (13/16) and 50% (1/2) resp.), however, these differences are relatively small. The only parameter showing similar percentages in both species is adiposity with 80% (4/5) of studies showing an effect of the condition. Generally, choosing a certain type of rodent for an experiment is based on the genetic background of an animal, the similarities between the human situation/disease and the features the animal model displays, the surgical techniques that need to be carried out, the type of behavioural tests that have to be performed or other specific reasons. To this point, shift work models have been performed with both species and it is important to keep in mind that when creating a shift work model, behavioural conditions are manipulated. Often we tend to think that behavioural manipulations have similar effects in different species, but we should be aware that mice and rats might respond very differently. Causality of the dissimilar effects between mouse and rat studies is yet unknown. Hypothetically, the difference in body size and associated metabolic rate could play a role in these differences, but this remains to be investigated.

In our opinion, an important, but lacking, model is exposure of a diurnal species to shift work conditions. Day-active animals are considered more similar to human when it comes to circadian research and therefore in principle would be a better model to study the metabolic consequences of shift work. Moreover, if similar effects on metabolism are found between nocturnal and diurnal species, this would support the translatability of animal models for human shift work simulations.

### ***Possible mechanism of health consequences of shift work***

Mimicking human shift work conditions in an animal model ultimately aims to study and understand the underlying mechanism of shift work leading to health problems. The predominant current theory stresses the process of desynchronization. In general, it is thought that desynchronization leads to a suboptimal functioning of many bodily



processes. Observed effects range from shifts in gene expression and altered hormone secretion (i.e., leptin, insulin, melatonin and corticosterone) to modified behavioural output (i.e., food intake rhythm, activity levels and rhythm) and changes in whole body physiology (i.e., body weight, food intake, RER, energy expenditure, glucose and lipid metabolism). Metabolic processes within and between important metabolic tissues such as liver and muscle should cooperate in a proper timely manner to control optimally, for instance, glucose and lipid metabolism. If not, this may lead to metabolic problems.

In principal, shift work can cause desynchronization at different levels, which in general all result from desynchronization between the environment and the (complete circadian system within an) organism. Within the organism, we distinguish four separate levels. The first level (1) concerns the desynchronization between the central clock and the peripheral clocks. It is well known that light is the most important Zeitgeber for the SCN, while food and activity are such for the peripheral clocks. During shift work, these two Zeitgebers present conflicting information resulting in an opposite phase for the central and peripheral oscillators. Question is if and how these disturbances affect downstream processes.

Besides this possible top-down desynchronization between central and peripheral clocks, desynchronization may also occur between anatomically separated organs, the second level (2). Shifting the timing of food intake has been shown to differentially affect liver and muscle clocks [37, 41]. Desynchronization between peripheral clocks supposedly originates from tissue-specific sensitivity to entrainment signals such as activity, energy levels (e.g. periods of fasting/feeding), responses to hormone secretion, input from autonomic nervous system etc. An additional type of desynchronization at level 2 occurs between anatomical parts of the SCN. Clock gene expression and electrical activity resynchronize differently between the dorsal and ventral part of the SCN after 6h phase shifting [88, 89]. Hypothetically, temporal desynchronization and thereby suboptimal functioning of (parts of) the SCN may lead to a malfunctioning of SCN-mediated downstream mechanisms.

The third level (3) encompasses desynchronization between the molecular clock mechanism and the clock-induced genes. Many genes involved in metabolism display a circadian rhythm in their expression. Several studies have described that a manipulation of SCN output

signals, by for instance adrenalectomy or denervation of autonomic inputs, induces a loss of rhythmicity in the expression of clock-induced genes in white adipose tissue, liver and bone, while clock genes remain rhythmic [90–94]. This suggests that although the molecular clock machinery is still intact, the rhythmic expression of clock-induced genes is disturbed. It is likely that this level of desynchronization indeed also takes place during shift work and a first suggestion was made by Salgado-Delgado *et al.* [46]. They showed that in their forced-activity shift work model the effect on the rhythmicity of metabolic genes ( $NAD^+$ , *Nampt*, *Ppara*, *Ppar $\gamma$*  and *Pgc1 $\alpha$* ) did not resemble the effects on clock genes rhythmicity.

The fourth level (4) of desynchronization concerns desynchronization within the molecular clock itself, i.e., different parts of the molecular clockwork are affected to a different degree within one tissue. Studies in which animals are exposed to phase shifts of the light dark cycle to induce experimental jet lag, a proper method to induce temporal circadian desynchrony, report dissimilar resynchronization speeds of different parts of the molecular clock. For instance, expression of the clock gene *Cry1* appeared to resynchronize slower than *mPer* expression in the SCN after a 6h phase advance [95]. Although this level of desynchronization has not yet been shown in studies using a shift work model, the aforementioned jetlag studies resemble studies in category 4 (i.e., shifts in timing of light exposure). Despite the body's ability to adapt to challenging conditions, this obviously becomes metabolically problematic if this occurs every few days or weeks as is the case in most working schedules of employees who are shift workers.

However, up to now desynchronization is mostly studied at the level of communication between central and peripheral clocks (level 1). The other three levels of desynchronization were not or only marginally studied in the aforementioned models but potentially contribute significantly to the causal link between circadian desynchronization and negative health outcomes. Therefore, we encourage future studies to also focus on possible desynchronization at levels 2, 3 and 4.

### **Interaction of shift work aspects**

Most studies discussed in this review used either one of four manipulations (food, activity, sleep, and light) as a model for shift work. Tackling shift work conditions by manipulating one aspect is a good approach

when studying the effects of that particular aspect of shift work. This gives insight in how food, activity, sleep and light manipulations contribute to the associated negative health effects. However, the mentioned aspects of shift work are strongly intertwined and cannot easily be separated. For example, forcing an animal to consume its daily food at an unusual time inevitably also disturbs its activity and sleep pattern, which in itself also affects metabolism. Effects found of a manipulation are rapidly assigned to the main manipulation but it is often not very well considered whether and if so, how the main manipulation affects other aspects and its consequences. For instance, sleep behaviour is hardly ever monitored by EEG recordings or high-resolution actimetry, thus information about sleep duration and sleep quality is usually missing. In addition, in order to translate results obtained in animal studies properly to humans, also more knowledge regarding these parameters in human shift work is required. Thus, we propose more elaborate measurements on the main aspects of shift work (Fig. 1) in animal as well as in human studies.

## CONCLUSION

This review provides an overview of animal models for shift work to investigate metabolic health effects. This overview indicates the large variety present in models used as well as a substantial amount of indecisive results. Ideally, we would have concluded this review with suggestions for a more standardized model including a number of factors to manipulate and different possible outcome measures. Standardization would reduce the heterogeneity between studies for both methods and outcome parameters. Unfortunately, at this point our mechanistic knowledge on the effects of shift work is not sufficient yet to draw firm conclusions and thereby put a certain model forward or eliminate others. Furthermore, human shift work conditions are highly variable and not outlined well enough to propose an ideal animal model. For now, we plead for more awareness of the interactions between the aspects of shift work, which are intentionally and unintentionally manipulated. Shift work and the type of manipulations used in animal models are multi-aspects exposures (Fig. 1). Therefore, it is important to measure additional parameters apart from the ones directly related to the

**Table 6. A summary of 7 most frequently measured parameters in the 5 categories of shiftwork models (food, activity, sleep, L/D shifts and LL/LDim).** The most right columns represent the results of all studies together. All results are presented as the number of studies in which an effect of the manipulation was found (when compared to the control condition) / the number of studies in which the parameter was measured. Between brackets, the number of studies showing an effect is depicted as a percentage. *EE* energy expenditure; *L/D* light/dark; *LL* continuous light; *LDim* dim light at night

MODEL TYPE	FOOD	ACTIV- ITY	SLEEP	L/D shift	LL/Dim	ALL		
	RAT + MOUSE	RAT + MOUSE	RAT + MOUSE	RAT + MOUSE	RAT + MOUSE	RAT	MOUSE	TOTAL
BODYWEIGHT	7/11 (63.6%)	5/6 (83.3%)	3/5 (60%)	5/9 (55.5%)	6/11 (54.5%)	12/22 (54.5%)	14/20 (70%)	26/42 (61.9%)
TOTAL FOOD INTAKE	5/11 (45.4%)	2/6 (33.3%)	2/4 (50%)	3/6 (50%)	2/9 (22.2%)	6/16 (37.5%)	8/20 (40%)	14/36 (38.9%)
TOTAL ACTIVITY	3/7 (42.9%)	4/6 (66.7%)	0/3 (0%)	2/2 (100%)	0/5 (0%)	7/11 (63.6%)	2/12 (16.7%)	9/23 (39.1%)
TOTAL EE	3/3 (100%)		0/1 (0%)		2/2 (100%)	1/2 (50%)	4/4 (100%)	5/6 (80%)
ADIPOSITY	5/7 (71.4%)	2/2 (100%)			1/1 (100%)	4/5 (80%)	4/5 (80%)	8/10 (80%)
GLUCOSE METABOLISM	6/9 (66.7%)	3/3 (100%)	5/5 (100%)	5/6 (83.3%)	6/7 (85.7%)	13/16 (81.2%)	12/14 (85.7%)	25/30 (83.3%)
LIPID METABOLISM	5/7 (71.4%)	2/2 (100%)	2/2 (100%)	1/3 (33.3%)	1/2 (50%)	5/7 (71.4%)	6/9 (66.7%)	11/16 (68.8%)

manipulation. For example, measuring sleep behaviour when using a model with light shifts. Other examples are circadian parameters, such as gene expression in several organs, hormones, activity, body temperature, sleep behaviour and metabolic parameters. More insights into these parameters will be beneficial for comparing different outcomes when different types of manipulations are used.

Where possible these parameters should be measured in human shift work-studies as well to allow for more insight into translatability of findings. Furthermore, experiments ideally should cover both short- and long-term effects, ranging from days to years, to study details of underlying mechanisms in the development of the unfavourable health outcomes caused by shift work. The perfect model is as yet non-existent but ideally combines several aspects of shift work to mimic the human situation best (e.g. when manipulating activity and light, changes in food intake will follow and this should be monitored).

Only by properly studying the effects of shift work conditions solely and combined, this research eventually will help the general community to learn how to deal with shift work conditions best, prevent shift workers from becoming disturbed and possibly prevent and treat negative health outcomes.

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