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The circadian clock, shift work and tissue-specific insulin resistance

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Abstract

Obesity and type 2 diabetes (T2D) have become a global health concern. Prevalence of obesity and T2D is significantly higher in shift workers compared to people working regular hours. An accepted hypothesis is that the increased risk for metabolic health problems arises from aberrantly timed eating behaviour, *i.e.* eating out-of-synchrony with the biological clock.

The biological clock is part of the internal circadian timing system, which controls the sleep/wake- and feeding/fasting-cycle, but also many metabolic processes in the body, including timing of our eating behaviour, and processes involved in glucose homeostasis. Rodent studies have shown that eating out of phase with the endogenous clock results in desynchronization between rhythms of the central and peripheral clock systems and between rhythms of different tissue clocks (e.g. liver and muscle clock).

Glucose homeostasis is a complex process that involves multiple organs. In the healthiest situation, functional rhythms of these organs are synchronized. We hypothesize that desynchronization between different metabolically active organs contributes to alterations in glucose homeostasis.

Here we summarize the most recent information on desynchronization between organs due to shift work and shifted food intake patterns and introduce the concept of phenotypic flexibility, a validated test to assess the contribution of each organ to insulin resistance (IR) in humans. We propose this test as a way to provide further insight into the possible desynchronization between tissue clocks. Since different types of IR benefit from different therapeutic approaches, we also describe different chronotherapeutic strategies to promote synchrony within and between metabolically active organs.

Introduction

In healthy subjects, glucose homeostasis is a tightly regulated process, involving the pancreas (producing the glucoregulatory hormones glucagon and insulin), liver (glycogenolysis and gluconeogenesis upon glucagon stimulation, or glycogenesis and glycolysis upon insulin stimulation), and muscle and fat tissue (glucose uptake from the blood via insulin). Lastly, the incretins gastric inhibitory polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) are secreted from the upper gastrointestinal tract in a glucose-dependent matter and augment insulin release from the pancreas (1).

In order to maintain normoglycemia, it is of importance that these different processes act in synchrony. The internal circadian timing system controls the timing of our eating behaviour, as well as the timing of insulin secretion from the pancreatic beta cells, glucose production by the liver, insulin-dependent glucose transporter GLUT 4 expression in skeletal muscle, and GIP and GLP-1 secretion from the gastro-intestinal tract (2, 3). Rodent studies have shown that eating out of phase with the endogenous circadian clock can result in desynchronization between rhythms of the central and peripheral clock systems (4, 5), between rhythms of different tissue clocks (e.g. between liver and muscle clock), and even between rhythms of clock genes and clock-controlled genes within one organ (6, 7).

Due to the rotation of the earth around its axis, most organisms experience a daily change in the exposure to sunlight. Consequently, in a broad range of species, a light-sensitive circadian clock evolved as an autonomous timekeeping system that permits anticipation of and entrainment to the ever-changing environmental light conditions (8, 9). In mammals, the central brain clock is located within the suprachiasmatic nucleus (SCN) in the anterior hypothalamus. The SCN functions as a master clock that aligns behavioural patterns with the solar day and supports vital functions by anticipating and coordinating the required metabolic programmes (8, 10). This anticipation is evolutionarily advantageous, as species have a greater chance of survival when they are active and searching for

food during the period in which the chances for encountering prey and avoiding predators are highest (8, 10). The SCN generates an approximate ("circa") 24 hour ("diem") rhythm by means of a transcriptional-translational feedback loop (9). The core of this circadian oscillator is formed by the transcription factors circadian locomotor output cycles kaput (CLOCK) and brain and muscle ARNT-like 1 (BMAL1), which drive the expression of three *Period (Per 1-3)* and two *Cryptochrome (Cry 1-2)* genes. PER and CRY proteins heterodimerize and subsequently suppress their own transcription by interacting with the CLOCK:BMAL complex. An additional, stabilizing loop is formed by nuclear receptors that either activate or repress the transcription of *Bmal1* (11, 12). This circadian rhythm is entrained to the exact 24-hour rhythm of the environment mainly by light and is transmitted to other brain areas and the periphery via endocrine, autonomic and behavioural signals, hence synchronizing peripheral clocks, most of which cannot receive photic input themselves (13). For many peripheral clocks, the most important external time giver, or "Zeitgeber", in addition to the SCN, is the feeding/fasting cycle (14).

In the most healthy situation, rhythms from the central and peripheral clocks are aligned. Epidemiological studies have shown that shift workers are at higher risk for developing obesity, and obesity-related pathologies, including type 2 diabetes (T2D) (15-17), presumably due to eating out of synchrony with the endogenous biological clock (18). Yet, in the contemporary Western 24/7 society, many more people than only shift workers might be at risk for similar metabolic derangement, since eating moments are distributed over a great part of the day, including food ingested late in the evening (19). Short-term misalignment, for example in case of a long-haul flight, can result in jet lag, fatigue and bowel problems. However, chronic misalignment can be seen as a sustained stressor leading to an ongoing conflict between the endogenous central clock, peripheral clocks and the environment (20). Therefore, it is not surprising that circadian misalignment contributes to a wide variety of medical conditions (21).

It can be hypothesized that asynchrony between different organs plays an important role in the etiology of T2D, because such a circadian misalignment will cause a mismatch of glucose and lipid fluxes between the various organs. Otherwise, disrupted tissue clocks may possibly cause insulin resistance at the tissue level (22). Yet, the exact mechanisms involved in the metabolic derangements resulting from circadian disruption remain to be resolved.

In the current mini-review, we provide a summary of what is known today on organ desynchronization in the context of glucose metabolism, by evaluating studies that focused on shift-work and shifted food intake patterns. We introduce the concept of tissue-specific insulin resistance (IR) that could be a consequence of organ desynchronization. However, in humans it remains difficult to study organ desynchronization by assessing tissue-specific clock gene expression. We therefore propose the concept of phenotypic flexibility as a validated way to assess the contribution of each organ to insulin resistance in humans. Finally, we provide an overview of different interventions that specifically target organ desynchronization or tissue-specific IR. We conclude that in addition to personalized lifestyle interventions, timing of those interventions is important as well and should be considered for the next generation interventions.

Search strategy

A non-structured PubMed search was performed, searching for articles related to shift work and insulin resistance, circadian variation in glucose tolerance, shifts in clock gene expression in different organs, as well as tissue-specific insulin resistance. Known literature was used to search for ‘similar articles’ within PubMed. In addition, Google was used to search for specific topics, in order to find important papers on PubMed.

Clock control of insulin sensitivity

116 It is the interplay of clock-controlled glucose uptake and release processes in different organs,
117 including pancreas, liver, muscle and adipose tissue that ultimately maintains blood glucose
118 homeostasis throughout the day.
119 The central brain clock (SCN) is responsible for the 24h rhythm in plasma glucose concentrations
120 peaking at the start of the active period, independent of feeding conditions (23, 24). Furthermore,
121 glucose transporters and glucagon receptors fluctuate with the circadian cycle, and synthesis of
122 glucose via gluconeogenesis is highly rhythmically controlled (10). The SCN also regulates the daily
123 variation in glucose tolerance, which is highest at the beginning of the activity period, followed by a
124 gradual reduction towards the end of the activity period (25). The higher morning glucose tolerance
125 is partly the result of increased beta-cell responsiveness, accompanied by a tendency to improved
126 insulin action and lower hepatic insulin extraction as compared to later in the day (25). Skeletal muscle
127 has a day-night rhythm in mitochondrial respiratory capacity (26), thereby contributing to the daily
128 rhythm of carbohydrate and lipid oxidation. Besides the crucial role of the central brain clock in
129 regulating glucose homeostasis, (timing of) food intake is also an important determinant for glucose
130 regulation, as feeding at “inappropriate” times of the day causes hyperglycemia and evokes insulin
131 release at a phase opposite to the phase of other physiological rhythms dictated by the SCN master
132 clock, and hence contributes to metabolic imbalance (23, 27, 28).
133 Over the past decades, it has become apparent that all organs involved in glucose homeostasis contain
134 a functional clock (29) and studies with organ-specific clock-gene knockouts show that a functional
135 clock is crucial for maintaining normal glucose homeostasis. SCN-lesioned mice completely lack daily
136 rhythms of plasma glucose (23), and whole-body loss of clock function (e.g. *Clock* or *Bmal1* knockout
137 animals) leads to hyperglycaemia, glucose intolerance and ultimately obesity and metabolic syndrome
138 (reviewed in (10)). Ablation of the liver clock results in fasting-induced hypoglycaemia due to impaired
139 glycogenesis and in reduced hepatic glucose production (29), whereas ablation of the pancreas clock
140 can induce diabetes due to beta-cell failure, indicating opposing consequences of clock dysfunction in
141 liver and pancreas (20). Furthermore, specific disruption of the muscle clock results in diminished

insulin sensitivity in the muscle, causing hyperglycaemia in the non-fasting condition and glucose intolerance (30).

In subjects with T2D, the daily rhythm of glucose tolerance is abolished or even inverted compared to healthy subjects, with an increase in insulin sensitivity towards the evening (31, 32). The peripheral clock may be impaired in (obese) individuals with T2D, as dampened daily rhythms were found in clock gene expression in peripheral leukocytes (33) and subcutaneous adipose tissue (34) compared with non-diabetic controls. However, whether the impaired clock is the cause or the result of impaired glucose regulation remains unanswered.

Problems in glucose homeostasis arising from misalignment/shift work

Temporary circadian misalignment introduced in a controlled laboratory setting, similar to what occurs during jet lag or chronically during shift work, results in decreased glucose tolerance and insulin sensitivity, lower leptin levels, higher mean arterial pressure, lower sleep efficiency and a complete reversion of the cortisol profile. The abnormally high cortisol levels at the beginning of the sleep period have been proposed to contribute to the development of IR and hyperglycemia (37, 38). Circadian misalignment affected postprandial glucose more than fasting glucose levels, suggesting that misalignment impacts fat/muscle metabolism or beta-cell function more than hepatic gluconeogenesis (37). During a short-term misalignment study, Wefers *et al*. found that endogenous glucose production (EGP), measured with a two-step hyperinsulinaemic euglycemic clamp, was not affected. In contrast, circadian misalignment resulted in decreased muscle insulin sensitivity (39), indicating that indeed the process of glucose uptake, rather than glucose production is disturbed during misalignment.

In healthy rotational shift workers, higher post-prandial glucose peaks, accompanied with a lower first-phase insulin response were observed during a simulated night shift than during a simulated day shift,

suggesting reduced beta-cell responsivity during the night shift. This resembles the pattern observed in people with impaired glucose tolerance or diabetes, hence indicating how circadian misalignment during shift work may contribute to the development of T2D. The shift work conditions cause people to consume their food at a time of day when the beta-cell response is reduced due to the normal daily variation (40). Notably, it is of importance to realize that factors such as gastrointestinal absorption, hepatic glucose production suppression, and non-insulin-dependent glucose metabolic pathways will also contribute to the circadian misalignment effects on glucose tolerance (41).

Morris and co-workers showed that the detrimental effects of shift work (*i.e.* being awake and consuming food at night) on glucose tolerance are the result of two different mechanisms: the internal circadian timing system (*i.e.* lower glucose tolerance in the biological evening due to lower beta-cell response) as well as circadian misalignment (lower glucose tolerance when eating during night time than during day time, due to lower insulin sensitivity) (41). It is likely that an internal desynchronization between tissue clocks in liver, muscle and pancreas further contributes to the observed effects of circadian misalignment on glucose metabolism (41).

Desynchronization between organs

In rodents, the rhythms of the SCN and peripheral clocks can be uncoupled by restricting food access to an inappropriate time of day, as peripheral clocks rapidly entrain to the reversed feeding schedule, whereas the central clock does not (5, 42). Moreover, not all peripheral clocks may entrain at the same pace to the shifted food availability (6, 43, 44) and not all organs are synchronized by the SCN in the same manner (45), all of which may contribute to a desynchronization between organs.

Recently, it was shown that desynchronization between the central clock and peripheral clocks also occurs in humans. Shifting mealtime resulted in a shift in the phase of plasma glucose rhythms and caused a delay in the phase of the clock gene PER2 in white adipose tissue, but did not shift rhythms

of melatonin and cortisol (output parameters of the SCN), indicating misalignment between the SCN and peripheral rhythms (46). By applying a constant routine protocol (in which participants are kept in constant conditions and receive no photic or timing information) after either 3 days of simulated night-shifts or day-shifts, Skene *et al.* showed that traditional markers of SCN phase (melatonin, cortisol and expression of the core clock gene PER3) remained relatively stable, whereas 95% of the studied plasma metabolites dissociated from the SCN rhythm and aligned with the shifted behavioural cycles of feeding/fasting and sleep/wake. This disruption in the circadian organization might represent a pathway through which shift work is associated with metabolic disease (47). The observation that these altered rhythms persisted under constant routine conditions in the absence of any externally imposed rhythm suggests an after-effect of night-shift work on metabolism, indicating the long-term negative effects of shift work.

Next to dissociation of peripheral rhythms from the SCN, shifting food intake may cause internal desynchronization between organs, leading to disruption of otherwise coordinated processes. Core clock gene expression in muscle tissue did not shift upon a short-term misalignment protocol, resulting in a misalignment of the skeletal molecular clock relative to the shifted behavioural cycle (39). In addition, shifting mealtime resulted in a shift in the circadian phase of the adipose tissue molecular clock and affected glucose homeostasis and lipid metabolism differentially, thereby dissociating the temporal regulation of these key processes (46). These distinct differences in tissue responses to the timing of food cues suggest discrete routes of entrainment in different organs.

Phenotypic flexibility to study metabolic dynamics

Metabolic flexibility is the ability to respond or adapt to conditional changes in metabolic demand. Recent evidence shows that circadian variation in the molecular metabolic machinery also influences metabolic flexibility (20).

Phenotypic flexibility is the broader concept of metabolic flexibility and can be defined as the metabolic adaptation to a disturbance of homeostasis. The magnitude of the amplitude and duration of the homeostatic disturbance of these "adaptive response systems" determine to what extent a person can adequately respond to a standardised external perturbation and can be used to quantify the individual's health status. Phenotypic flexibility is thus the orchestration of all mechanisms and processes involved in the adaptation capacity to maintain a healthy metabolic phenotype (48).

An important aspect of health is the ability to maintain homeostasis under a large variety of continuously changing environmental conditions, including dietary perturbations. In order to quantify health as a function of the resilience to daily stressors, a standardized mixed meal challenge test was developed named PhenFlex test (PFT) (49), which can be regarded as an extension of the oral glucose tolerance test (OGTT). The Phenflex drink is a 400 ml beverage consisting of high amounts of fat (60 g), glucose (75 g) and protein (20 g), corresponding to an intake of 920 kCal. Subjects drink the PFT in the morning after an overnight fast, following which blood is drawn at t=0, 0.5, 1, 2, 4 and, optionally, 6 and 8 h (49). With the PFT, a total of 132 markers over a maximum of 8-h time course can be quantified, reporting on 26 metabolic processes originating from seven organs (gut, liver, adipose tissue, pancreas, vasculature, muscle, and kidney), as well as determination of a systemic stress response (*i.e.* systemic IR, chronic low grade inflammation, oxidative stress, and metabolic flexibility). In this way, the PFT can reveal the adaptive capacities of a broad set of metabolic processes (50, 51). In patients with T2D, quantification of the PFT response was more sensitive in demonstrating disturbances in metabolic health as compared to overnight fasting measures (50, 51). Furthermore, the PFT test showed additional and new markers that were different between individuals with T2D and healthy controls, revealing new processes underlying metabolic health (51).

Phenotypic flexibility and tissue-specific insulin resistance (IR)

T2D refers to a metabolic glucose dysregulation resulting from defective insulin action (also referred to as insulin resistance (IR)), insulin secretion, or both. IR is the most powerful predictor of future development of T2D. The primary pathophysiological defects in T2D are IR of the liver, muscle and/or adipose tissue, and a failing pancreatic beta-cell function resulting in reduced and/or impaired insulin secretion (reviewed in (52)). Although skeletal muscle and liver are the main insulin-sensitive target tissues (53), the development of IR can originate from many more tissues, including adipose tissue or brain (54, 55). The pathophysiology of T2D also differs between individuals, because persons differ in terms of their genetics, phenotype, lifestyle and environment. Additionally, the severity of IR may differ among various tissues (56). Recently, it has been shown that T2D subtypes could be identified based on IR of muscle, liver or a combination of those, (57) and that these IR phenotypes have a different etiology (58, 59).

Through determination of phenotypic flexibility using the standardised PFT, the orchestration, as well as disruptions, of glucose metabolism by different organs and processes can be quantified (51), allowing for assessment of the degree of β-cell function (BCF) and IR of the primary involved organs. Establishing the diabetic subtype can be achieved by measuring glucose and insulin plasma levels at baseline and in the 30-minute intervals up to 2h in response to the PFT or OGTT, following which several indices indicative for BCF and IR of different tissues/organs can be calculated. A combination of a high fasting plasma glucose (FPG) level with a high plasma insulin level is indicative of hepatic IR (56), whereas muscle insulin sensitivity can be derived from the decline in glucose levels especially in the second half of the two hour response curve (56). The BCF can be defined as the mathematical product of glucose absorption and insulin secretion during the acute phase of the metabolic challenge test corrected for whole body insulin sensitivity (60-62), and adipose tissue IR is calculated as fasting plasma insulin × fasting plasma non-esterified fatty acids (NEFA) concentrations (63, 64). These indices are all validated against the glucose clamp, the gold standard to determine organ specific IR and very well described in literature (60, 63).

Phenotypic flexibility and the circadian clock

In light of the differential effects of shift work and shifted meal timing on the different metabolic organs (e.g. circadian misalignment affected liver and muscle function differentially (39, 41)), it can be hypothesized that differences in tissue IR are related to changes in the circadian clock.

As desynchronization in clock gene expression is difficult to assess in living humans, determining tissue-specific IR could be a way to get more insight into the possible desynchronization between organs. For this, it is important to quantitate which tissues (muscle, liver, pancreas, and/or adipose tissue) are resistant to insulin (56). Yet, assessing tissue-specific IR can be laborious. For example, IR of adipose tissue is an important feature of obesity-related metabolic disease, but assessment of lipolysis in humans requires labour-intensive and expensive methods (63). Using the PFT or OGTT is a relatively easy and minimally invasive strategy to quantify IR within different organs. Another beneficial aspect of the PFT and OGTT is the aspect of dynamic testing. Circadian clock disruption mainly affects postprandial glucose metabolism and beta-cell responsiveness (37, 65), highlighting the importance of a dynamic test (meal tolerance test) when studying and diagnosing circadian clock involvement in IR and T2D.

Interventions to shift or repair the clock

Depending on the tissue in which IR is most abundant, specific therapeutic approaches may be more or less beneficial. Therefore, it is important to identify different subclasses of T2D, as well as the magnitude of the IR, in order to improve the efficacy of personalized, tissue-specific interventions.

These interventions include increasing physical activity in case of muscle IR, polyunsaturated *vs.* saturated fatty acid balance for adipose IR, energy restriction for hepatic IR, and weight loss and thiazolidinediones (PPAR-γ agonists) for muscle and hepatic IR (67). In terms of dietary interventions, a low-fat diet was beneficial for hepatic IR, whereas individuals with muscle IR benefited more from a

Mediterranean diet (57). Interestingly, caloric restriction was not effective in individuals with T2D with an impaired beta-cell function (66).

As both food intake and exercise can affect clock genes and clock-output genes in various organs, it can be hypothesized that these interventions, when timed correctly, can be used to re-synchronize a disrupted clock. Improving these dysregulated daily rhythms might help ameliorate negative metabolic consequences, for example in shift workers who are at risk of metabolic disease (68). Increased amplitudes of the circadian rhythm might result in increased tolerance to shift work.

Although it can be hypothesized that individuals with muscle IR may respond better to timing of exercise as a lifestyle intervention than those with liver insulin IR, to date there is no information on differential responses to timed lifestyle interventions specifically tailored for T2D subclasses. Thus, this could be another angle of treatment to consider in persons with IR or T2D, especially for persons that regularly experience misalignment, such as night-shift workers.

Eating in line with circadian time

Timing the majority of calorie consumption to coincide with the time that the endocrine system is most responsive, *i.e.* during daytime, seems most beneficial for health. As described previously, in non-diabetic subjects, glucose tolerance is highest in the morning, and decreases throughout the day. This coincides with the finding that carbohydrate oxidation was highest during the biological morning and lowest during the biological evening, whereas for lipid oxidation the pattern was inverted (69). This means that the largest portions of carbohydrates can be best consumed during the morning, as opposed to the evening. In lean men, breakfast consumption resulted in higher diet-induced thermogenesis than dinner consumption, which further encourages an high caloric breakfast over large dinner (70). In line with this, meal timing has been associated with weight loss effectiveness, both during regular weight loss (71) and after bariatric surgery (72).

Interestingly, although the diurnal variation in glucose tolerance is altered in patients with T2D (31, 32), increasing the caloric load of breakfast and reducing that of dinner improved insulin sensitivity

and decreased body weight, glucose excursions and HbA1c among patients with obesity and T2D (3, 73, 74). Furthermore, in individuals with T2D, a high-energy breakfast and low-energy dinner led to overall increased GLP-1 and insulin levels and reduced hyperglycaemia throughout the day compared with a reverse meal schedule, despite a similar total caloric content (3). The other way around, skipping breakfast results in an increased postprandial glycaemic response and is associated with an increased risk of T2D (75, 76), and a meta-analysis showed a trend between BMI and evening energy consumption (77). In late eaters, IR was more prevalent than in early eaters (71). Thus, timing food intake within the day may be a therapeutic strategy to improve glycaemic control in patients with T2D. However, causality, timing and direction of the underlying mechanism, *i.e.* do timed treatments repair desynchronization and/or do timed treatments directly repair IR in the context of desynchronization, is still unclear and merely speculation.

Intermittent fasting (IF)

Intermittent fasting (IF) is a broad term including a spectrum of eating regimens that intentionally prohibit energy consumption for extended periods. This includes time-restricted eating in which energy intake is restricted to a short period of the day, as well as full fasting during several days a week, alternate-day modified fasting, the 5:2 diet, fasting-mimicking diet, etc. (78). IF has been shown to improve insulin sensitivity, reduce glucose and/or insulin levels, lower blood pressure, improve plasma lipid profiles and reduce markers of inflammation and oxidative stress, but contradictory results have also been shown, and the beneficial effects of IF could often not be seen independently from weight loss (reviewed in (78)).

Only a few randomized clinical trials have been published on IF in patients with T2D. Although IF reduced body weight and improved HbA1c, fasting glucose levels, quality of life, and blood pressure, it did not affect HOMA-IR, but most importantly, the results were not different from the calorie-restricted group (79). Thus, it remains the question whether the observed effects of IF are due to the timing of food intake or due to calorie-restriction.

Time-restricted feeding (TRF)

Time-restricted feeding (TRF) without caloric reduction in rodents has shown to prevent obesity and obesity-related diseases in a fasting-duration-dependant manner (80-82), and has been shown to prevent the deleterious effects of shift work (being active and awake during the rest phase) on glucose homeostasis (83). Furthermore, dark-phase TRF normalized clock gene expression in diabetic mice of which the daily rhythm in locomotor was dampened and clock gene rhythms were shifted (84). TRF can help adapt to a changing light-dark schedule, hence providing a promising strategy to expedite adaptation of the circadian system (85) or to prevent shifting during shift-work.

Restricting the time of food intake to 10-12h per day in overweight subjects reduced body weight and improved sleep, due to self-chosen reduced time-frame of caloric intake (86). Even in young, healthy, trained participants, TRF to a period of 8h decreased fat mass, increased fat-free mass and improved several health related biomarkers (87).

Early TRF (eTRF), with an eating period of 6 hrs and completing all meals before 3 PM, as compared to eating periods of 12h in men with prediabetes, improved beta-cell responsiveness, blood pressure, oxidative stress and appetite, independent of weight loss (78). This suggests the importance for timing of food intake, irrespective of caloric intake. eTRF did not improve glucose levels, but it reduced mean and peak insulin values, improved insulin sensitivity and beta-cell responsiveness (78).

Although it would intuitively be healthier to shift food intake with the shifted (work) activity (in order for them to be aligned) during night work, this appears not the case. Eating during a simulated night shift, as opposed to not eating during the night shift, in healthy males led to higher glucose AUC, indicating that withholding food intake at night may limit impairment in glucose tolerance (65). This suggests that rhythms in different organs shift with different paces, which has been shown in rodents (43, 88), with indeed the muscle clock adapting much slower to the new feeding situation than the

372 liver clock. Timing the moment of food intake could be especially beneficial for individuals with liver
373 IR.
374
375 *Physical activity*
376 In addition to eating, exercise is another well-known non-photic phase-shifting cue (89). As exercise is
377 known to re-set clock genes in skeletal muscle and other tissues, it could be hypothesized that
378 appropriately, and recurrently, timed exercise can help to re-set the daily clock and improve
379 pathologically deteriorated circadian rhythms. In rodents, exercise can advance the phase of the
380 circadian rhythm in peripheral clocks such as liver and gastrocnemius muscle (88), and exercise-
381 induced re-entrainment depends on the timing of exercise and differs between peripheral tissues (90).
382
383 Several studies have demonstrated that exercise modifies the rhythm of the clock machinery in
384 skeletal muscle, however, the optimal timing of exercise for health and the potential of exercise
385 training to ameliorate the effects of disrupted circadian rhythms have not been fully elucidated yet
386 (reviewed in (68)). Using a constant routine protocol, differential effects of high-intensity exercise on
387 the melatonin rhythm were observed, with a phase advancing effect of acute exposure to evening
388 exercise on the human circadian system (91, 92). Exercise in the morning and afternoon, *i.e*. at a time
389 when higher levels of physical activity often occur in real life, appeared to have no consistent effect
390 on circadian phase (91). Exercise in very dim light resulted in a phase delay of the human circadian
391 pacemaker, showing a non-photic pathway that facilitates phase delays of the human endogenous
392 circadian pacemaker (93). Exercise has been shown to facilitate adaptation to nightshift-work in a field
393 study that exposed subjects to a 9h phase delay with daytime sleep (93).
394 Another important factor to take into consideration when evaluating the phase-shifting effects of
395 exercise is chronotype, *i.e.* whether someone is a morning-type ('early lark') or evening-type ('night
396 owl'). In healthy, young sedentary adults, morning exercise induced greater phase shifts than evening
397 exercise. Late chronotypes experienced phase advances following morning or evening exercise, while

early chronotypes had phase advances from morning exercise, but phase delays from evening exercise (94). This is an important finding, as it demonstrates that it is possible to shift someone's circadian phase by timed exercise, which may be a way to alleviate circadian misalignment. As evening chronotype is associated with higher obesity, timed exercise may be a strategy to adopt an earlier chronotype, hence diminishing the deleterious effects on metabolism of the late chronotype.

Timed exercise as a way to shift the circadian timing system and/or improve blood glucose levels in individuals with T2D has not been examined often yet. In a short randomized controlled trial it was shown that afternoon high-intensity interval training (HIIT) was more efficacious than morning HIIT at improving blood glucose in men with T2D. Strikingly, morning HIIT had an acute, deleterious effect, increasing blood glucose (95). This is an area that should be investigated more in the coming years, as it may be a relatively easy way to improve glucose control.

Conclusions and further research

In this review, we reported that the circadian timing system is important for the daily variations in all aspects of metabolism. Living out of sync with the normal daily rhythm can lead to metabolic alterations, possibly due to desynchronization between peripheral rhythms and the central clock, as well as desynchronization between different organs, leading to a mismatch in the otherwise well-coordinated system of, for instance, glucose homeostasis.

Studies in rodents and humans have shown metabolic problems as a result of desynchronization (e.g. obesity and T2D in shift workers), as well as alterations in peripheral clock gene rhythms in metabolic tissues. However, to date there is little literature on desynchronization between different organs, especially in humans. We hypothesized that misalignment between organs involved in glucose homeostasis could lead to tissue-dependent insulin resistance, e.g. muscle IR rather than hepatic IR. Since desynchronization in clock gene expression between organs is very difficult to assess in living humans, we proposed the Phenflex test as a way to assess desynchronization between organs. Further studies are needed to assess how tissue-specific IR is related to clock gene alterations in those tissues.

It will also be interesting to assess the effect of shift work or misalignment on tissue-specific IR. Determining the tissue that contributes most to the IR could help adjusting a personalized lifestyle intervention (e.g. focus on physical activity vs. focus on caloric intake). In addition to personalized lifestyle interventions, timing of those interventions is important as well and should be considered for the next generation interventions.

All in all, we have come a long way, but there also still lies a long way ahead in optimizing lifestyle intervention for shift workers, with or without T2D.

Data Availability

Data sharing is not applicable to this article as no datasets were generated or analysed for this review.

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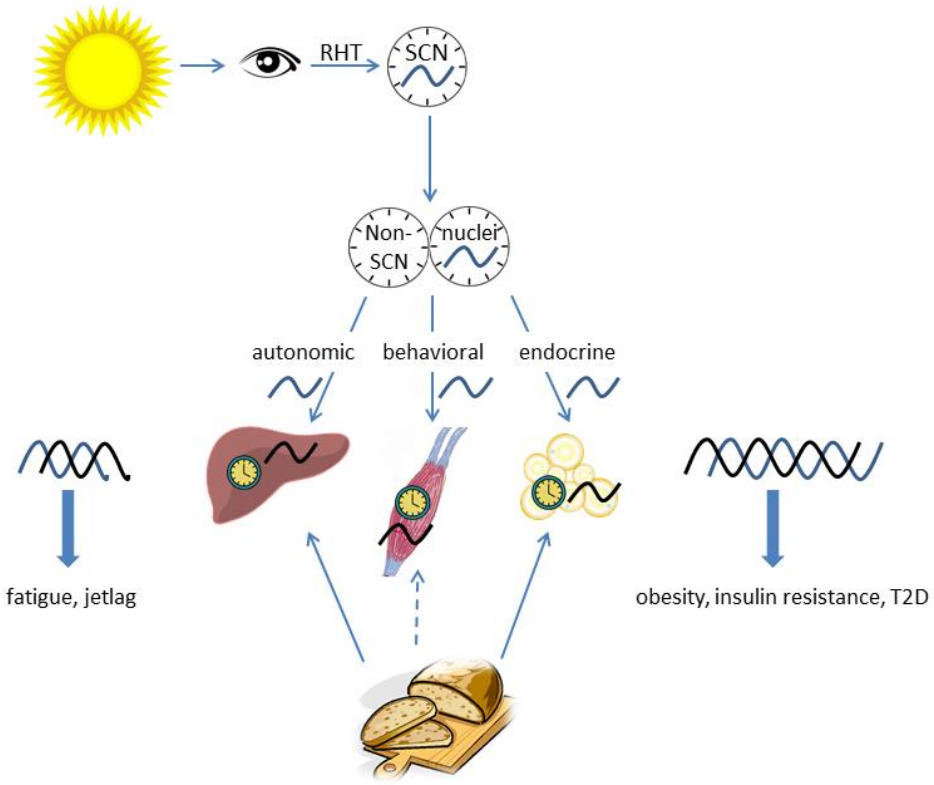
677 **Figure**

678



679

680 **Figure 1: The circadian timing system.** The SCN generates an approximate 24 h (*i.e.* circadian) rhythm,
681 which is set to exact 24 h by the light/dark cycle. Light information is transmitted from the retina to
682 the SCN through the retinohypothalamic tract (RHT). Subsequently, the SCN transmits its rhythmic
683 information to other hypothalamic nuclei and peripheral organs, which also contain endogenous
684 clocks. These clocks are synchronized not only by the SCN, but also by external signals, of which the
685 feeding/fasting cycle is the most important. Conflicting signals between SCN-driven and feeding-
686 driven rhythms may result in desynchronized rhythms, leading to jetlag, and when regularly present,
687 contribute to the development of obesity and T2D. Reproduced from Oosterman et al., *Am J Physiol*
688 *Regul Integr Comp Physiol,* 2015, 308(5):R337-50 with permission from APS Journals.