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REVIEW ARTICLE

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The importance of 24-h metabolism in obesity-related metabolic disorders: opportunities for timed interventions

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Abstract

Various metabolic processes in the body oscillate throughout the natural day, driven by a biological clock. Circadian rhythms are also influenced by time cues from the environment (light exposure) and behaviour (eating and exercise). Recent evidence from diurnal- and circadian-rhythm studies indicates rhythmicity in various circulating metabolites, insulin secretion and -sensitivity and energy expenditure in metabolically healthy adults. These rhythms have been shown to be disturbed in adults with obesity-related metabolic disturbances. Moreover, eating and being (in)active at a time that the body is not prepared for it, as in night-shift work, is related to poor metabolic outcomes. These findings indicate the relevance of 24-h metabolism in obesity-related metabolic alterations and have also led to novel strategies, such as timing of food intake and exercise, to reinforce the circadian rhythm and thereby improving metabolic health. This review aims to deepen the understanding of the influence of the circadian system on metabolic processes and obesity-related metabolic disturbances and to discuss novel time-based strategies that may be helpful in combating metabolic disease.

Introduction

Already 77.000 years ago, the Maya's were able to keep track of time using naturally occurring, predictable events such as the rising and setting of the sun, and adapted their behavioural rhythms accordingly [1]. As a result, the Maya's ate and were active during the day when there was sunlight, whereas they slept during the night. Indeed, the human body has an internal biological clock that is tuned by recurring events such as light exposure, food intake and exercise. Fascinatingly, even without these external cues, clocks in various tissues of the human body appear to generate a rhythm. This internal rhythm takes ~24 h to complete and is referred to as the circadian rhythm ("circa" *lat* about, "diem" *lat* day) [2, 3]. Environmental time cues (or Zeitgebers), i.e., light exposure, entrain the internal clock and synchronise the biological rhythm of numerous processes in the human body to the 24-h geophysical day. Behavioural time cues can also act as Zeitgebers and further modulate internal biological rhythms. The biological clock has been hypothesised to anticipate predictable external cycles that occur during the day in order to generate an optimal homoeostatic response [3, 4].

In current Western society, food intake and physical activity are often no longer attuned to the natural light–dark cycle, since technology makes it possible to eat and be active at any time of the day. As a result, misalignment of the behavioural rhythm with the internal circadian rhythm can occur, i.e., people may eat and be physically (in)active at times when the body is not optimally prepared for it. Indeed, it has been shown that most people spread their food intake over a 15-h time period, which also includes evening hours [5]. Furthermore, approximately one in five people of the European working population works in shifts, including work during the night when the body is prepared for sleep [6]. In addition to shift work, people also experience circadian misalignment when travelling across different time zones (jetlag) or even by staying up late and sleeping in longer during the weekend as compared to weekdays (social jetlag) [7].

Epidemiological studies show that shift work is associated with body-weight gain, impaired glucose tolerance and an increased risk for type 2 diabetes (T2D) [8, 9]. In addition, social jetlag has been associated with impaired

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metabolic health, metabolic syndrome and T2D [10–12]. Moreover, a 12-h shift in day and night rhythm, imposed in an experimental setting, already led to a transient decrease in skeletal muscle insulin sensitivity and an increase in blood pressure and inflammatory markers of otherwise healthy adults [13, 14]. Therefore, it is evident that disturbances in the circadian system are negatively linked to metabolic health.

This review aims to provide insight into the working mechanisms of the biological clock and its role in the pathogenesis of obesity-related metabolic disturbances in humans. Furthermore, opportunities to reinforce the circadian system thereby improving metabolic health, for example by adjusting the timing of lifestyle factors, will be discussed.

The working mechanism of the biological clock

In the human body, circadian rhythms are generated by a master pacemaker (or central clock) that is located in the suprachiasmatic nucleus (SCN) of the posterior hypothalamus and that works in close connection with auxiliary clocks located in peripheral tissues (Fig. 1) [15]. The SCN uses environmental light to synchronise the body's approximate 24-h circadian rhythm with the actual 24-h geophysical day [15]. Light is sensed by a small number of retinal ganglion cells in the eyes that express the photopigment melanopsin. Subsequently, the light signal travels to the SCN via the retino-hypothalamic tract [16]. The SCN entrains the rhythms of the peripheral tissues to the light–dark cycle via the autonomic nervous system, hormonal signals (including melatonin and cortisol), body temperature and by its influence on e.g., food-intake behaviour [15, 17–19]. Next to the central clock in the SCN, peripheral clocks are present in virtually all tissues of the body and, even when disconnected from the SCN, they exhibit their own, internal rhythms [2].

On a molecular level, circadian rhythms are driven by transcriptional–translational feedback loops. The primary feedback loop comprises the transcription factors brain and muscle ARNT-Like1 (BMAL1) and Circadian Locomotor Output Cycles (CLOCK) that form a heterodimeric complex, which drives the transcription of the repressor proteins PER (Period) and CRY (Cryptochrome) by activating the Per and Cry genes [20]. In turn, PER and CRY form an inhibitory complex that, in sufficient concentrations, inhibits the activity of CLOCK-BMAL1. In turn, lower activity of CLOCK-BMAL1 results in a decreased PER and CRY expression. When PER and CRY protein levels are below a certain threshold, they stop inhibiting CLOCK-BMAL1 thus allowing a new cycle of PER and CRY accumulation (Fig. 1) [20]. A secondary feedback loop is formed by the expression of genes encoding REV-ERBα (nuclear receptor subfamily 1 group D) and RORα (RAR-related orphan receptor), which bind to the same element within the Clock and Bmal1 promotor regions and thus drive the rhythmic expression of Bmal1 [21, 22]. In peripheral clocks, signals emanating from the behavioural rhythm, e.g., feeding/fasting, can also modulate the clock machinery by means of post-translational modification [22, 23]. For example, AMP-activated protein kinase, the cellular energy sensor, has been shown to modulate the clock mechanism by phosphorylation of PER and CRY [24, 25].

Taken together, the internal timekeeping system of the body consists of both a central clock and peripheral clocks

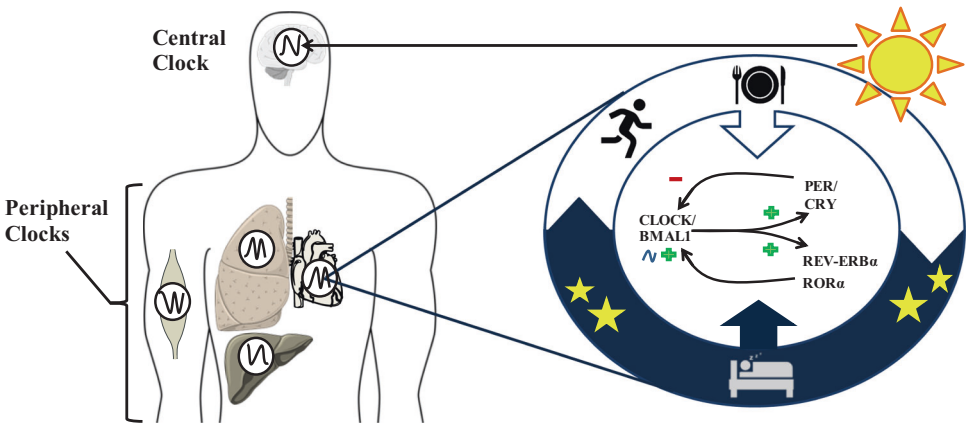

Fig. 1 Organisation of circadian rhythms in the human body. The master pacemaker is located in the SCN of the hypothalamus and its main time cue is light. In turn, intrinsic peripheral clocks in the body are present, which are aligned to the 24-h environment by both the SCN and behavioural rhythms, including sleeping, activity and eating. The molecular driver for circadian rhythms is a transcriptional–translational feedback loop with a positive limp consisting of the BMAL1/CLOCK complex and a negative limp consisting of PER and CRY. An auxiliary feedback loop consisting of REV-ERBα and RORα adds to the robustness of the oscillatory mechanisms by driving the rhythmic BMAL1 expression.

that are molecularly driven by transcriptional–translational feedback loops (Fig. 1). The end result of this molecular machinery is an overt rhythm in physiology and behaviour, which is further modulated by the interplay of the body clocks and environmental and behavioural signals. This timekeeping system enables the body to respond efficiently to predictable disturbances in homoeostatic processes [3, 4].

Out of tune? Circadian rhythmicity in relation to metabolic perturbations

The influence of the circadian system in metabolism is visible through diurnal rhythms that have been found in various important metabolic parameters, including circulating metabolite levels, insulin secretion and -sensitivity as well as energy expenditure [26–31]. Thus, over a 24-h period, glucose levels in both healthy lean and overweight individuals have been reported to be highest in the morning [28, 31]. In healthy lean males, fasting insulin was found lowest in the early morning compared to noon and midnight when assessed at four different time points on separate occasions with fasting time standardised before each measurement [32]. However, other studies did not find a clear difference between morning vs. afternoon levels of fasting insulin in lean and overweight adults [31, 33], not even when fasting time was standardised [31]. The discrepancy in results might be explained by the time points at which fasting insulin levels were measured, since also insulin secretion has been shown to be rhythmic [26]. Thus, using a 68-h hyperglycaemic clamp, healthy lean participants exhibited the highest insulin secretion during the day and the lowest during the night [26]. In addition, meal tests or glucose/insulin tolerance tests performed at different times of day revealed that glucose tolerance of healthy individuals is highest in the morning and lowest in the afternoon and evening, and that this rhythm appears to be at least partially mediated by the rhythms of both insulin sensitivity and β-cell responsivity [27, 29, 33–40]. Interestingly, when assessed in the evening, glucose tolerance in metabolically healthy individuals appeared to be metabolically equivalent to the pre-diabetic state (as measured in the morning) [39, 40].

Fasting levels of free fatty acids have also been reported to show rhythmicity and appeared to be lower in the morning than in the afternoon [33] and evening [27], even when the fasting time before measurements was standardised [27]. Triglycerides, on the other hand, have been shown to rise during the day and fall during the night when lean male participants received three main meals during daytime and measurements were performed throughout the 24-h day [30]. Using lipidomics approaches, diurnal oscillations of lipid metabolites have also been shown in skeletal muscle tissue from healthy nonobese adults [41–43], which also persisted in primary muscle cell cultures [42]. Besides oscillations observed in glucose homoeostasis (also reviewed extensively elsewhere [29, 44]) and various metabolites, studies also observed day–night rhythmicity in energy metabolism. Thus, the energetic cost of food intake, i.e., diet-induced thermogenesis (DIT), of a test meal identical in caloric value and macronutrient composition, was found to be highest in the morning and lowest during the night in lean and overweight individuals when two [45] or three [46] different clock times of meal intake were compared on separate occasions [45, 46]. This response appeared to be irrespective of the fasting duration prior to the meal ingestion since fasting time was standardised [45]. Furthermore, we found that healthy lean men displayed a relatively higher glucose oxidation during the day and switched toward more fat oxidation during the night. In addition, energy expenditure was highest during the late evening and lowest after midnight and this rhythm was partially paralleled by oscillations in oxidative capacity of the mitochondria, the energy producing organelles of the cells, which appeared to peak in the evening and had its trough just after noon [30]. Together, these results show that both fat and glucose metabolism, as well as energy expenditure, are not static processes but rather oscillate over the course of a natural day.

Although these diurnal studies on 24-h metabolic rhythms suggest the presence of circadian control, this cannot be concluded from the abovementioned studies since they do not eliminate the possibility that the findings are the result of (differences in) the environmental and/or the behavioural rhythm. Thus, the modulating effects of light exposure, food intake and physical activity on the circadian system complicates the assessment of the contribution of the internal, independent circadian rhythm (see Fig. 1). To disentangle the behavioural and environmental rhythms from the internal circadian rhythm, specific study designs can be employed including the constant routine protocol, the inverted sleep–wake cycle protocol, the forced desynchrony protocol and the misalignment protocol (see Fig. 2 for more information on these study designs). Thus, using a 26-h constant routine protocol (Fig. 2) in which healthy, lean female participants were seated in dim light and received hourly equicaloric drinks, endogenous circadian rhythms were found for glucose and triglyceride levels (both with higher levels during the night), but not for postprandial free fatty acids [47]. A nocturnal peak in glucose levels, as well as in insulin secretion, was also found when healthy lean males, after a night of sleep, were being kept awake for 28 h and this period of wakefulness was followed by sleep during daytime (the inverted sleep–wake cycle protocol, Fig. 2) [48]. This study, in which participants were subjected to continuous glucose infusion while remaining fasted otherwise, also demonstrated that the sleep

Fig. 2 Various study protocols to examine circadian rhythmicity in humans. The different study protocols are used to reveal the independent circadian rhythm of metabolic processes by filtering out the modifying effects of the behavioural and environmental rhythm.

Constant routine protocol: The participant remains awake and at rest, in a fixed posture and receives isocaloric meals at equal intervals under dim light conditions. The invariability of behavioural and environmental conditions allows to reveal the endogenous circadian rhythm.

Forced desynchrony protocol: Participants undergo behavioural cycles that are not 24 hrs (instead they are e.g. 28 or 20 hrs) and remain in dim light conditions. Since these cycles are outside the range of entrainment, the circadian system will express its internal circadian period. Behavioural rhythms are spread evenly across the circadian day-night cycle and this permits assessment of independent circadian rhythmicity.

Inverted sleep-wake cycle protocol: The participant remains awake for an extended time period and sleep is displaced to the daytime. In addition to the circadian rhythm, this protocol also allows to assess the effects of sleep irrespective of the time of day. Sometimes, participants are instructed to remain in a constant body posture, have minimal physical activity and are continuous supplied with nutrients via e.g. constant glucose infusion. This protocol takes advantage of the slow adaptation of the body to changes in environmental and behavioural rhythms and allows observation of independent circadian rhythms. The protocol is sometimes viewed as a form of forced desynchrony.

Misalignment protocol: The normal daily routine is shifted with a certain amount of hours often reflecting shift work and performed as part of a cross-over study that also entails an intervention arm reflecting the aligned situation. By using this protocol, the environmental and behavioural rhythms are uncoupled from the internal circadian rhythm, which enables the researcher to distinguish the behavioural effects, circadian phase effects and circadian misalignment effects.

Of note, in both the inverted sleep-wake and the constant routine protocol the forced wakefulness builds up sleep pressure which may affect the study variable of interest.

onset results in elevation of levels of plasma glucose and serum insulin and increases insulin secretion rate, irrespective of the time of day when sleep occurs [48]. Furthermore, a 38-h constant routine protocol found the peaks of insulin and glucose of healthy and overweight males to be around the usual time of awakening [49].

In addition, endogenous circadian rhythms have also been found for energy expenditure. Thus, using a forced desynchrony protocol (Fig. 2) in which participants were subjected to a 28-h behavioural rhythm, it was found that resting energy expenditure and substrate oxidation of healthy, lean to obese participants oscillates during the day with resting energy expenditure being highest during the day and lowest during the late night [50]. Fasting carbohydrate oxidation proved to be highest in the biological morning whereas fasted fat oxidation peaked in the biological evening [50]. Finally, a circadian misalignment protocol (Fig. 2) that shifted the day and night rhythm with 12 h showed that DIT of healthy lean and overweight adults was found to be highest in the morning and lowest in the evening in response to identical test meals [51]. This circadian rhythmicity in DIT is in line with previous diurnal rhythm studies, indicating that the rhythm of DIT is not (entirely) driven by changes in behavioural rhythms [45, 46].

Taken together, various metabolic factors appear to exhibit specific day–night rhythmicity of which several are under endogenous circadian control, indicating that the time of day at least partially determines metabolic responses to homoeostatic disturbances. The question then remains if obesity-related metabolic disturbances may be associated

with disturbed circadian rhythmicity or inappropriate alignment of these (internal) circadian rhythms with external timing of behavioural/environmental rhythms.

In this context, day–night rhythmicity has indeed been shown to be disturbed in people with impaired metabolic health. Cross-sectional studies revealed that the nocturnal peak in glucose level was twofold higher in T2D patients in comparison with healthy matched controls during a prolonged 34-h fast, a finding that could be replicated in some [28, 52], but not all [53], studies. In addition, both obese people with a normal fasting glucose as well as individuals with hyperglycaemia appear to lack rhythmicity of glucose tolerance since they do not display the typical higher glucose tolerance in the morning compared to the evening that is found in healthy lean individuals [38, 54]. Furthermore, in obese patients with T2D a lack of rhythmicity in insulin secretion was observed during a 72-h hyperglycaemic clamp [55], although insulin sensitivity showed a similar rhythm as reported for healthy lean adults [56]. In addition, using continuous infusion of labelled glucose, it has been shown that the rates of endogenous glucose production were higher throughout the night in participants with T2D compared to the healthy controls, indicating an altered rhythm in hepatic glucose production in T2D [57, 58]. These findings suggest altered rhythmicity of glucose metabolism in metabolically comprised individuals, which could be indicative of a disturbed circadian system.

Indeed, there is some evidence to suggest that the putative differences in metabolic rhythmicity between people with obesity-related metabolic disturbances and healthy people relate to alterations in the central circadian system, although more research is needed on the nature of the altered rhythmicity of metabolism in metabolic diseases. In rodents, a lesion in the SCN resulted not only in the disappearance of rhythms in glucose uptake and insulin sensitivity, but also resulted in insulin resistance within 8 weeks after the SCN lesion was made, emphasising the role of the central clock in regulating metabolic rhythmicity and insulin sensitivity [59, 60]. Furthermore, mice studies showed that knockout of core clock genes, such as Bmal1, in peripheral metabolic tissues can lead to e.g., hypoglycaemia during fasting [61] or diminished glucose-stimulated insulin secretion [62], which suggests that the peripheral clocks are also important in regulating metabolic processes. In humans, a recent study suggests that the SCN of people with T2D has a decreased number of arginine vasopressin and vasoactive intestinal polypeptide neuropeptides and astroglial cells as compared to healthy controls, which might underlie an altered metabolic circadian rhythmicity in T2D [63]. In addition, the circadian rhythm in cortisol levels, reflecting the direct output of the SCN, appears to be blunted in T2D patients as compared to individuals without T2D [64]. Moreover, it is becoming increasingly evident that misalignment between the endogenous circadian rhythm and environmental and behavioural rhythms, i.e., eating and being active at times that the body is not prepared for it, is likely to hamper metabolic health. In this context, several meta-analyses showed that shift work, i.e., work performed during the nightly hours, is associated with a high BMI and an increased risk for impaired glucose tolerance and T2D [8, 65, 66]. The probability to develop T2D appears to be higher when performing rotating night-shift work as compared to rare-shift work or permanent-shift work, and a higher number of night shifts per month associates with higher probability of developing T2D [9]. A large cohort study further found that the risk for T2D increased with 5% for every 5 years with a least three nights per month of performing rotating shift work [67]. Shift workers diagnosed with T2D also displayed higher Haemoglobin A1c levels, body mass index and daily caloric intake as compared to diabetes patients not involved in shift work [68]. Interestingly, cohort studies have shown that the typical shift in day–night rhythm that occurs during the weekend (social jetlag) already associates with a worsened glucose regulation and lower metabolic health and increases the risk on metabolic syndrome and T2D [11, 12, 69]. More direct evidence for a causal relation between circadian misalignment and metabolic health comes from experimental studies that artificially shifted the normal day and night rhythm of healthy participants under strictly controlled conditions (see Fig. 2). Such circadian misalignment studies revealed that a disturbed circadian rhythm results in a decreased total daily energy expenditure but elevated sleeping metabolic rate, and increased levels of glucose, insulin, ghrelin, free fatty acids, triacylglycerol and inflammatory markers as well as an increased 24-h systolic and diastolic blood pressure [10, 13, 14, 70–73]. Moreover, several independent studies reported that a simulated night shift decreases glucose tolerance and insulin sensitivity of healthy participants [14, 34, 70, 74–76]. Thus, in a cross-over design, healthy lean and overweight participants followed a controlled 8-day laboratory protocol with a circadian alignment and misalignment arm. Both post-prandial glucose and insulin excursions were higher after misalignment compared to alignment condition, both after 1 and after 3 days of misalignment, indicating slow adjustment of glucose tolerance upon circadian misalignment [34]. Follow-up research found that the decreased glucose tolerance was not related to differences in beta-cell responsiveness but could mainly be attributed to a decrease in insulin sensitivity [75]. Another circadian misalignment study suggested that it takes healthy participants at least 2 days to adapt to a simulated night shift. In this study, a meal test was performed under normal conditions (baseline), directly after a simulated night shift and 2 days after the night shift, and various metabolites were measured

in response to the meal. Plasma free fatty acid levels were decreased in response to the night shift as compared to baseline whereas postprandial triglycerides were increased, and these values did not appear to return to baseline levels 2 days after the simulated night shift [77]. Interestingly, decreased glucose tolerance upon experimentally induced circadian misalignment was also observed in individuals performing regular-shift work, suggesting no long-term adaptive responses [74, 78], although it is unclear if the decrease in glucose tolerance is different in magnitude between shift workers and non-shift workers.

One aspect that complicates the interpretation of circadian misalignment studies is the loss of sleep that is associated with circadian misalignment, and sleep loss has also been reported to decrease insulin sensitivity and glucose tolerance in healthy volunteers [79–83]. Interestingly, a parallel intervention study in two groups of healthy lean males undergoing either sleep deprivation (5 h of sleep opportunity) or sleep deprivation plus circadian misalignment, showed significantly lower glucose tolerance in the misalignment group [84]. These findings indicate that the detrimental metabolic effects during circadian misalignment cannot be fully attributed to sleep loss [84].

Hence, eating and being active at a time that the body is not prepared for it impairs metabolic health beyond the effects of experienced sleep loss. Furthermore, the body does not adapt quickly to disturbed rhythms and does not become impervious to frequent challenges of changes in circadian rhythmicity, as is the case with shift workers.

The potential of timed lifestyle interventions

Since several key metabolic processes have proven to be rhythmic, and disturbances in the timing of behavioural/environmental rhythms hamper metabolic health, it can be hypothesised that properly aligning the external behavioural and environmental rhythms with the circadian system is able to improve metabolic health. In this context, timing of lifestyle factors such as food (intake) and exercise have been given considerable attention over the past years.

Recent studies showed that both in the United States and in Europe energy consumption is distributed over the largest part of the day [5, 85]. This means that these populations eat at time points that the body is not optimally prepared for it and also results in a lack of a true nocturnal fast. This in itself can be viewed as circadian misalignment [5, 85]. Furthermore, since food intake is an important Zeitgeber, eating at the wrong time of day may desynchronise peripheral rhythms involved in metabolism from the central rhythm generated by the SCN, thereby leading to aberrant metabolic rhythmicity [3]. Time-restricted eating (TRE), i.e., consumption of the habitual diet within a limited time window (usually ≤12 h) without (deliberately) attempting to change the nutritional or caloric content of the diet, has been proposed to improve metabolic health by restoring fasting-feeding cycles allowing the body to utilise nutrient stores [86]. Indeed, mice fed an *ad libitum* high-fat diet, confined to a limited period during the active phase (night time for mice), displayed an improved circadian rhythm in macronutrient utilisation [87] as well as an improved glucose homoeostasis as compared to non-time-restricted fed mice [87, 88]. In addition, time-restricted feeding also protected mice against high-fat diet-induced weight gain, despite caloric intake being similar between both groups [87, 88].

In humans, restricting food intake of healthy overweight and obese adults to a self-selected time frame of 10–12 h per day for 16 weeks induced 3.8% weight loss, without any dietary recommendations [5]. Later studies also reported that TRE was able to induce weight loss in overweight and obese adults [89–92]. In overweight/obese males at risk for T2D, a 5-week fully supervised 6-h TRE (8 a.m. to 2 p.m.) regime improved insulin sensitivity, β-cell responsiveness, blood pressure, oxidative stress and appetite as compared to the control group who ate in a 12-h eating time frame, despite similar caloric intake and meal frequency [93]. Consistently, a 9-h TRE improved glucose tolerance and reduced fasting triglycerides in obese males at risk for T2D [94]. In this study, it was also shown that the time period during which the 9 h of eating was planned (early vs. later in the day) had no effect on the beneficial outcome of TRE [94]. In addition, it has been found that a 6-h TRE for 4 days increased fat oxidation in overweight and obese adults as compared to energy consumption spread out over 12 h, even though total energy intake was similar between the two arms [95]. A more recent randomised crossover study in overweight and obese men reported that a 5-day 8-h TRE regime reduced nocturnal glucose levels compared to spreading the intake of the same amount of calories over a 14-h time frame [96]. The main outcomes of recent human TRE studies, performed in overweight and obese participants, are summarised in Table 1 and collectively show that restricting food intake to a limited time frame improves metabolic health in both rodent and humans.

Besides limiting the time frame of caloric intake, TRE regimes often distribute food intake to the earlier part of the day, which in itself may confer metabolic benefits. Thus, several studies report a greater weight loss efficacy of hypocaloric diets [97, 98], hypocaloric diets in combination with moderate exercise [99, 100] and bariatric surgery [101] in overweight and obese participants when the majority of calories are consumed earlier in the day, typically before 3 a.m. [97, 99, 101], even when total energy intake is similar between early and late eaters [98–102]. Please note that these are not TRE studies, as not all energy intake was consumed before 3 a.m. Conversely, consuming more

Table 1 Time-restricted eating studies performed in humans.

Study	Population	Eating hours	TRE time frame	Control arm	Period	EI	Weight loss	Outcome
Chow et al. [89]	Overweight and obese adults	8 h	Self-selected	Unlimited eating time	12 weeks	↓	Yes	Body weight ↓ Lean mass ↓
Gabel et al. [90]	Obese adults	8 h	10 a.m. to 6 p.m.	Habitual eating times	12 weeks	↓	Yes	Body weight ↓ Blood pressure ↓
Gill and Panda [5]	Overweight and obese adults	10–12 h	Self-selected	No	16 weeks	↓	Yes	Body weight ↓ Sleep satisfaction ↑
Hutchison et al. [94]	Men at risk for T2D	9 h	8 a.m. to 5 p.m. vs. 12 p.m. to 9 p.m.	No	7 days	=	Yes, both arms	Glucose tolerance ↑ Fasting triglycerides ↓ Similar in both TRE interventions
Jamshed et al. [92]	Overweight and obese adults	6 h	8 a.m. to 2 p.m.	Eating from 8AM-8PM	4 days	=	Yes	24-h glucose ↓
Parr et al. [96]	Overweight and obese males	7 h	10 a.m. to 5 p.m. vs. 7 a.m. to 9 p.m.	Eating from 7AM-9PM	5 days	=	?	Nocturnal glucose ↓
Ravussin et al. [95]	Overweight and obese adults	6 h	8 a.m. to 2 p.m.	Eating from 8AM-8PM	4 days	=	Yes	Energy expenditure = Fat oxidation ↑ Hunger ↓
Sutton et al. [93]	Men at risk for T2D	6 h	8 a.m. to 2 p.m.	Eating from 8AM-8PM	5 weeks	=	No	Insulin sensitivity ↑ Blood pressure ↓ Oxidative stress ↓
Wilkinson et al. [91]	Adults at risk for T2D	10 h	Self-selected	No	12 weeks	↓	Yes	Body weight ↓ Blood pressure ↓ Atherogenic lipid ↓

↓ denotes a decrease, = denotes no change, ↑ denotes an increase, and ? denotes that there was no information provided on the variable.

calories in the evening [103] or eating closer to the biological night [104] has been associated with higher adiposity compared to eating early during the day [103, 104]. In an experimental setting, a study in young lean men delayed meal times with 5-h without changing meal content and found that circadian rhythms (measured using the constant routine protocol, see Fig. 2) in plasma glucose were delayed by the late meals without changes in rhythmicity of circulating insulin and triglycerides levels [105]. In addition, healthy lean females eating lunch (47% of total energy intake) at 4.30 p.m. vs. 1 p.m. for a week displayed a decreased glucose tolerance, resting energy expenditure and fasting carbohydrate oxidation [106]. Several randomised controlled trials also suggest that skipping breakfast, thereby consuming the majority of calories later during the day, worsens metabolic health in healthy adults [107, 108], although not all studies agree [109, 110]. A randomised crossover study in healthy lean and overweight adults compared the effect of omitting breakfast and eating a late-night snack (at 10 p.m.) with eating breakfast (at 8 a.m.) and omitting the snack [111]. The study found that the typical nocturnal switch to fat oxidation that was found in the breakfast arm was absent in the late snack arm, despite similar meals, fasting times and total energy expenditure [111]. Moreover, when participants with T2D consumed a high caloric breakfast for 7 days an overall lower postprandial hyperglycaemia was found compared to consuming a high caloric dinner, even though total daily energy was equal [97]. Hence, although there is still some controversy on the most optimal meal timing, accumulating evidence suggests that consumption of the majority of calories earlier in the day leads to metabolic benefits. This has also lead to a recommendation issued by the American Heart Association [112], which was based on a meta-analysis of the effects of timing of food intake on metabolic health.

Next to manipulating food intake, exercise is traditionally viewed as another powerful lifestyle intervention, since it increases metabolic flexibility and skeletal muscle and whole-body insulin sensitivity [113]. Importantly, exercise acts as a Zeitgeber for skeletal muscle [114, 115] and it is tempting to suggest that timing of exercise could help to reset disturbances due to circadian misalignment. The importance of the skeletal muscle molecular clock in metabolism is illustrated by the finding that knockout of the Bmal gene in skeletal muscle of mice not only resulted in a disturbed skeletal muscle glucose homoeostasis but also disrupted whole-body glucose homoeostasis [116]. In humans, both skeletal muscle cells and tissue from lean and overweight donors have been shown to exhibit rhythms in

genes involved in glucose homoeostasis and insulin resistance [117]. In mice, 4 weeks of involuntary exercise during the inactive phase (daytime) resulted in an altered rhythm of the skeletal muscle molecular clock [114]. Moreover, in humans, an acute bout of exercise already modulated the rhythmicity of the expression of molecular circadian clock components in skeletal muscle tissue [115]. To further examine the role of regular exercise training on the molecular clock, synchronised skeletal muscle cells obtained from people with a sedentary lifestyle and from athletes have been obtained, but these cells do not show any difference in rhythmicity of molecular clock components [118]. However, it was shown that only skeletal muscle cells taken from athletes exhibited rhythmicity in nicotinamide phosphoribosyl transferase and SIRT1 (sirtuin 1), factors involved in regulating cellular energy status, suggesting that regular exercise training may improve some rhythmicity of skeletal muscle energy metabolism [118]. However, it should be noted that these studies have been done in synchronised primary myotubes and that culture conditions may affect the outcome.

Next to effects on skeletal muscle, exercise has also been suggested to have effects on whole-body circadian rhythmicity. Thus, an in vivo study in healthy young males showed that exercise affects the oscillations normally seen in melatonin levels, generated by the central clock [119, 120], whereas nightly bouts of cycle ergometry exercise resulted in a phase delay of the melatonin rhythm [119]. Interestingly, regularly breaking the sedentary time with light-intensity walks during daytime has been shown to improve the nocturnal glucose levels of overweight and obese T2D patients as compared to prolonged sitting [121]. Based on these promising results, it can be anticipated that exercising at a particular time of day could be more or less effective with respect to the physiological and metabolic outcomes of exercise training. Indeed, mice exercising on a treadmill at the beginning of the active phase show a higher skeletal muscle carbohydrate and ketone body utilisation than mice exercising at the beginning of the rest phase, indicating that timing of exercise differentially affects metabolism [122]. To date, only a few human studies have been performed that specifically looked into the effects of exercise timing on metabolic outcomes. In that context, timing of exercise can be interpreted as the clock time of day when exercise is performed, but also the time of exercise relative to the time of a meal. Thus, a study in obese insulin resistant adults showed that three short (6×1 min) intense exercise bouts (walking incline) per day, immediately before breakfast, lunch and dinner, reduced postprandial and 24 h glucose levels more effectively when compared to a single 30-min bout of moderate continuous exercise performed before dinner [123]. Furthermore, a low intensity walk after a meal has been shown to decrease postprandial glucose excursions in healthy participants and participants with type 1 diabetes as compared to meals followed by inactivity [124]. It should be noted that—despite similar meal times and content—differences in workload exist in the abovementioned studies. These differences in exercise intensity are likely to contribute to the different metabolic benefits observed, which hampers conclusions on the true effect of the timing of the exercise.

The effect of exercising at different clock times of day has been investigated in only a few human studies so far. It has been found that male cyclists have increased levels of insulin and cortisol in response to a morning cycling test as compared to cycling in the evening, and these changed hormone levels may also affect substrate metabolism [125]. In young lean participants, an acute 1-h submaximal aerobic exercise test performed in the evening decreased glucose levels and led to a relatively higher glucose oxidation when compared to a similar exercise bout performed in the morning [126]. Similar findings were reported in overweight men with T2D who performed high-intensity interval training (HIIT) either in the morning or in the afternoon, with more effective reductions in 24-h blood glucose levels when exercise was done in the afternoon compared to morning HIIT [127]. Taken together, rodent and human studies have convincingly showed rhythmicity in skeletal muscle metabolism and modifying these rhythms to improve metabolic health by means of (timed) exercise may be a promising future approach.

Conclusions

The human body possesses a circadian system that anticipates predictable disturbances in homoeostasis. Both diurnal- and circadian-rhythm studies show that also metabolic processes of healthy, lean adults fluctuate during the day. People with obesity-related metabolic disturbances, however, have shown an altered rhythm in glucose homoeostasis as well as disturbances in (output of) the SCN, indicating a hampered circadian system. Moreover, experimentally induced circadian misalignment by a rapid switch of the day and night deteriorates metabolic health of otherwise healthy volunteers. Combined, these findings indicate that metabolic disturbances are characterised and possibly caused by a disturbed circadian rhythm. The intimate relationship between the circadian system and metabolism also opens up new opportunities to improve metabolic health by means of reinforcement of the circadian rhythm. Both food intake and exercise have been shown to be important Zeitgebers for peripheral clocks in metabolic tissues, indicating that they can modulate the circadian rhythms in these tissues. Therefore, eating and exercising at a time that the body is prepared for it does not only lead to

the appropriate homoeostatic response, but also reinforces the circadian rhythm. Interventions aimed at improving metabolic health by adjusting the timing of lifestyle factors have shown promising results, although the amount of human studies addressing this topic is still limited. Therefore, human studies investigating the effects of timed lifestyle interventions on metabolism, including the underlying mechanisms, are highly encouraged.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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