



## Conference on 'Improving nutrition in metropolitan areas' Symposium 2: Chrono-nutrition in the urban environment

### Shift-work: is time of eating determining metabolic health? Evidence from animal models

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The circadian disruption in shift-workers is suggested to be a risk factor to develop overweight and metabolic dysfunction. The conflicting time signals given by shifted activity, shifted food intake and exposure to light at night occurring in the shift-worker are proposed to be the cause for the loss of internal synchrony and the consequent adverse effects on body weight and metabolism. Because food elicited signals have proven to be potent entraining signals for peripheral oscillations, here we review the findings from experimental models of shift-work and verify whether they provide evidence about the causal association between shifted feeding schedules, circadian disruption and altered metabolism. We found mainly four experimental models that mimic the conditions of shift-work: protocols of forced sleep deprivation, of forced activity during the normal rest phase, exposure to light at night and shifted food timing. A big variability in the intensity and duration of the protocols was observed, which led to a diversity of effects. A common result was the disruption of temporal patterns of activity; however, not all studies explored the temporal patterns of food intake. According to studies that evaluate time of food intake as an experimental model of shift-work and studies that evaluate shifted food consumption, time of food intake may be a determining factor for the loss of balance at the circadian and metabolic level.

#### Circadian disruption: Shift-work: Scheduled feeding: Light at night: Obesity: Metabolic syndrome

Shift-work and night work require individuals to labour outside conventional daytime hours, imposing shifted activity and sleep schedules<sup>(1)</sup>. It is estimated that worldwide about 20 % of individuals participate in some form of shift-work. Besides shift- and night workers, another 25 % of young adults worldwide are exposed voluntarily to shifted sleep-activity habits<sup>(2)</sup>, mainly due to the modern life style that promotes social or leisure activities at normal rest times. Individuals engaged in nocturnal leisure activities shift their sleep-activity patterns differentially between weekdays and weekends, leading them to similar conditions as shift-workers. This shifted sleep timing is now referred as social jet-lag<sup>(3)</sup>. Both social jet-lag and shift-work share similar features because in

both conditions individuals are awake and active outside conventional daytime hours; they suffer from altered sleep-activity habits, shifted eating patterns and are exposed to light at night<sup>(4,5)</sup>. Such conditions cause a conflict with the internal biological clock and promote circadian disruption triggering loss of homeostasis<sup>(6)</sup>.

While we know little about the short- or long-term consequences of social jet-lag, a vast number of studies provide information about the short- and long-term effects of night and shift-work. Shift-workers are identified as a population with higher risk to develop adverse health effects including myocardial infarction, ischemic stroke and CVD<sup>(7,8)</sup>. Shift-workers complain of disturbed sleep and excessive fatigue<sup>(1,9)</sup>, which have adverse

**Abbreviation:** LL, constant light intensity throughout 24 h; SCN, suprachiasmatic nucleus.

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consequences on their work performance, leading to high levels of stress<sup>(10,11)</sup> to a reduced reaction time and sleepiness-related accidents<sup>(12,13)</sup>. In the long term, shift-work is associated with psychiatric disorders, depression and substance abuse<sup>(14–16)</sup>.

Metabolically, shift-work is associated with a higher propensity to develop overweight or obesity<sup>(17,18)</sup> and it is a risk factor for metabolic syndrome<sup>(17,19)</sup>, insulin resistance<sup>(20)</sup>, dyslipidaemia and type 2 diabetes<sup>(21,22)</sup>. Due to the worldwide increasing incidence of obesity and metabolic disease, attention has focused on individuals at risk, especially shift-workers and groups exposed to disrupted sleep-activity patterns. In regard to social jet-lag, studies are needed to confirm this association; however, in individuals presenting overweight, a higher BMI was associated with a higher number of shifted hours between weekdays and weekends<sup>(2)</sup>. Clinical and experimental studies have indicated that shift-work and other conditions that cause circadian disruption prime individuals for a higher vulnerability to lose metabolic balance and obesity<sup>(18,23,24)</sup>.

Circadian rhythms are relevant in order to adjust the intensity and efficiency of the organism's response to the daily challenges required by the day–night cycles. Disrupted circadian rhythms will result in a time-deficient response that in the long term will lead to loss of homeostasis and disease. Circadian rhythms are driven by the circadian system, which is a complex internal timing system constituted by a biological clock, the suprachiasmatic nucleus (SCN), and by peripheral oscillators<sup>(25)</sup>. The circadian system fluctuates, synchronised to the external light–dark cycle, which is the main environmental time reference; however, other inputs, relevant for the group or the individual's survival, can also provide time information, including the internal energetic state and food availability.

At the cellular level, clock mechanisms are driven by transcription–translation feedback loops of several interacting genes better known as clock genes<sup>(26)</sup>. Clock genes impose a temporal order to the transcription of other genes necessary for metabolic functions in the cells. The SCN coordinates such rhythms by means of hormonal and autonomic mechanisms, allowing in this way the time signal to reach cells that are not directly exposed to light<sup>(27)</sup>. However, other internal stimuli that provide time information to the cells are elicited by feeding cycles that induce metabolic rhythms. Peripheral organs respond to the changing levels of glucose, insulin, temperature and corticosterone, in different ways, which depend on their function and involvement in metabolic balance<sup>(26)</sup>. Therefore, time of food intake has shown to be a powerful signal for the circadian system, driving brain and peripheral oscillators as well as behaviour<sup>(28)</sup>. When time of food does not coincide with the normal sleep–activity cycle, driven by the biological clock, food creates an internal conflict with temporal signals driven by the SCN for the regulation of metabolic efficiency favouring weight gain, obesity and metabolic syndrome<sup>(29–31)</sup>.

Several studies demonstrate that shift-workers develop shifted food intake patterns, with increased consumption

towards late at night<sup>(32,33)</sup>, moreover during their shifts they show a preference for high-energetic and high-fat food<sup>(34,35)</sup> creating a shifted time pattern of energy signals to the cells, organs and components of the circadian system. Thus, it is possible that the shifted mealtime may trigger an internal conflict promoting internal desynchrony, leading to a deficient temporal response by organs involved in digestion and metabolic balance, and to a loss of homeostasis. Food intake currently of the normal activity phase may be the solution to counteract the adverse physiological consequences frequently observed in human shift-workers. Founded on this assumption, studies based on chrono-nutrition suggest implementing time-organised eating schedules for shift- and night workers in such a way that food does not represent a conflicting temporal signal with the normal light–dark cycle. More information is necessary in order to dissect the contribution of the time of eating for circadian disruption and to verify if restricted feeding schedules can be a possible intervention for individuals at risk of circadian disruption.

Experimental models in rodents have been used to better understand how shift-work impacts the circadian system, and to uncover factors associated with circadian disruption that exert adverse effects on behaviour and metabolic efficiency. Therefore, experimental protocols have implemented conditions of shifted timing of sleep, shifted timing of activity or shifted timing of food intake, and the exposure to light at night<sup>(36)</sup>. The advantage of experimental models is that variables can be better controlled and causal relations can be determined. A limitation is that mainly rats or mice, which are nocturnal animals, have been used for these models.

In this review, we have searched in the experimental protocols modelling shift-work whether the time of food intake could be the cause of circadian disruption and metabolic disease.

Data bases used for this review were Google scholar and PubMed, and keywords used for the bibliographic search were (shift work circadian disruption metabolism rat mice obesity) including 'sleep deprivation' and not review = 271 articles; (shift work circadian disruption metabolism rat mice obesity) including 'forced activity' and not review = 37 articles; (shift work circadian disruption metabolism rat mice obesity) including 'light at night' and not review = 131 articles; (shift work circadian disruption metabolism rat mice obesity) including 'restricted feeding' and not review = 262 articles.

Studies published in languages different from English were not considered. Reviews were discarded. Citations referring to human studies were discarded; only studies using repeated manipulations (usually more than 3 d) as a model of shift-work were included, leaving out numerous studies that explore acute effects (manipulations for a single occasion). For models of experimental shift-work, we identified the following protocols: shifted timing of sleep, shifted timing of activity or shifted timing of food intake, as well as the exposure to light at night. From these models, only studies describing circadian disruption and/or overweight and/or metabolic dysfunction were analysed, discarding studies that



explored other physiological systems or other mechanisms. Models using shifted light–dark cycles that resemble a jet-lag condition were not included as well as models using knock out or GM mice. For the condition of shifted food intake, a big spectrum of diets and timing schedules were observed ranging from 2 to 16 h food access<sup>(29)</sup>, which led to diverse metabolic outcomes. In this analysis, we have included studies using 8–16 h food access with a regular chow or a high-fat, high energetic diet, because shorter access to food promotes a hypoenergetic condition leading animals to lose weight. Considering all criteria, a total of fifty-four studies were included in this review.

### Experimental models of shift- and night work: what do they indicate?

#### *Experimental models of chronic sleep disruption*

The most well-known feature of shift-work in human subjects is the disruption of the normal sleep-activity patterns; therefore, animal models aimed at mimicking shift-work have used protocols in rodents to chronically reduce or shift the sleep timing. [Table 1](#) summarises studies that explored the consequences of chronic sleep disruption, and the effects on the circadian system, on body weight and/or metabolic function. Studies causing chronic sleep disruption vary in their strategies and in the time employed to produce a chronic sleep deprivation, some (ten studies) reduce total sleep, others inhibit rapid eye movement sleep (seven studies) or induce sleep fragmentation (two studies). In general, all strategies led to a redistribution of sleep–wake phase<sup>(37–40)</sup>, suggesting a circadian disturbance. However, the majority of such studies have not assessed circadian rhythms.

Due to sleep deprivation, food ingestion was decreased (seven out of fifteen studies), or not changed (four out of fifteen), while in some studies (four studies) this behaviour was not monitored ([Table 1](#)). In the majority of the studies, a decrease in body weight gain was observed, which is suggested to be the result of increased energy expenditure<sup>(40)</sup> due to the exhausting conditions and physiological stress imposed by the extended protocols (from 18 to 20 h) of sleep deprivation<sup>(40–47)</sup>. Studies observing a reduced body weight reported metabolic changes indicating a catabolic state or fasting like state, with reduced levels of glucose, low TAG, low cholesterol, low leptin levels, and in some studies accompanied by high levels of ghrelin and corticosterone<sup>(38,40–50)</sup>. Thus, an anabolic state was associated with sleep deprivation.

Importantly, studies that have implemented a milder strategy of sleep restriction by using randomised loud noise or reducing the period and hours of sleep restriction observed increased body weight using a regular diet<sup>(39,45)</sup>. Using gentle handling for 6 h at the start of the rest phase resulted in disrupted circadian rhythmicity of clock and metabolic genes in the liver<sup>(51)</sup>, altered glucose and TAG blood levels, as well as modifications in adipocytes transcription profile<sup>(52)</sup>. While some studies did not find a significant effect on body weight, they observed glucose intolerance, insulin insensitivity<sup>(41,42,53,54)</sup> and increased

circulating insulin<sup>(44)</sup>. An important feature of protocols using milder strategies for sleep deprivation is that animals were able to maintain a normal feeding rate. An example is the study by Caron and Stephenson<sup>(45)</sup> in which, by using milder sleep deprivation and giving rats the opportunity to have short recovery sleep bouts, this reduced the sleep debt and improved temperature regulation, food intake and metabolic efficiency.

The relevance of the time of food intake on body weight and the metabolic outcome was assessed in five studies<sup>(23,38,43,51,52)</sup> combining sleep deprivation with daytime feeding, where food consumption was shifted to the hours that animals were kept awake. The effects of shifted food intake are not consistent; it did not increase body weight using a regular diet<sup>(38,51)</sup>; in two studies it resulted in decreased bodyweight<sup>(43,52)</sup>, and in one study it promoted overweight when combined with a highly palatable energy-dense diet, better known as cafeteria diet<sup>(23)</sup>. Overweight remained during the recovery period after chronic rapid eye movement sleep deprivation combined with high-fat diet<sup>(44)</sup>. Metabolic effects were also worsened when combining cafeteria diet with sleep deprivation leading to a metabolic syndrome<sup>(23,53)</sup>, and in aged mice, the combination of a high-fat diet with sleep deprivation led to damage to the pancreas<sup>(55)</sup>.

All together, chronic disrupted sleep causes metabolic alterations favouring in some cases a metabolic syndrome and in others reflecting a fasted state, even when body weight is reduced or not affected. Only a few studies using milder strategies for sleep disruption report increased and shifted food intake towards the forced hours of wake time and this was associated with indicators of metabolic syndrome.

#### *Experimental models of forced activity*

Animal models that shift activity to the resting phase are scarce ([Table 2](#)). A main difficulty in defining such models is that some protocols that shift activity coincide with manipulations used for sleep deprivation.

The strategies used to keep animals awake and active mainly consist of motorised wheels that vary in their construction. Similar to the studies for sleep deprivation, we found a variety of studies requiring from the animals different intensities of activity and effort ([Table 2](#)), which is reflected by the number of revolutions per minute, by the frequency of locomotor adjustments or the number of responses of the animal. The majority of studies required from the animals a strong effort during their forced activity schedules, keeping continuous alertness and emitting effortful movements that mimic more of an exercise routine<sup>(56)</sup>. Some schedules represented a stressful condition<sup>(57)</sup> or even drove animals to exhaustion and to a torpor state due to the negative metabolic state driven by the exhausting protocol<sup>(58)</sup>. Models of forced activity induced disrupted circadian rhythms mainly by shifting activity towards the rest phase.

Body weight was decreased (five out of eight studies)<sup>(56–60)</sup>, in two studies body weight was mildly (7%) increased<sup>(61,62)</sup> and one study did not assess it<sup>(63)</sup>.

**Table 1.** Experimental models of chronic sleep disruption

Protocol				Findings				
Photoperiod	Feeding schedules	Diet	Duration (d)	Circadian disruption	Body weight	Metabolic findings	Effects on food intake	Reference
12/12 LD	<i>Ad libitum</i>	Standard chow	21	NE	↓	Reduced leptin and leptin receptor, deficient glucose clearance, increased TNF- $\alpha$ and IL-6, macrophage infiltration in white adipose tissue, reduced retroperitoneal fat	↓	Venancio & Suchecki <sup>(41)</sup>
12/12 LD	<i>Ad libitum</i>	Standard chow	4	NE	↓	Decreased leptin and insulin levels, no change in glucose, increased leptin receptors in the hypothalamus	↑ Food intake in the light phase	Moraes <i>et al.</i> <sup>(43)</sup>
12/12 LD	<i>Ad libitum</i>	Standard chow	4	NE	↓ Adipose tissue	Decreased glucose, TAG, leptin and VLDL	NE	Rosa Neto <i>et al.</i> <sup>(47)</sup>
12/12 LD	<i>Ad libitum</i>	Standard chow, HFD or liquid diet	4	NE	↓	Fasting like metabolic profile: increased ketone bodies, reduced liver glycogen and TAG	↓	Martins <i>et al.</i> <sup>(46)</sup>
12/12 LD	<i>Ad libitum</i>	Standard chow	90	NE	↓	Glucose intolerance with a GTT and insulin insensitivity	NE	Xu <i>et al.</i> <sup>(42)</sup>
12/12 LD	<i>Ad libitum</i>	Standard chow	7	NE	↓	Low glucose levels and TAG levels, low insulin and leptin, increased ghrelin and corticosterone. Effects proportional to the duration of sleep deprivation	↓	Brianza-Padilla <i>et al.</i> <sup>(50)</sup>
12/12 LD	<i>Ad libitum</i>	Standard chow followed by HFD during sleep recovery	21	NE	↓	Increased insulin, resistin and inflammation indicators	=	de Oliveira <i>et al.</i> <sup>(44)</sup>
12/12 LD	<i>Ad libitum</i>	Standard chow and HFD	9	NE	↓ With Chow diet and body weight loss was ameliorated with HFD	High-fat feeding in combination with sleep disruption impairs glucose tolerance	↓ With regular chow ↑ When exposed to HFD	Ho <i>et al.</i> <sup>(53)</sup>
12/12 LD	<i>Ad libitum</i>	Standard chow	14	NO The daily peak of corticosterone remained in the same phase	=	Glucose intolerance with a GTT	↑	Baud <i>et al.</i> <sup>(54)</sup>
12/12 LD	<i>Ad libitum</i>	Standard chow	7	NE	NE	High glucose levels and low insulin with low insulin sensitivity, UPR protein in endocrine pancreatic cells	NE	Naidoo <i>et al.</i> <sup>(55)</sup>
12/12 LD	<i>Ad libitum</i>	45 % Fat diet	8	NE Microsleeps during sleep deprivation	↓	Decreased glucose, insulin and leptin levels, increased energy expenditure	=	Barf <i>et al.</i> <sup>(40)</sup>



12/12 LD	<i>Ad libitum</i>	Standard chow	60	NO Overall diurnal pattern of sleep was not affected. Total wake time correlated with total cell loss in the VLPO	↓	40 % sleep reduction reduced glucose TAG, cholesterol, C-reactive protein, increased ghrelin and lower leptin	=	Vetrivelan <i>et al.</i> <sup>(48)</sup>
12/12 LD	<i>Ad libitum</i>	Standard chow	9	YES Increased wake time in the light phase and more sleep in the night, decreased SWS and REM time, more phase transitions	↑	NE	↑	Mavanji <i>et al.</i> <sup>(39)</sup>
12/12 LD	<i>Ad libitum</i>	Standard chow	4	YES Pronounced redistribution of sleep/wake cycle, with sporadic microsleep episodes during the activity phase	NE	NE	NE	Gronli <i>et al.</i> <sup>(37)</sup>
12/12 LD	<i>Ad libitum</i>	Standard chow	5	YES Compensatory redistribution of sleep stages, activity increased 11 % during hours of sleep restriction	NE	Ghrelin and corticosterone increased during sleep deprivation, leptin levels were not changed	↓	Bodosi <i>et al.</i> <sup>(38)</sup>
12/12 LD	<i>Ad libitum</i>	Standard chow	10	NE	↓	Metabolic rate and body temperature related to the rate of accumulation of sleep deprivation	=	Caron and Stephenson <sup>(45)</sup>
12/12 LD	<i>Ad libitum</i>	Standard chow	5	YES Redistribution of activity levels in the 24 h cycle, low activity in the last half of the night	↓	Leptin 3-fold higher, lipid and carbohydrate metabolism transcripts overexpressed in white adipose tissue, indicating increased lipogenesis	↓ During the hours of sleep restriction. Total daily food =	Husse <i>et al.</i> <sup>(52)</sup>
12/12 LD with high light intensity 500 lux during the day	<i>Ad libitum</i>	Standard chow	10	YES Shifted corticosterone peak, gradual decline of nocturnal activity. In DD no change in daily activity patterns or in SCN clock genes	=	Loss of rhythmicity in genes involved in carbohydrate metabolism in the liver	↑ Food intake during the light phase	Barclay <i>et al.</i> <sup>(51)</sup>
12/12 LD	<i>Ad libitum</i>	Standard chow and cafeteria diet	70	YES Shifted acrophases and low amplitude of general activity	↓	Sleep restriction combined with cafeteria diet promoted criteria for metabolic syndrome	↓With regular chow ↑With cafeteria diet	Espitia-Bautista <i>et al.</i> <sup>(23)</sup>

DD, constant dark; GTT, glucose tolerance test; HFD, high-fat diet; LD, light-dark; NE, not explored; REM, rapid eye movement; SCN, suprachiasmatic nucleus; UPR, unfolded protein response; VLPO, ventrolateral preoptic nucleus; =, similar to controls; ↑ increased; ↓ decreased.

Shift-work: is time of eating determining metabolic health?

**Table 2.** Experimental models of forced activity

Protocol							Findings			
Forced activity model	Animal model	Photoperiod	Feeding schedules	Diet	Duration (d)	Circadian disruption	Body weight	Metabolic findings	Effects on food intake	Reference
Timed forced activity with automatic slow rotating (0.3 rpm) wheels 8 h/5 d/ week	Male Wistar rats	12/12 LD	<i>Ad libitum</i>	Standard chow	35	YES Nocturnal activity decreased, temperature and metabolic rhythms shifted to the workhours	↑ 7 %	Dyslipidaemia, steatosis, glucose intolerance	Shifted feeding patterns to the light phase	Salgado-Delgado <i>et al.</i> <sup>(62,61)</sup>
Timed forced activity with automatic slow rotating (3 rpm) wheels 8 h	Male Wistar rats	12/12 LD	<i>Ad libitum</i>	Standard chow	4	NO Core body patterns not changed, feeding patterns shifted	↓	In the liver disturbed genes encoding for insulin sensitivity and lipid metabolism	Shifted feeding patterns to the light phase	Marti <i>et al.</i> <sup>(59)</sup>
Floor rotates bidirectionally at varying speed. 8 h/5 d/week	Male Wistar rats	12/12 LD	<i>Ad libitum</i>	Standard chow	35	NO Core body patterns not changed, daily activity patterns not changed, nocturnal activity decreased	↓	NE	NE	Leenaars <i>et al.</i> <sup>(57)</sup>
Access to activity wheel + food only in the day or only in the night	Male Long Evans rats	12/12 LD	12 h restricted food access	Standard chow	22	YES Nocturnal activity decreased, altered temperature rhythms shifted to the work hours	↓	NE	=	Murphy <i>et al.</i> <sup>(60)</sup>
Shifted LD cycles combined with 12 h exercise in a running wheel	Male F344 rats	12/12 LD and shifted	<i>Ad libitum</i>	Standard chow	91	YES The combination of LD shifts + wheel running altered temperature rhythms	↓	NE	Exercised rats ate more	Tsai & Tsai <sup>(56)</sup>
Motorised wheels 10 h in the light phase	Male Syrian hamsters	14/10 LD	<i>Ad libitum</i>	Standard chow	21	YES Reduced daily running with 6 lux light, shifted activity to the light phase	NE	NE	NE	Dallman & Mrosovsky <sup>(63)</sup>
'Work for food' protocol increasing the number of revolution for a reward pellet	Male CBA mice	12/12 LD	<i>Ad libitum</i>	Standard chow	40 (approximately)	YES Shifted activity towards the day	↓	Decreased temperature, torpor	↓ Food intake	Hut <i>et al.</i> <sup>(58)</sup>

LD, light-dark; NE, not explored; rpm, revolutions per minute; =, similar to controls; ↑ increased; ↓ decreased.



Interestingly, only two groups have explored the metabolic outcome of shifted forced activity using a mild strategy to enforce activity. Both groups report shifted feeding patterns towards the rest phase and a resulting disrupted metabolism<sup>(59,61,62)</sup>. The study by Marti *et al.*<sup>(59)</sup> imposed this protocol for only 4 d and could already observe in the liver a disturbed pattern of genes encoding insulin sensitivity and lipid metabolism. A series of studies by our group, using slow rotating wheels, reported that activity during the resting phase for 4 weeks induced rats to gain more bodyweight and to develop abdominal obesity accompanied by liver steatosis and glucose intolerance<sup>(61,62)</sup>.

With this protocol, rats developed disrupted circadian rhythms characterised by a shift in core body temperature, in general activity and serum TAG, a loss of the rhythm in glucose, in the rhythm in clock genes in the liver and no change in corticosterone rhythm.

Importantly, forced activity in the slow rotating wheel induced a shift in the timing of food consumption to the light phase, suggesting this as a possible factor inducing circadian disruption and loss of metabolic balance<sup>(64)</sup>. To confirm this association, rats were prevented from ingesting food during the forced activity hours and only had access to food during the night (which is the normal activity phase for rats). This procedure prevented circadian disruption and the adverse metabolic effects observed in rats exposed to the working schedule. Moreover, daytime food access alone recapitulated the effects of this working schedule on metabolism<sup>(61,64)</sup>. A possible effect of food intake in other protocols of forced activity in the rest phase was not assessed.

All together, most of the experimental models using forced activity in the rest phase have not provided evidence that this factor may cause circadian disruption and metabolic dysfunction. This is probably due to the exhausting protocols used to induce activity. Similar as observed with the sleep deprivation studies, milder protocols favouring rats to eat during the rest phase induced increased body weight and adverse metabolic changes in the direction of metabolic syndrome, pointing out shifted food intake as an important risk factor for circadian and metabolic disruption.

#### *Experimental models for light at night*

In rodents, light exposure at night has been used as a strategy to mimic one of the most disrupting and common conditions experienced by human shift-workers. Light is a signal that immediately activates the SCN; however, for nocturnal rodents, light is a rest signal, and for human subjects, light is associated with activity. **Table 3** summarises studies that explored the metabolic consequences of continuous light exposure in mice and rats either implementing constant light intensity throughout 24 h (LL) or alternating bright light during the day with dim light exposure at night (L/DL).

From studies involving LL, seven out of eight reported clear circadian disruption based on arrhythmic locomotor activity patterns, on low SCN neuronal activation or disturbed corticosterone and melatonin rhythms<sup>(65–71)</sup>.

From the other two studies, we may assume that the circadian system was also affected because they report low melatonin levels, which is also an indicator of disruption at the level of the biological clock<sup>(71,72)</sup>. To note is that two studies using alternating bright light during the day with dim light at night did not induce circadian arrhythmicity at least in general activity and corticosterone levels<sup>(65,73)</sup>, and a third study did not explore it<sup>(74)</sup>.

The effects of LL on body weight are inconsistent: in two studies body weight was increased<sup>(65,66)</sup>, in four studies animals remained similar to controls<sup>(67,68,70,71)</sup> and in two studies body weight gain is not reported<sup>(69,72)</sup>. Interestingly, metabolic dysfunction was consistently reported in all studies independently of the body weight outcome<sup>(65–72)</sup>. Rodents in LL developed increased glucose levels, glucose intolerance, decreased insulin sensitivity, increased fat mass deposition, elevated plasma fatty acids, decreased activity of brown adipocytes, higher RER during the subjective day and decreased energy expenditure<sup>(65–71)</sup>. Adverse effects of LL are also described in organs involved in energy balance. Qian *et al.*<sup>(68)</sup> reported disrupted pancreatic islet architecture as well as increased apoptosis due to LL. Loss of circadian rhythmicity in clock genes in the liver and the colon were also reported while rhythmicity in the duodenum was preserved<sup>(69,72)</sup>.

In the three studies alternating bright light with dim light at night, consistent increased body weight gain was observed, together with higher RER, glucose intolerance, increased insulin levels during the light phase and decreased energy expenditure<sup>(65,74,75)</sup>, similar as observed in LL.

Interestingly only two of the cited studies using LL (**Table 3**) assessed the 24 h pattern of food consumption. Polidarova *et al.*<sup>(69)</sup> reported loss of circadian rhythms in feeding behaviour, Wideman and Murphy<sup>(72)</sup> indicated that rats in LL consumed less food throughout the 24 h cycle, however attained a positive feed efficiency value (g body weight change/g food intake), which suggests that this condition may favour body weight gain. Contrasting, only the study by Fonken *et al.*<sup>(65)</sup> reported that mice exposed to alternating bright light during the day with dim light at night shifted their feeding patterns and consumed a higher amount of food in the day.

Altogether, studies using constant light report a consistent disruptive effect of light on metabolism leading to increased adiposity and disruptive glucose balance. Because few studies assessed patterns of food ingestion, the contribution of food timing to metabolic dysfunction is not clear. Nevertheless, eating while the nocturnal animal is exposed to light suggests a circadian conflict, which requires further studies.

#### *Experimental models of shifted food timing*

Restricting food access to the rest phase has been used in rodents as a strategy to reproduce the shifted feeding schedule of human shift-workers. Studies that explored the metabolic consequences of shifted feeding schedules are summarised in **Table 4**. The majority of the studies used a protocol of restricting food access to 12 h during

**Table 3.** Experimental models for light at night

Protocol						Findings				
Shift-work Model	Animal model	Photoperiod	Feeding schedules	Diet	Duration (d)	Circadian disruption	Body weight	Metabolic findings	Effects on food intake	Reference
Constant light	Male Swiss-Webster mice	L/L constant light (150 lx)	<i>Ad libitum</i>	Standard chow	56	YES Locomotor activity arrhythmicity, blunted corticosterone levels	↑	Weight gain, increased epididymal fat deposition, glucose intolerance	↑ During the subjective day	Fonken <i>et al.</i> <sup>(65)</sup>
Constant light	Male C57Bl/6 J mice	L/L constant light (180 lx)	<i>Ad libitum</i>	Standard chow	30	YES Locomotor activity arrhythmicity. Decreased SCN neuronal activity (electrophysiological recordings). Blunted corticosterone levels	↑	Weight gain, glucose intolerance. More energy intake during the subjective day period, RER during the subjective day period. Decreased total energy expenditure levels. Abolishment of circadian variation in insulin sensitivity	NE	Coomans <i>et al.</i> <sup>(66)</sup>
Constant light	Male C57Bl/6 J mice	L/L constant light (about 85 μW/cm <sup>2</sup> )	<i>Ad libitum</i>	Standard chow	35	YES Loss of circadian rhythm in behavioural activity	=	Increased body fat mass, reduced uptake of TAG-derived fatty acids and glucose by BAT, decreased activity of brown adipocytes	NE	Kooijman <i>et al.</i> <sup>(67)</sup>
Constant light	Male Sprague-Dawley rats	L/L constant light (>100 lx)	<i>Ad libitum</i>	Standard chow	70	YES Locomotor activity arrhythmicity	=	Loss of diurnal variations in insulin plasma concentrations, damped Per-1 amplitude in isolated pancreatic islets from LL rats, disrupted islet architecture, increased B-cell apoptosis	NE	Qian <i>et al.</i> <sup>(68)</sup>
Constant light	Male Sprague-Dawley rats	L/L constant light (300 lx)	<i>Ad libitum</i>	Standard chow	42	YES Low melatonin concentrations and loss of metabolic rhythms	=	Constantly elevated plasma fatty acid levels	=	Dauchy <i>et al.</i> <sup>(71)</sup>
Constant light	Diabetic prone HIP rats	L/L constant light (>100 lx)	<i>Ad libitum</i>	Standard chow	70	YES Locomotor activity arrhythmicity. Disrupted melatonin rhythms	=	Increased glucose levels, decreased glucose and arginine stimulated insulin secretion.	NE	Gale <i>et al.</i> <sup>(70)</sup>
Constant light	Male Long Evans rats	L/L constant light (450 lx)	<i>Ad libitum</i>	Standard chow	17	AMBIGUOUS Free-running locomotor activity but low melatonin concentrations during the night	NE	More positive fed efficiency value and increased visceral adiposity	↓	Wideman & Murphy <sup>(72)</sup>



Constant light	Male Wistar rats	L/L constant light (50-300 lx)	Ad libitum	Not mention	30	YES Locomotor activity arrhythmicity.	NE	NE	Loss of circadian rhythm in feeding behaviour	Polidarova et al. <sup>(69)</sup>
Dim light at night	Female Swiss Webster rats	L/DL 16-8 (150 lx/14 h-5 lx 8 h)	Ad libitum	Standard chow	56	NE	↑	Weight gain	↓	Aubrecht et al. <sup>(74)</sup>
Dim light at night	Male Swiss-Webster mice	L/DL 14-10 (150 lx/14 h-5 lx 10 h)	Ad libitum	Standard chow	14	NO Dim light did not cause arrhythmic activity	↑	Less O <sub>2</sub> consumption, higher RER values, decreased energy expenditure	NE	Borniger et al. <sup>(75)</sup>
Dim light at night	Male Swiss-Webster mice	L/DL 16-8 (150 lx/16 h-5 lx 8 h)	Ad libitum	Standard chow	56	NO Typical circadian rhythm in locomotor activity and corticosterone levels	↑	Glucose intolerance, increased insulin levels during the light phase	↑ During the day (55-5 %)	Fonken et al. <sup>(65)</sup>

BAT, brown adipose tissue; HIP, human islet amyloid polypeptide; L/DL, light/dim light; L/L, light/light; lx, lux; NE, not explored; SCN, suprachiasmatic nucleus; =, similar to controls; ↑ increased; ↓ decreased.

the day, which is the rest phase, and compared this with food access for 12 h during the night. Diets mainly consist of standard chow; however, some studies also have used high-fat diet or high-fat and high-sucrose diets. There are also some studies using shortened food access for 4 or 5 h during the day; however, we have not included them in this review because this daily brief access to food can lead to food entrainment, resets circadian metabolism and induce energy restriction resembling a fasted day<sup>(29)</sup>.

From the studies examined here, twelve out of fourteen reported circadian disturbances, while the other two studies did not explore this feature<sup>(76, 77)</sup>. Disrupted circadian rhythms were observed in the fluctuations of clock genes in the liver, muscle and heart<sup>(61,78-83)</sup>. Studies also report loss of temperature rhythms, shifted locomotor activity towards the rest phase, shifted glucose, TAG, leptin and ghrelin rhythms and decreased leptin immunoreactivity rhythms in the organum vasculosum of the lamina terminalis<sup>(61,64,78-86)</sup>. In the study of Ramirez-Plascencia et al.<sup>(87)</sup>, authors described shifted or blunted activity rhythms of orexin, melanin-concentrating hormone and α-melanocortin-stimulating hormone neurons in the hypothalamus. Therefore, it is a consistent finding that shifted food access to the rest phase affects brain and peripheral clocks involved in metabolic regulation. Such findings emphasise the importance of food as a powerful circadian synchroniser.

The effect of shifting food to the light period on body weight gain is quite clear. In ten studies using 12 h day feeding, animals gained significantly more body weight<sup>(61,64,76-78,81-84,87)</sup>; in four studies, animals remained similar to controls<sup>(79,80,85,86)</sup>. In two studies, where authors did not find differences in body weight gain<sup>(79,80)</sup>, this was associated with decreased food consumption. In the case of the studies by Shamsi et al.<sup>(85)</sup> (8 or 16 h day feeding) and Rocha et al.<sup>(86)</sup>, animals' body weight remained similar to controls; however, animals developed metabolic alterations (Table 4).

Among rodents that gained weight, metabolic dysfunction was reported, mainly fat mass accumulation, dyslipidaemia, high glucose levels and glucose intolerance, decreased insulin sensitivity, lower RER, decreased energy expenditure and disrupted circadian rhythms of metabolic genes in the liver and muscle<sup>(61,76-78,82-84,87)</sup>.

All together, studies using restricted food access to the rest phase reported circadian disruption in organs related with metabolic function. This loss of temporal order among organs regulating metabolism may be the cause of a disturbed metabolism that can have potential health consequences, such as metabolic syndrome, obesity and diabetes<sup>(6,88)</sup>.

### What do experimental models indicate about the association between circadian disruption, overweight and metabolic disturbance?

All models described here induce circadian disruption and affect the metabolic state in one or another direction (Table 5), indicating a clear association between



**Table 4.** Experimental models of shifted food timing

PROTOCOL						FINDINGS				
Shift-work model	Animal model	Photoperiod	Feeding schedules	Diet	Duration (d)	Circadian disruption	Body weight	Metabolic findings	Effects on food intake	Reference
Shifted feeding	Male C57BL/6 J mice	12/12 LD	Day (12 h) or Night (12 h)	HFD	42	NE	↑	Slightly increased in fat mass accumulation	NE	Arble <i>et al.</i> <sup>(76)</sup>
Shifted feeding	Male Wistar rats	12/12 LD	Day (12 h) or night (12 h)	Standard chow	28	YES Loss of temperature rhythms. disrupted rhythms of clock genes in the liver	↑	Dyslipidaemia. Increased fat mass accumulation. High glucose levels and glucose intolerance	=	Salgado-Delgado <i>et al.</i> <sup>(61,64)</sup>
Shifted feeding	Male Wistar rats	12/12 LD	Day (12 h) or <i>ad libitum</i>	Standard chow	21	YES Shifted activity towards the light phase. Decreased nocturnal activity	↑ Only in 24 months old rats	NE	↓ In young rats ↑ In old rats	Reddy & Jagota <sup>(84)</sup>
Shifted feeding	Male Wistar rats	12/12 LD	Day (12 h) or night (12 h)	Standard chow	21	YES Shifted activity of ORX neurons in the LH-PeF. Blunted daily rhythm of MCH neuron activation. Loss of circadian activity of α-MSH neurons in the arcuate nucleus	↑	NE	↓	Ramirez-Plascencia <i>et al.</i> <sup>(87)</sup>
Shifted feeding	Male Wistar rats	12/12 LD	Day (12 h) or night (12 h)	Standard chow, fat/sugar diet	31	NE	↑ Only with high-sugar diet in the day	Hyperphagia, lower RER in the light period. Decreased food efficiency, increased fat pads	↑ Consumption of energy during the light period	Oosterman <i>et al.</i> <sup>(77)</sup>
Shifted feeding	Male wild-type mice (on FVB/N background)	12/12 LD	Day (12 h) or night (12 h)	Standard chow	9	YES Disrupted rhythms in clock and metabolic genes expression in the liver, epididymal fat, muscle and heart	↑	Food consumption during the day induced: lower energy expenditure, higher RER	↑ Food intake at the beginning of the day	Bray <i>et al.</i> <sup>(78)</sup>
Shifted feeding	Male Wistar rats	12/12 LD	Day (12 h) or night (12 h)	Standard chow	56	YES Disrupted circadian rhythms in leptin levels. Altered rhythms of clock genes in the liver	=	Increased perirenal fat mass deposition. Increased feed efficiency. Increased RER	↓	Opperhuizen <i>et al.</i> <sup>(79)</sup>



Shifted feeding	Male Wistar rats	12/12 LD	Day (12 h) or <i>ad libitum</i>	Standard chow or HFD	21	YES Disrupted rhythms in insulin, leptin and NEFA levels. Disrupted circadian rhythms of clock genes in the liver and muscle. Decreased nocturnal activity and oxygen consumption	=	Increased day corticosterone. Shifted RER profile to the day. Increased glycogen in the muscle	↓ in food intake (3–5 d)	Reznick <i>et al.</i> <sup>(80)</sup>
Shifted feeding	Male C57BL/6 J mice	12/12 LD	Day (12 h) or night (12 h)	Standard chow	15, 30 and 90	YES Disrupted clock genes rhythms in the liver and pancreas	↑	Increased glucose, free fatty acids levels and corticosterone in the circulation. Increased peritoneal fat mass deposition. Glucose intolerance. Decreased insulin levels. Increased triacylglycerols and cholesterol in the liver	NE	Mukherji <i>et al.</i> <sup>(81,82)</sup>
Shifted feeding	Male C57BL/6 mice	8/16 LD 16/8 LD	Day (16 h) or day (8 h)	Standard chow	56	YES Altered rhythms of clock and metabolic genes in the liver	=	Increased insulin levels. Decreased TAG. Increased glucose tolerance. Decreased liver weight	NE	Shamsi <i>et al.</i> <sup>(85)</sup>
Shifted feeding	Male C57BL/6 J mice	12/12 LD	Day (8 h) night (8 h)	High-fat and high-sucrose diet	7	YES Disrupted circadian rhythms of metabolic genes in the liver. Shifted rhythms of ghrelin, insulin and leptin and reduced nocturnal activity	↑	Hyperphagia. Physical inactivity. Increased adiposity, insulin, leptin, total cholesterol and ghrelin levels. Increased hepatic lipids	↑	Yasumoto <i>et al.</i> <sup>(83)</sup>
Shifted feeding	Male Wistar rats	12/12 LD	Day 80 % of their total food intake Night 20 %	Standard chow	60	YES Shifted daily feeding and glucose patterns	=	Glucose intolerance, fat mass accumulation, accumulation of fat in the liver	↑ hyperproteic and hyperglucidic diet = total 24 h intake	Rocha <i>et al.</i> <sup>(86)</sup>

HFD, high-fat diet; LH-PeF, lateral hypothalamus-perifornical area; LD, light-dark; MCH, melanin-concentrating hormone;  $\alpha$ -MSH,  $\alpha$ -melanocyte-stimulating hormone; NE, not explored; ORX, orexin; OVLT, organum vasculosum lamina terminalis; ↑ increased; ↓ decreased.

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conditions that affect daily cycles and the development of an adverse metabolic condition. In some studies due to the lack of circadian assessment or metabolic follow-up, this association is not always evident. However, protocols using light exposure at night or shifted food to the rest phase highlight the adverse effects of food intake at the wrong time or light at night on metabolic health. Both factors are present in the modern life style and affect the shift-worker.

Metabolic effects largely depended on the specific manipulations by each model, i.e. duration, intensity of activity, feeding schedule and type of diet. Regarding sleep deprivation and forced activity models, the majority of the studies report decreased body weight gain associated with a catabolic metabolism which, as previously discussed, may be related to the physical requirements and stressful conditions of the protocols. Importantly, studies using milder protocols for sleep disruption or for enforced activity are scarce, however report metabolic changes that suggest the development of metabolic syndrome<sup>(51,52,61,64)</sup>. Contrasting, light exposure at night has proved to be an effective and consistent model to mimic the metabolic alterations observed in human shift-workers. Most of the studies using light at night reported increased body weight gain and all of them described alterations reflecting a dyslipidaemia and criteria for a metabolic syndrome. Likewise models using shifted food to the rest phase found consistent metabolic dysfunction and showed to be effective to produce body weight gain, in spite of a few reports that observed no difference from the control.

#### **The relevance of shifted food intake as a cause of circadian and metabolic disorder in shift-work model**

When looking at all models of shift-work, it is clear that many studies did not explore the possible role of the time of food intake as a risk factor for circadian disruption and metabolic alterations (Table 5). Evidently, the models implementing shifted food access to the rest phase have paid attention to this factor and reported consistently changes in the metabolic state towards dyslipidaemia and glucose intolerance. Other models that observed shifted food consumption to the normal rest hours have provided strong evidence about this association. In the studies by Salgado-Delgado *et al.*<sup>(61,64)</sup>, animals shifted their food intake to the rest phase and this had an adverse metabolic outcome. When animals were not allowed to eat at the wrong phase, the adverse metabolic effects were prevented, pointing out the relevance of food timing as a main risk factor for metabolic problems as observed in human shift-workers. One of the studies using dim light at night also observed a shift in meal patterns, and this shift was associated with overweight and metabolic changes<sup>(65)</sup>.

Food has proved to be a powerful entraining signal for the circadian system. Metabolic signals elicited by food intake impact organs at the cellular level and provide timing to cellular processes and genes involved in glucose and lipid metabolism<sup>(28)</sup>. Under shift-work conditions

several external and internal timing signals are shifted due to abnormal exposure to activity, to light and to food, resulting in an internal conflict with time signals from the SCN transmitted to organs and cells via the autonomic nervous system. This affects differentially organs and regulatory genes depending on their dependence on metabolism, endocrine or autonomic signals<sup>(28)</sup>. The loss of an internal temporal order among different regulatory systems leads to incorrect or deficient adaptive responses to external demands, which can be the cause of a loss of homeostasis and a higher propensity to disease<sup>(25)</sup>.

#### **Main internal signals that influence metabolic function**

##### *Food intake as a time signal for the circadian system*

Studies implementing shifted feeding schedules towards the rest phase described shifted rhythms of clock and metabolic genes in organs involved in metabolic balance, especially in the liver<sup>(61,79,82,83,85)</sup>, in the muscle and adipose tissue<sup>(78,80)</sup>. As we have mentioned earlier, clock genes impose a temporal order to the transcription of other genes necessary for metabolic functions in the cells.

In the study by Salgado-Delgado *et al.*<sup>(64)</sup>, where forced activity in slow rotating wheels induced a shifted food intake towards the day, also shifted and blunted clock genes in the liver were reported, suggesting a circadian disruption at the cellular level. Such studies indicate the relevant effect of the time of food intake as a potent disrupting factor, when time of food does not coincide with the light–dark cycle. The disruption of clock gene expression in the liver is associated with disturbed liver metabolism and development of liver steatosis (see for review<sup>(89)</sup>).

The circadian conflict at the cellular level is suggested to occur between the shifted food-related signals (glucose, insulin) and the biological clock transmitting light–dark information to peripheral organs and cells<sup>(27)</sup>. Melatonin and corticosterone are the main hormonal pathways used by the biological clock to transmit time information of the light–dark cycle to peripheral organs<sup>(90,91)</sup> and may be the source of conflict with the food-entrained rhythms.

##### *Corticosterone*

Corticosterone is proposed as an internal timing signal for a variety of organs<sup>(92)</sup> and reaches peak levels at the beginning of the active phase, which in rodents corresponds to the beginning of the night. In the liver<sup>(93,94)</sup>, corticosterone influences gluconeogenesis as demonstrated *in vivo* while *in vitro* exerts synchronizing effects on fibroblasts<sup>(95)</sup> and adipose tissue<sup>(96)</sup>.

In rats, sleep deprivation induced increased levels of corticosterone during the protocol<sup>(38)</sup>, mild forced activity, as well as exposure to food restriction to the resting phase, induced a peak of corticosterone at the beginning of the schedule in addition to the normal peak at the beginning of the night<sup>(38,64,80)</sup>. Similarly, animals exposed to LL exhibit increased levels of corticosterone

**Table 5.** Proportion of studies providing evidence for the relationship between circadian disruptions and adverse metabolic function

Shift-work model	Animal model	Circadian disruption	Body weight	Metabolic findings	Effects on food intake
Sleep deprivation (19 studies)	63.2 % Male rats	YES 31.6 %	= 10.5 %	31 % Metabolic syndrome	↑ 26.3 %
	31.5 % Male mice	NO 10.5 %	↓ 68.4 %	37 % Catabolic state	= 21.1 %
	5.2 % Female rats	NE 57.9 %	NE 15.8 %	16 % No effects 16 % NE	↓ 31.5 % NE 21.2 %
Forced activity (8 studies)	75 % Male rats	YES 75 %	↑ 25 %	37.5 % Metabolic syndrome	↑ 12.5 %
	12.5 % Male mice	NO 25 %	NE 14.3 %	12.5 % Catabolic state	= 12.5 %
	12.5 % Male hamsters		↓ 62.7 %	50 % NE	↓ 12.5 % NE 25 % Shifted to the light phase 37.5 %
Light exposure at night (11 studies)	45.4 % Male rats	YES 63.6 %	↑ 45.4 %	100 % Metabolic syndrome	↑ 18.2 %
	45.4 % Male mice	NO 18.1 %	= 36.4 %		= 9.1 %
	9.1 % Female rats	NE 9.1 %	NE 18.2 %		↓ 18.2 %
		AMBIGUOUS 9.1 %			NE 45.4 % 9.1 % No loss of rhythm in feeding behaviour
Shifted feeding (14 studies)	42.8 % Male mice	YES 85.7 %	↑ 71.6 %	85.7 % Metabolic syndrome	↑ 35.7 %
	57.2 % Male rats	NE 14.3 %	= 21.4 %	14.3 % NE	= 14.3 % ↓ 21.4 % NE 28.5 %

Metabolic syndrome = overweight or obesity, increased adipose tissue, glucose intolerance, increased TAG, increased cholesterol; NE, not explored; =, similar to controls; ↑ increased; ↓ decreased.

along the 24 h period with a loss of the circadian rhythmicity<sup>(65,66)</sup>. Such high levels of glucocorticoids affect glucose homeostasis and promote gluconeogenesis in the liver<sup>(97)</sup>. It is also known that high doses of glucocorticoids result in increased weight gain, glucose intolerance and high insulin and TAG levels<sup>(98)</sup>. Elevated corticosteroid levels and dampened or disrupted glucocorticoid rhythmicity have been reported in obese adults and in genetically obese Zucker rats and db/db mice<sup>(92)</sup>. Moreover, the shifted timing of corticosterone release may function as an altered time signal and exert a disruptive effect on the circadian system.

### Melatonin

It is suggested that the SCN uses the nocturnal melatonin secretion to distribute circadian signals within the brain or the periphery, to organs and cells possessing melatonin receptors<sup>(91)</sup>. In rodents, daily administration of melatonin entrains activity rhythms in free-running rats<sup>(99–101)</sup>, and some studies suggest that melatonin can entrain adipocytes<sup>(102)</sup> and protein synthesis in hepatocytes<sup>(103)</sup>. In three of the experimental models of light at night, altered timing and/or levels of melatonin were observed<sup>(70–72)</sup>.

It is well described that low light intensities at night are sufficient to inhibit melatonin<sup>(71)</sup>; however, this hormone under the dim light at night schedule has not been evaluated. Likewise in animal protocols that restrict food access to the day, melatonin has not been evaluated. Importantly, the three studies involving LL that reported low melatonin levels associated the hormone levels with metabolic alterations<sup>(70–72)</sup>.

Melatonin in addition to being an important regulator of circadian rhythms is also involved in the regulation of glucose metabolism<sup>(104)</sup>. The relevance of melatonin for metabolic balance is further demonstrated under conditions of low hormone concentrations such as ageing, in which, melatonin supplementation effectively decreased fat mass accumulation, body weight gain and restored insulin and leptin levels<sup>(105,106)</sup>. In diabetic rats and mice, melatonin treatment also improved glucose and TAG levels, diminished body weight and insulin levels<sup>(107,108)</sup>. Based on this evidence, we suggest that low melatonin levels resulting from circadian disruption may promote the metabolic dysfunction; however, more data from the experimental models will be necessary to support this association.

### Main contributions of experimental models, limitations and perspectives

The analysis of the four experimental models for shift-work indicates that the strategies and variables assessed by different groups are diverse with respect to intensity, time of exposure and variables used to assess circadian and/or metabolic state, leading in some studies to contrasting or inconclusive findings. It is important to indicate that studies here reported have included a significant number of subjects in their experimental designs, which permits to draw conclusions; however, the variability in the use of factors has led to different outcomes. Moreover, not all studies performed a circadian screening, not all determined the temporal order of food intake and only a few studies have explored the association

between the time of food intake as the cause of circadian and metabolic disruption. Importantly, studies that explored this relationship report that the time of food intake is indeed essential for the development of metabolic disturbances.

A growing body of epidemiological evidence in human populations indicates that short sleep is a risk factor for the development of obesity and metabolic disturbance. Animal studies exploring the effects of restricted sleep indeed observed a reduction in insulin sensitivity and changed levels of hormones involved in appetite and neuroendocrine regulation, such as ghrelin, leptin and insulin<sup>(109,110)</sup>. However, experimental models using sleep deprivation have not provided conclusive effects of the contribution of shifted feeding schedules because very few have explored the possibility of a shifted food intake. Moreover, models for sleep deprivation or forced activity require more uniformity, using mild protocols in order to provide conclusive results about metabolic mechanisms.

As a model of shift-work, light at night exposure has proved to be an effective and a consistent model to mimic the metabolic alterations observed in human shift-workers, highlighting the importance of being exposed to dark nights in order to promote metabolic health. The fact that light at night will activate neurons in the SCN that are normally inactive and inhibit melatonin secretion when melatonin is normally high indicates that light at night is a strong circadian disruptive signal. In such conditions, scheduled food has shown to ameliorate metabolic conditions and to exert strong entraining signal for metabolic genes in the liver<sup>(111)</sup>. More evidence is needed to determine the role of food timing under constant light. The majority of studies exploring experimental shift-work have been performed in nocturnal animals, which is a limitation when translating their findings to the diurnal human species. As indicated earlier, melatonin is involved in the regulation of glucose metabolism, and in nocturnal rodents, melatonin treatment improved the metabolic state in obese mice<sup>(108)</sup> suggesting that low melatonin levels resulting from circadian disruption may be partly the cause of metabolic dysfunction. In nocturnal rodents, melatonin release coincides with their active phase and with the time of food intake, while in human subjects, melatonin release coincides with the rest and sleep phase and not with the moments of maximal digestion and food absorption. This important difference requires a better understanding for the role that melatonin could play linking circadian disruption and metabolism. In a similar way, light at night has shown to be a disruptive signal for the circadian system, and while nocturnal rodents are normally active at night, human subjects sleep, thus the disrupting effect on sleep-activity patterns is inverted. Therefore, a next necessary step for the experimental models of shift-work is to use diurnal species in order to confirm such adverse effects and to better translate experimental results to the problem of the human shift-worker.

Interestingly, the majority of studies here reported have used male animals and only two studies were found that explored the response in females<sup>(42,74)</sup>. In the study by Xu *et al.*<sup>(42)</sup>, authors do not discuss a

possible difference in their outcomes associated with the sex of the animals. In this regard, Aubrecht *et al.*<sup>(74)</sup> discuss their previous work using male animals and stated that dim light affects in a similar way body mass and likely metabolic function in both males and females. Other studies testing a high-fat diet in male and female rodents indicate a differential influence associated with sex in rats<sup>(112,113)</sup>, mice and hamsters<sup>(114,115)</sup>. This is partly explained by a differential response to metabolic signals in brain areas involved with metabolic regulation<sup>(116)</sup>. Since women are also exposed to shift-work, further studies exploring the effects of circadian disruption and shifted food timing in female rodents are necessary.

We conclude that so far experimental evidence confirms the association of circadian disruption with metabolic alterations, hereby some models indicate that the shifted time of food intake may be a determining factor for the loss of internal synchrony because of the differential response of individual organs to internal entraining signals. Thus, the adverse consequences of shift-work on metabolism may be explained by a loss of coordinated rhythmicity among different organs due to shifted food elicited signals, low melatonin and shifted or increased corticosterone levels<sup>(117)</sup>.

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#### Conflicts of Interest

None.

#### Authorship

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