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Review article

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Circadian regulation of liver function: from molecular mechanisms to disease pathophysiology

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Abstract

A wide variety of liver functions are regulated daily by the liver circadian clock and via systemic circadian control by other organs and cells within the gastrointestinal tract as well as the microbiome and immune cells. Disruption of the circadian system, as occurs during jetlag, shift work or an unhealthy lifestyle, is implicated in several liver-related pathologies, ranging from metabolic diseases such as obesity, type 2 diabetes mellitus and nonalcoholic fatty liver disease to liver malignancies such as hepatocellular carcinoma. In this Review, we cover the molecular, cellular and organismal aspects of various liver pathologies from a circadian viewpoint, and in particular how circadian dysregulation has a role in the development and progression of these diseases. Finally, we discuss therapeutic and lifestyle interventions that carry health benefits through support of a functional circadian clock that acts in synchrony with the environment.

Sections

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Key points

- Liver physiology and metabolism exhibit daily rhythmicity under the governance of the circadian clock.
- Circadian clocks are implicated in metabolic diseases, such as obesity and diabetes, through hepatic and hepatic-associated mechanisms.
- Clock components have a role in the pathophysiology of a wide variety of liver pathologies, from nonalcoholic fatty liver disease to hepatocellular carcinoma.
- The microbiome and the immune system are emerging clock-controlled regulators of metabolic health.
- Circadian-based medicine in the form of time-of-day nutritional and pharmacological interventions carries health benefits.

Introduction

Most organisms on Earth need to cope with rhythmic environmental changes due to our planet's rotation around its axis. Light-sensitive organisms have evolved a mechanism that enables them to adjust to these rhythmic changes in a proactive manner through an internal clock. This clock measures the time span of one complete light–dark cycle, which is approximately 24 h, and is therefore called the circadian clock (*circa diem* in Latin means 'around one day'). The circadian clock regulates a wide variety of processes at multiple levels, from cells to organs to whole organisms, and synchronizes behavioural (for example, sleep–wake cycle, fasting–feeding), physiological (for example, hormonal secretion) and molecular functions with geophysical time to maintain a 24-h rhythmicity[1–3].

The mammalian circadian system is hierarchically structured. A central clock is situated in the suprachiasmatic nucleus (SCN) of the brain. This central pacemaker is predominantly entrained by light signals perceived through the retina and transmitted by the retinohypothalamic tract. The SCN synchronizes subsidiary oscillators in peripheral tissues through a wide variety of time signals (or zeitgeber – 'time giver' in German) that can be neuronal, hormonal and metabolic. Importantly, circadian clocks are present in most if not all cells of the body and were shown to function in a self-sustained and cell-autonomous manner in cultured mammalian cells ex vivo[1–3].

Misalignment between the internal clock and the environment, as occurs as a result of shift work or frequent jetlag, is associated with various conditions, ranging from obesity and metabolic syndrome to malignancy and neurological disorders. Furthermore, internal misalignment between the central clock and peripheral clocks as well as between clocks in different peripheral tissues has emerged as a factor contributing to various pathologies. Hence, a circadian clock that functions in synchrony with the environment and throughout the body is considered to confer health benefits[4,5].

Mounting evidence supports the notion that there is an interplay between nutrition, metabolism and the circadian clock. On the one hand, various metabolic pathways and their intermediates exhibit daily oscillations and, in turn, feed back to the clock to modify its phase and amplitude[6]. For example, nicotinamide adenine dinucleotide (NAD) levels are controlled by the circadian clock and modulate clock function in mammalian cell lines and mouse models[7–10]. In addition, meal timing and diets, such as time-restricted eating (TRE), have been shown both in animal models and clinical trials to confer metabolic and cardiovascular health benefits[11–13]. This intimate interaction between nutrition, metabolism and the circadian clock is implicated in the function of the gastrointestinal tract in general and in the liver specifically, both in physiological and pathological conditions[14–16].

In this Review, we survey the literature on the circadian nature of the gastrointestinal tract, with an emphasis on molecular mechanisms in the liver that are involved in various liver-related pathologies such as nonalcoholic fatty liver disease (NAFLD), type 2 diabetes mellitus (T2DM) and hepatocellular carcinoma (HCC).

Molecular clockwork

Most if not all of the cells in our body harbour a molecular clock, which oscillates in a self-sustained and cell-autonomous manner. The molecular clockwork consists of genes and proteins, known as core clock components, that generate 24-h rhythms in expression of clock genes as well as many output genes (that is, clock-controlled genes) through transcription–translation feedback loops (Fig. 1). The expression of *PER1* and *PER2* as well as of *CRY1* and *CRY2* (encoding Period (PER) and Cryptochrome (CRY) proteins, respectively) is driven by the transcriptional activators clock circadian regulator (CLOCK) (or neuronal PAS domain-containing protein 2 (NPAS2)), which heterodimerizes with basic helix–loop–helix ARNT-like 1 (BMAL1). As PER and CRY proteins accumulate and translocate to the nucleus, they repress transcription at their own gene loci[17]. Degradation of PER and CRY is mediated by F-box proteins such as F-box/WD repeat-containing protein 1A (BTRC; also known as βTrCP), F-box and WD repeat-containing protein 11 (FBXW11; also known as βTrCP2) and F-box and LRR-repeat proteins 3 and 21 (FBXL3 and FBXL21), respectively. Together, these proteins control the pace of the clock[18]. An auxiliary feedback loop consists of the nuclear receptor subfamily 1 group D members 1 and 2 (NR1D1 and NR1D2; also known as REV-ERBα and REV-ERBβ) and the RAR-related orphan receptor proteins (RORA, RORB and RORC; also known as RORα, RORβ and RORγ)[19] (Fig. 1). Notably, these so-called core clock components are not exclusively implicated in clock function but are also involved in a wide variety of other cellular processes unrelated to the clock oscillator such as the cell cycle[20] or autophagy[21].

Circadian zeitgeber and communication between body clocks

Cell-autonomous pacemakers are present not only in the SCN but also in cells in peripheral organs, including in the gastrointestinal tract, for example, the liver, colon and pancreas. All of these clocks oscillate in unison, suggesting the presence of mechanisms that maintain phase coherence between them. As previously mentioned, this coherence is achieved through various routes of communication such as neuronal, endocrine and metabolic signalling. Several studies suggest that, although clocks in peripheral tissues share a similar molecular makeup, they respond to zeitgeber signals in a time-specific and tissue-specific manner[22–24]. Thus, it is conceivable that input pathways to the clock are tissue specific and relate to the function of the tissue.

The predominant zeitgeber for the central clock in the brain is light, whereas clocks in peripheral tissues are dominated by different signals whose identity has been examined in a number of studies. The best-documented example is the liver, the core clock gene expression and rhythmic transcriptome levels of which have been widely characterized. Experiments in rodents in which food availability was limited to the light

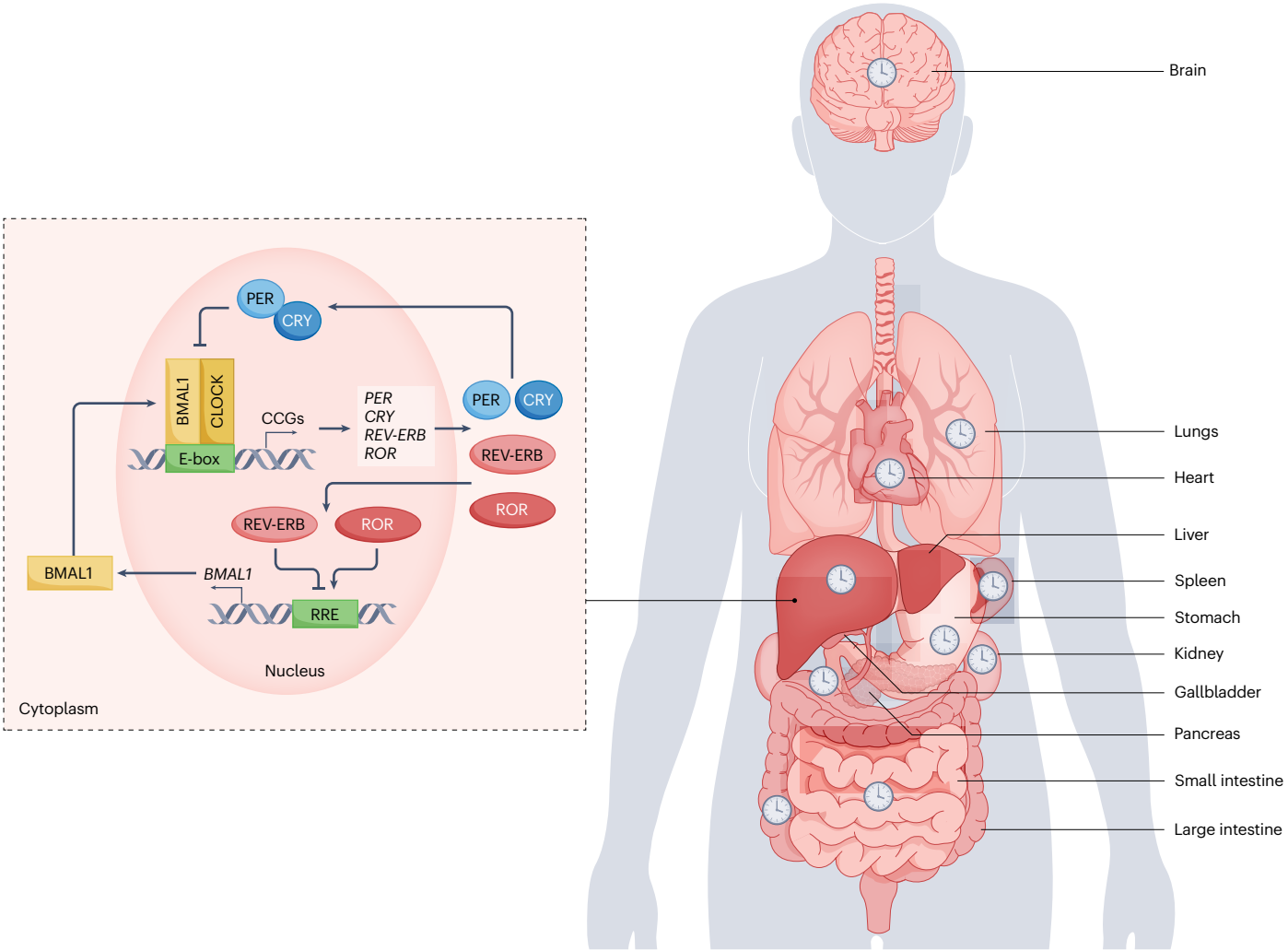

Fig. 1 | The circadian clock system. The mammalian circadian clock system consists of a central pacemaker in the suprachiasmatic nucleus of the brain that coordinates all other clocks present in peripheral organs[1]. The molecular clock machinery relies on a transcription-translational feedback loop in which a heterodimeric complex of basic helix–loop–helix ARNT-like 1 (BMAL1) and clock circadian regulator (CLOCK) activates the expression of repressors known as period (*Per1* and *Per2*) and cryptochrome (*Cry1* and *Cry2*) genes through binding at E-box sequences in their promoters. The period (PER) and cryptochrome (CRY) proteins enter the nucleus, and their complexes inhibit BMAL1–CLOCK activity, thereby inhibiting their own expression. An additional feedback loop consists of the nuclear receptor subfamily 1 group D members 1 and 2 (NR1D1 and NR1D2, also known as REV-ERBα and REV-ERBβ) and RAR-related orphan receptor proteins (RORA, RORB and RORC). These proteins compete for binding at ROR–REV-ERB-response elements (RRE) on the *Bmal1* promoter and support (ROR) or repress (REV-ERB) *Bmal1* transcription[17]. CCGs, clock-controlled genes.

phase (daytime feeding, when they normally sleep and do not ingest food) revealed that the liver clock is phase inverted compared with the SCN clock, that is, the peak times of most physiological and molecular rhythms differ by 12 h between ad libitum and daytime feeding. Moreover, the expression phases of most liver genes were shifted by 12 h upon daytime feeding, suggesting that both the liver clock and the rhythmic part of the liver transcriptome are directed by feeding-related signals[24,25]. Notably, feeding rhythms are sufficient to drive rhythmicity in liver gene expression in circadian clock-deficient mice (*Cry1*$^{-/-}$;*Cry2*$^{-/-}$)[26], further highlighting the prominent role of feeding-derived signals on liver rhythmicity. Of note, feeding has the most prominent effect on clock rhythmicity in the liver. In other peripheral tissues (for example, kidney and heart), the effect is much less pronounced, and the effects are very minor in the lung[24,27]. Hence, although peripheral clocks rely on a similar molecular makeup, their responses to various zeitgebers differ greatly. It will be interesting to examine in future studies whether the circadian rhythmicity of other organs within the gastrointestinal tract, besides the liver, is also dominated by feeding-related signals[28] or substantially responds to other zeitgebers in a tissue-specific manner. Furthermore, studies that addressed the autonomous role of the liver clock using clock-mutant mouse models in which the clock is restored exclusively in hepatocytes suggest that hepatocytes can maintain synchrony in the absence of clocks in other organs and that their rhythmicity can be further enhanced by

the timing of food intake[29–31]. Thus, defining the functional roles of clocks in different organs within the gastrointestinal tract as well as their prominent zeitgeber signals is of great interest as they might be relevant for disease pathophysiology and the development of future therapeutic interventions[32,33].

Circadian regulation of liver physiology

The liver is a highly metabolically active organ that is strongly influenced by feeding and fasting cycles. Nutrient availability during the behaviourally active phase enables energy storage in the form of glycogen, triglycerides and proteins whereas, in the rest phase or upon increased demand (for example, exercise), these energy reservoirs are consumed. Several interdependent mechanisms work in concert to drive rhythmic liver physiology and metabolism. The systemic and cellular energy and metabolite levels are signalled to the molecular oscillator through diverse metabolic sensors such as silent mating-type information regulation 2 homologue (SIRT)[7,8,10,34], poly(ADP-ribose) polymerase 1 (PARP1)[35], sterol regulatory-element binding protein (SREBP; also known as SREBF)[36], AKT serine–threonine kinase (AKT)[37], AMP-activated protein kinase (AMPK)[38] and peroxisome proliferator-activated receptor-γ coactivator 1α (PPARGC1A; also known as PGC1α)[39]. Moreover, these sensors of cellular metabolic states interact with core clock proteins and modulate their functions while, at the same time, their activity is regulated by the cellular circadian clock. This bidirectional relationship sustains proper liver function[6].

Over the past two decades, high-throughput analyses performed in the mouse liver have highlighted the broad rhythmicity of the hepatic transcriptome[40–42], cistrome[43], proteome and phosphoproteome[44–48], acetylome[49], metabolome[50] and lipidome[51,52], further suggesting that circadian rhythmicity in the liver is regulated at multiple levels, both transcriptionally and post-transcriptionally[53,54].

Owing to the pervasive role of circadian rhythmicity for liver functions and the involvement of the circadian clock in their regulation, the circadian clock is considered a key modulator of several liver and liver-related pathologies such as obesity, NAFLD and T2DM, as detailed in the next sections.

Obesity

Obesity is, in most cases, caused by an excess uptake of macronutrients relative to energy expenditure via resting metabolism and physical activity. However, other diet-independent factors, such as genetic predisposition, endocrine disorders, medication and stress, also have a role[55,56]. In the liver, surplus macronutrients, in particular free fatty acids and carbohydrates, are converted to triglycerides, packaged into very-low-density lipoprotein (VLDL), secreted into the bloodstream and delivered to adipocytes for storage. In this way, the liver provides a 'bottleneck' mechanism for fat deposition[57]. In addition, short-chain fatty acids can serve as substrates for hepatic de novo lipogenesis, connecting liver metabolism with gut microbial metabolism[58] (Box 1). Moreover, high blood sugar levels trigger insulin release, which suppresses lipolysis[59] and promotes lipogenesis by activating the synthesis of malonyl-CoA, the building block for fatty acid synthesis[60]. Over time, excessive adipose tissue, particularly in the abdomen, builds up and leads to the release of inflammatory mediators such as tumour necrosis factor (TNF), IL-6 and pro-inflammatory markers, known as adipokines[61]. As this condition progresses, inflammation and pathological metabolic changes enhance one another, causing a vicious cycle that leads to obesity, insulin resistance and T2DM.

The liver circadian clock is considered to be a strong factor in the development of obesity. Mounting evidence from animal studies as well as the association between genetic variations in core clock genes and an elevated BMI and obesity in rodents and humans suggest a major role for circadian clocks in the development of obesity[62]. One of the first examples of the role of clock genes in obesity was the *Clock*-mutant mouse model, which exhibits an obese phenotype that is caused specifically by visceral adiposity[63]. These mice exhibit disrupted circadian eating habits by shifting a substantial amount of their food intake to their inactive phase. These altered eating patterns are accompanied by an increased total energy uptake (as a result of hyperphagia) as well as a reduction in energy expenditure. As a result, the livers of *Clock*-mutant mice show signs of lipid overload and glycogen accumulation[63] and the mice develop NAFLD[64].

Additional evidence linking core clock gene expression to obesity comes from a mouse model with *Per2* deficiency. Similar to the *Clock*-mutant mice, *Per2*-deficient mice have a relative increase in food intake during the inactive phase as well as higher body weight. However, unlike the *Clock*-mutant mice, they consume the same daily amount of calories as their wild-type counterparts[65]. These changes in feeding behaviour lead to diminished fasting glycaemia and a reduced hepatic glycogen content, and are associated with an altered transcriptional response to food intake[66]. In addition, *Per2*$^{-/-}$ mice exhibit altered rhythmic accumulation of hepatic triglycerides[51,52].

Another relevant mouse model involves NR1D1. Like PER2 (ref. 67), NR1D1 is a regulator of adipocyte differentiation, adipogenesis[68], and carbohydrate[69] and lipid metabolism[70] in the liver. Mice that lack *Nr1d1* and its close homologue *Nr1d2* specifically in the liver develop nonalcoholic steatohepatitis (NASH)[71]. Metabolic regulation by NR1D1 in the liver and other tissues seems to be governed by both transcriptional and non-transcriptional functions of NR1D1. Association of NR1D1 with tissue-specific transcription factors, such as hepatocyte nuclear factor 4 and 6 (HNF4 and HNF6), can recruit NR1D1 to the target promoters of HNF4 and HNF6 and regulate transcription through binding to various cofactors, for example, histone deacetylase 3 (HDAC3)[72]. Additional evidence suggests that NR1D1 drives a diverse transcriptional programme exceeding the gene expression changes elicited by the interaction with HNF4 and HNF6 that protects the animals against metabolic perturbations such as mistimed feeding, a driver of obesity that is also observed in the *Nr1d1*$^{-/-}$ mouse model[73].

The role of BMAL1 in weight gain and obesity is less clear[74–76], which might be connected to the confounding role of the premature ageing phenotype of *Bmal1*-deficient mice[77]. Overall, whole-body knockout of *Bmal1* does not affect body weight[76–78], but *Bmal1*$^{-/-}$ mice exhibit a multitude of lipid and carbohydrate-related metabolic perturbations; they have a higher fat content[74–76] and reduced fatty acid oxidation and oxygen consumption rates[79], their glucose clearance and tolerance are perturbed, and they display hypoinsulinaemia[74,76] and insulin hypersensitivity[74,80]. *Bmal1*-deficient mice display additional metabolic disturbances that are possibly related to the regulation of adipogenesis by *Bmal1* (ref. 81). A study published in 2022 examined, in detail, the kinetics of the metabolic effect of a high-fat diet (HFD) on whole-body *Bmal1*-deficient mice and showed an early obesity phenotype with increased fasting glucose levels at an early stage (about 12 weeks) but the phenotype was lost at later stages[78]. Thus, it is possible that metabolic control by *Bmal1* is dependent on age and tissue type. Indeed, a comparison of whole-body with liver-specific disruption of the circadian clock paints a more complex picture of the potential role of the liver clock in obesity development.

Box 1

Circadian regulation of the gastrointestinal microbiome

The gastrointestinal tract hosts trillions of microorganisms such as bacteria, viruses, protozoa and fungi, collectively known as the gastrointestinal microbiome. Although, for a long time, these pathogens were considered harmful, accumulating evidence suggests that they have an important role in systemic metabolic health[202–204]. The composition of the gastrointestinal microbiome is highly complex and is responsive to systemic cues that originate from the host such as diet, exercise or medication[204]. Gut microorganisms are involved in the digestion and absorption of food and produce metabolites that can be used by the host. As a result, they have key roles in various aspects of human health, including immune and metabolic homeostasis[202]. Hence, it might be justified to call the gut microbiota an auxiliary metabolic organ[204].

The abundance and composition of the gut microbiota exhibit daily rhythms, both in mice and humans. For instance, Bacteriodetes and Firmicutes, the two major populations of commensal gut bacteria in mice, exhibit diurnal fluctuation in their abundance, with each having opposing peak hours[205]. Importantly, the rhythmicity of the gut microbiota is not autonomous but, rather, is driven by the host circadian clock, most likely through feeding rhythms[189]. Disruptions of the host circadian system, either through nutritional or genetic interventions, influence gut microbiome rhythmicity. In mice, both restriction of food availability during the active phase[206] and intermittent fasting[207] have been shown to alter the composition and rhythmicity of the gut microbiome. This observation indicates that gut microbiome rhythmicity is not only altered by the timing of food intake but also by the food composition.

Studies that have employed various clock-mutant mouse models support a role of the host circadian clock in controlling the gut microbiome. Mice that lack a functional circadian clock (that is, *Per1*$^{-/-}$*; Per2*$^{-/-}$) exhibit disrupted microbial rhythms; this is likely due to their irregular feeding pattern as rhythmicity in microbiota is restored upon a scheduled feeding regimen[188]. In *Bmal1*-deficient mice, the circadian changes in faecal microbiota abundance are altered compared with their wild-type littermates. Interestingly, the microbial abundance varies depending on the sex of the host[208]. Collectively, these studies indicate that both activators and repressors of the host internal clock machinery are fundamental to gut microbiota rhythmicity. On the other hand, the gut microbiome seems to modulate the molecular rhythmicity of the host, thereby establishing a mode of bidirectional regulation. The livers of mice that were housed in a germ-free environment exhibit altered expression levels of core circadian genes[209]. In addition, the expression of genes associated with rhythmic physiology differs strongly in germ-free mice[210]. This two-way interaction between the gut microbiome and the host circadian system likely has an important role in metabolic homeostasis.

In summary, dysfunction of either system, either by dysbiosis or disruption of a host clock, impacts homeostasis in a bidirectional manner and can contribute to metabolic disorders, including obesity, diabetes, nonalcoholic fatty liver disease, cirrhosis and hepatocellular carcinoma[211].

Liver-specific knockout of *Bmal1* leads to hypoglycaemia during the inactive phase[24,74], which suggests that *Bmal1* has a critical role in maintaining constant glucose levels throughout the day in the liver, likely via glycogen breakdown during the inactive or fasting state. These mice were also shown to accumulate oxidative damage and develop hepatic insulin resistance, which was attributed to mitochondrial dysfunction owing to the observation that *Bmal1* controls mitochondrial dynamics and biogenesis[82].

In humans, numerous connections between circadian clock genes and metabolic phenotypes have been identified. *CLOCK* gene polymorphisms were found to be associated with overweight and obesity[83–85], and these effects seem to be modified by sex, physical activity[86] and diet[87]. Interestingly, a meta-analysis in humans examining 13 studies with 17,381 individuals in total found an association between metabolic syndrome and *CLOCK* gene polymorphisms only in particular ethnic groups[88]. Additionally, *CLOCK* gene SNPs were reported to be associated with T2DM and stroke[89], which are both associated with obesity. In addition, polymorphisms of the core clock gene *NR1D1* are associated with obesity in various populations[90–93]. No associations between *BMAL1* polymorphisms and obesity have been reported in humans; however, *BMAL1* polymorphisms associated with T2DM, hypertension and metabolic syndrome have been identified[88,94]. Future studies in larger cohorts are required to confirm these findings and to distinguish specific effects of single clock genes from general disturbances of the circadian system.

In addition, it is notable that additional behavioural components of the circadian system can exacerbate obesity. For example, sleep is an important mediator between core clock genes, the circadian system and metabolic health. Large-scale epidemiological studies demonstrated a substantial role of sleep disturbance in adiposity, glycaemic control and T2DM[95,96]. In turn, circadian misalignment through shift work or social jetlag leads to sleep disturbance[97]. Moreover, chronotype, that is, the preference to be a morning or an evening person (a 'lark' or an 'owl'), can exacerbate circadian misalignment and is genetically modified by clock genes[98].

In summary, evidence from animal models and humans strongly support the involvement of disrupted circadian rhythmicity or core clock components in the pathophysiology of obesity. Hence, as detailed below, interventions that restore proper circadian rhythmicity are expected to serve as potent therapeutic modalities for the prevention and amelioration of obesity.

Type 2 diabetes mellitus

T2DM is associated with obesity and metabolic syndrome. One hallmark of T2DM is an inability to sufficiently clear sugar from the bloodstream due to an inadequate production of insulin combined with relative

insulin insensitivity in the main carbohydrate-storing organs such as skeletal muscle and liver. Complications of T2DM include, among others, restricted blood flow through the extremities, blindness and kidney failure, underscoring the vital importance of systemic carbohydrate regulation, in which the liver circadian clock has a fundamental role[6]. The expression levels of many enzymes involved in hepatic carbohydrate metabolism peak at different times during the day in order to ensure the breakdown, synthesis or storage of hexose sugars according to the daytime-specific requirements. CRY proteins, essential transcription factors in the cellular circadian clockwork, have an important role in hepatic gluconeogenesis[99,100], which is essential for glucose homeostasis during the rest phase when plasma glucose levels are not replenished through feeding[101]. Gluconeogenesis is regulated by glucocorticoids, and it has been demonstrated that CRY proteins interact directly with the glucocorticoid receptor[100] and modulate downstream glucagon signalling via interaction with the Gsα subunit of G protein-coupled receptors[99]. NR1D1 and NR1D2 are other core clock proteins that regulate hepatic glucose metabolism via various mechanisms[102]. On the one hand, NR1D1 and NR1D2 bind directly to the liver-specific transcription factors HNF4 and HNF6 and various corepressors to regulate transcription[103]. On the other hand, they recruit the glucocorticoid receptor to chromatin and establish active chromatin marks that assist (together with HNF4 and HNF6) in regulating target genes of carbohydrate and lipid metabolism[104].

The liver has another important role in the post-absorptive phase by maintaining constant plasma glucose levels, thereby counteracting the lack of macronutrient uptake imposed by the SCN through the rest–activity cycle. At the beginning of the active phase, when the body switches to a predominantly catabolic state, nutrient uptake peaks along with the expression levels of glucose transporters, the glucagon receptor and glycogen synthase[105,106]. Liver cells synthesize glucose through glycogenolysis and gluconeogenesis and simultaneously express the glucose transporter GLUT2, which is required for the secretion of glucose into the bloodstream. Liver-specific *Bmal1*-knockout mice lose the capacity to rhythmically express GLUT2 and thereby become hypoglycaemic in the post-absorptive phase, highlighting the interplay between the SCN and the liver clock in nutrient homeostasis[24,74].

Circadian clocks in pancreatic α-cells and β-cells also have a crucial role in glucose homeostasis by regulating rhythmic insulin and glucagon secretion. Patients with T2DM were demonstrated to have weakly synchronized cellular oscillators in pancreatic islets with low amplitudes that had defects in the granule docking and exocytosis steps of the insulin and glucagon secretion process[107]. Furthermore, due to altered lipid metabolism in the islet cells of patients with T2DM, their islet cell membranes were stiffer than the cell membranes of their healthy counterparts, which might, at least in part, explain the reduced capacity for insulin and glucagon secretion of islet cells of patients with T2DM[108].

Finally, epidemiological data also support a strong connection between the circadian system and T2DM in humans. 'Owl' patients with T2DM (with late chronotypes) have worse glycaemic control than their 'lark' counterparts[109], and an extreme chronotype and the resulting social jetlag due to misalignment of the endogenous clock with the social schedule is associated with metabolic syndrome and T2DM or a prediabetic state in the general population[110].

Taken together, these molecular and epidemiological studies outline a promising path to treatment of T2DM by targeting the activity of molecular oscillator components. However, additional studies on the relation between circadian clocks and the pathophysiology of T2DM at the molecular level are lacking.

Nonalcoholic fatty liver disease

NAFLD is an array of hepatic metabolic disorders that starts with nonalcoholic fatty liver or steatosis and progresses through NASH to cirrhosis, which can finally progress to HCC (Fig. 2). During the past three decades, there has been a dramatic increase in the global prevalence of NAFLD and it has emerged as the most common chronic liver disease[111]. NAFLD is strongly associated with features of metabolic syndrome, including abdominal obesity, insulin resistance, glucose intolerance or T2DM, and dyslipidaemia. The impaired capacity of hepatocytes to efficiently metabolize carbohydrates or lipids, such as free fatty acids, culminates in excessive fat accumulation in the liver, the hallmark of NAFLD.

As previously noted, a variety of hepatic functions are under circadian control, including hepatic glucose, triglyceride and cholesterol metabolism. In addition, altered lipid metabolism has been implicated in the disruption of clock function[112]. Hence, it has been suggested that circadian clock dysfunction can lead to metabolic imbalances that lead to a higher risk of developing NAFLD[113,114]. Furthermore, circadian misalignment in response to a modern lifestyle and western diet can further increase the prevalence of NAFLD and aggravate its progression[114,115].

Specifically, the circadian system regulates most aspects of lipid metabolism in the liver, ranging from lipoprotein synthesis to lipid uptake and utilization[15]. The temporal and net expression levels of many genes that are implicated in lipid metabolism are altered in mice with a disrupted clock and are associated with the dysregulation of various lipid species. For instance, whole-body *Per1*$^{-/-}$*;Per2*$^{-/-}$ mice (a dysfunctional circadian clock mouse model) exhibit altered hepatic triglyceride accumulation[52] and perturbed lipid accumulation in various subcellular compartments[51].

Major regulators of lipid homeostasis are the transcription factors SREBP1, SREBP2 and peroxisome proliferator-activated receptors (PPAR)[116]. Dysfunctional lipogenesis is one of the main reasons for lipid accumulation in NAFLD[117]. SREBPs have been shown in animal models to be expressed in a cyclic manner both at transcript and protein levels[118]. Genome-wide analyses evinced that SREBP1 rhythmically binds to some of its canonical target genes together with HNF4 and regulates their rhythmic expression. This rhythmicity is driven both by nutrient availability and the circadian clock[119]. Notably, the rhythmic expression of SREBP1C and PPARγ in mouse livers is upregulated during HFD, with robust oscillations compared with standard diet[120,121], suggesting that a HFD reprogrammes hepatic lipogenesis and β-oxidation pathways by inducing de novo oscillations in SREBP1C and PPARγ. Various clock components, such as BMAL1 (ref. 122), NR1D1 (refs. 118,119), and RORα and RORγ[123], have been reported to regulate SREBP1 activity in animal models. Reciprocally, SREBP1 can directly regulate the expression of clock genes. For example, a study published in 2016 indicated that *Cry1* gene expression is activated by SREBP1 in mouse primary hepatocytes[124]. This observation points towards the existence of crosstalk between the circadian system and the SREBP signalling pathway, which might be implicated in the pathophysiology of NAFLD.

Improper utilization or breakdown of lipids by the liver is another important mechanism of NAFLD progression. PPAR transcription factors are major regulators of the expression of lipolysis genes. For example, *Ppara* is rhythmically expressed and is regulated by BMAL1 and CLOCK[125] as mice lacking BMAL1 or CLOCK exhibit loss of *Ppara* rhythmicity in the liver. In addition, PPARα and PPARγ interact with

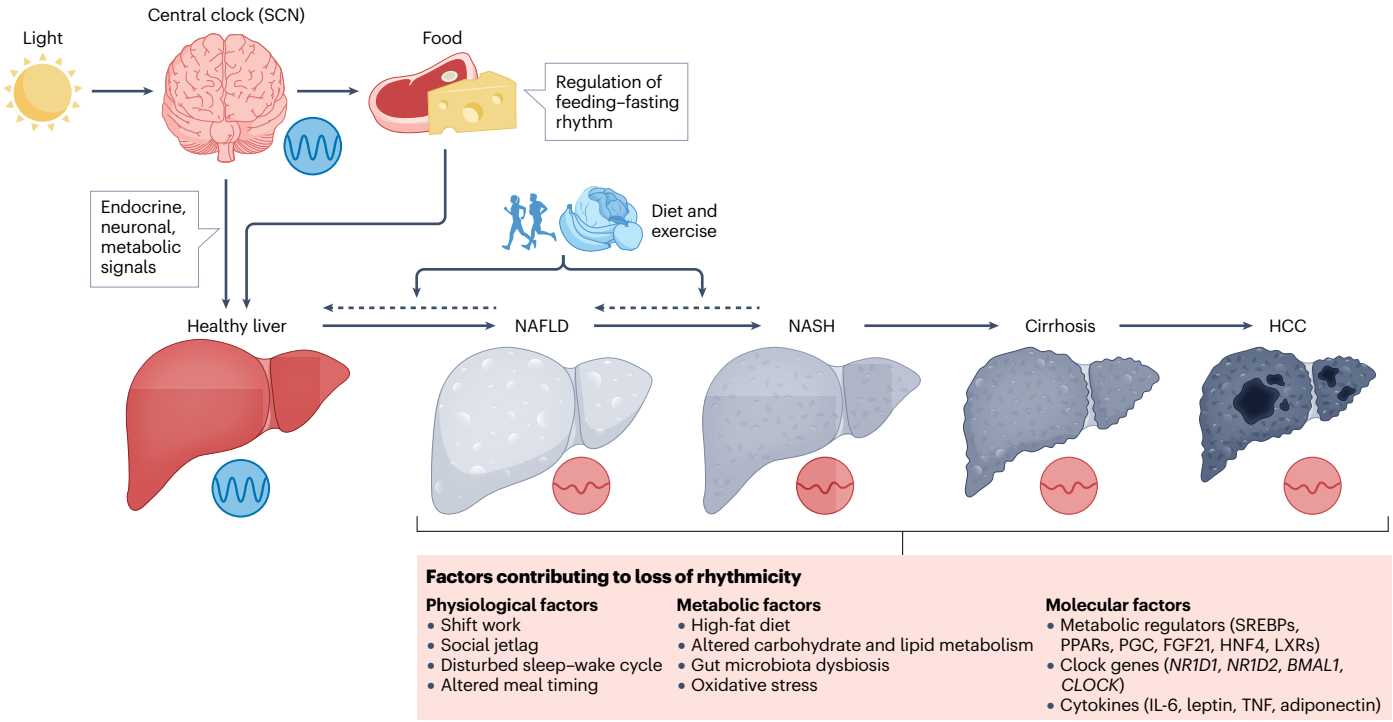

Fig. 2 | Involvement of circadian rhythmicity in the pathophysiology of liver diseases. A central circadian pacemaker is located in the suprachiasmatic nucleus (SCN) of the hypothalamus, which receives light as a synchronizing cue and communicates with the other clocks situated in peripheral organs, such as the liver, through endocrine, neuronal and metabolic signals. Food is considered a prominent zeitgeber for the liver clock[2]. Disruption of circadian rhythmicity, either directly or indirectly, due to physiological, metabolic or molecular factors, is associated with the pathophysiology and progression of liver diseases, including nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), liver cirrhosis and hepatocellular carcinoma (HCC), in animal models. FGF21, fibroblast growth factor 21; HNF4, hepatocyte nuclear factor 4; LXRs, liver X receptors; PGC, peroxisome proliferator-activated receptor-γ coactivator; PPAR, peroxisome proliferator-activated receptors; SREBPs, sterol regulatory-element binding protein; TNF, tumour necrosis factor.

core clock components. For instance, PPARα regulates *Bmal1*, *Per2* and *Per3* expression in mouse liver[126,127].

Circadian regulation of rate-limiting enzymes of key metabolic pathways, such as acetyl-CoA carboxylase (ACC) (fatty acid metabolism), hydroxymethylglutaryl-CoA reductase (HMGCR) (cholesterol metabolism) and cholesterol 7α hydroxylase (CYP7A1) (bile acid metabolism), can markedly contribute to NAFLD progression. ACC is a key enzyme in lipolysis and its phosphorylation and inactivation are regulated by fasting–feeding cycles and follow the circadian rhythm[128]. AMPK activation is clock controlled and represses ACC through phosphorylation, thereby decreasing levels of malonyl-CoA, which is a precursor for fatty acid biosynthesis[38,129]. HMGCR is the key enzyme involved in cholesterol homeostasis. Diurnal variations in its abundance and expression suggest that the enzyme is under circadian regulation. In the liver of *Clock*-mutant mice, *Hmgcr* expression is lower and does not exhibit notable rhythmicity[130].

Bile acid production is a key mechanism in cholesterol elimination. It takes place in the liver, and the rate-limiting enzyme CYP7A1 is cyclically expressed. *Cyp7a1* expression is regulated by clock components, such as NR1D1 and DEC2, and clock-controlled genes such as those that encode DBP and E4BP4 as well as the orphan nuclear receptor small heterodimer partner (SHP)[131].

Taken together, these studies highlight the intricate interplay between the molecular clockwork and principal components that participate in liver lipid metabolism and that are highly relevant to the pathophysiology of NAFLD. Future studies that address the molecular involvement of circadian clocks in NAFLD are expected to be instrumental in the development of therapeutic interventions for NAFLD.

Hepatocellular carcinoma

Epidemiological studies suggested the potential involvement of the circadian clock in the pathophysiology of various malignancies long before the molecular mechanisms of clock-regulated carcinogenesis began to unfold[132,133]. The expression of core clock genes is frequently dysregulated in tumour cells, and this dysregulation has been associated with poor prognosis[134,135]. Furthermore, circadian gene polymorphisms are associated with cancers in most human tissues[136].

Progression from NAFLD to NASH with concurrent fibrosis and cirrhosis is a major cause of HCC[137] (Fig. 3). Owing to the increasing prevalence of obesity, metabolic diseases associated with fatty liver diseases, which are exacerbated by circadian dysfunction, might already cause up to one-third of all cases of HCC in the United States and are expected to become the leading cause of this cancer type, which is also one of the deadliest[138]. A key event in HCC development is hyperactivation of the constitutive androstane receptor (CAR; also known as NR1I3), which is induced by detrimental bile acid signalling and excessive sympathetic nervous system activity during the sleep phase and is counteracted by the farnesoid X receptor (FXR)[139] (Fig. 3). In mice,

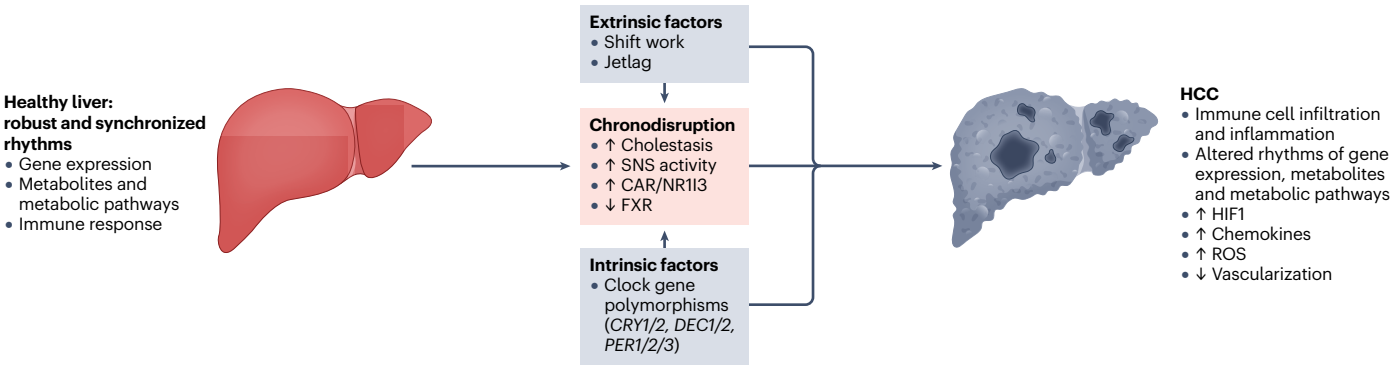

Fig. 3 | The role of circadian regulation in HCC development. The progression from healthy liver to advanced hepatocellular carcinoma (HCC) is accompanied by changes in clock-controlled molecular, metabolic and physiological functions that are vital for a healthy state and, once disturbed, likely promote progression to a disease state (arrows). Changes are driven both by intrinsic and extrinsic factors that promote tumour growth either directly or through circadian disruption. Evidence from human studies and mouse models is included. CAR, constitutive androstane receptor, also known as NR1I3; FXR, farnesoid X receptor; HIF1, hypoxia-inducible factor 1; ROS, reactive oxygen species; SNS, sympathetic nervous system.

chronic jetlag induces NAFLD, and cholestasis leading to the activation of CAR promotes the progression from NAFLD to NASH, fibrosis and, finally, HCC[139]. Moreover, in *Clk*$^{\Delta19/\Delta19}$*Apoe*$^{-/-}$ mice, NAFLD progresses more readily to HCC[64], and loss of PER2 or CRY1 and CRY2 predisposes animals to experimentally induced hepatocarcinogenesis[140,141].

In humans, the question of whether chronodisruption, such as that caused by shift work and social jetlag, is associated with NAFLD, and ultimately HCC, has been controversial[142,143]. However, a 2022 retrospective study of a large and diverse cohort with 286,825 participants indicates a significant association between irregular night shift work and extreme evening chronotypes and NAFLD[144], which confirms previous findings in mouse models in which chronic jetlag accelerated hepatocarcinogenesis, which could be slowed or reversed by limiting food access to a window within the activity phase[145].

Transcriptional regulators that interact with the molecular clock and have the potential to accelerate or inhibit tumour growth also influence HCC progression. One example is the liver-specific transcription factor HNF4, which, in addition to its role in programming circadian hepatic macronutrient metabolism[103,104], also switches isoform expression upon the development of liver carcinogenesis[146]. In healthy hepatocytes, the isoform P1-HNF4 is predominantly expressed. It is localized in the nucleus and exerts a tumour-suppressive role through the rhythmic suppression of cell cycle genes and epithelial–mesenchymal transition genes[146]. A switch to P2-HNF4 expression in HCC forces P1-HNF4 into the cytoplasm, which blunts its tumour-suppressor function and leads to the direct inhibition of BMAL1 expression by P2-HNF4. Consequently, ectopic overexpression of BMAL1 in HCC reduces tumour growth, presumably through the regulation of genes that lead to apoptosis or cell cycle arrest such as WEE1 G2 checkpoint kinase or cyclin-dependent kinase inhibitor 1A (CDKN1A; also known as P21)[146,147].

A common oncogenic driver in solid tumours that also influences aggressiveness and metastatic potential in HCC progression is hypoxia-inducible factor 1 (HIF1), which is activated in the hypoxic tumour tissue[148] (Fig. 3). HCC tumour cells consume oxygen at an elevated rate, and thereby create their own hypoxic environment, which is promoted by insufficient vascularization. These conditions are counteracted by upregulating HIF1, which transcriptionally reprogrammes HCC cells towards enhanced growth, metastasis formation and drug resistance, leading to faster proliferation and greater survival rates of tumour cells[149]. Notably, HIF1 activity is itself under circadian control and is ultimately driven by rhythmic tissue oxygenation[150–152]. Furthermore, the transcriptional response to hypoxia is daytime dependent and clock controlled[22].

The immune system also has a decisive role in the aetiology and progression of HCC (Fig. 3). Chronic liver inflammation that develops upon the transition from NAFLD to NASH triggers the infiltration of liver tissue by innate immune cells such as macrophages, dendritic cells, neutrophils and natural killer cells. Together with non-immune cells, such as fibroblasts and endothelial cells, and with the extracellular matrix, they form a tumour microenvironment that can both counteract and promote tumour development, growth and metastasis. The secretion of chemokines and cytokines and the production of reactive oxygen species in the newly remodelled liver and tumour microenvironment tissue lead to tissue damage, persistent inflammation and, ultimately, carcinogenesis[153]. In addition, infiltration of the liver by neutrophils is rhythmic and regulated by the circadian clock in non-cancerous tissue. Elastase secreted by neutrophils in the liver activates the stress-inducible kinase JNK, which in turn activates *Bmal1* and represses the expression of fibroblast growth factor 21 (FGF21)[154]. BMAL1 and FGF21 both regulate diverse metabolic programmes in hepatocytes such as lipid and hexose metabolism[80,155]. In addition, FGF21 is responsible for multiple beneficial outcomes for liver health that are still incompletely understood, including increased channelling of triglycerides towards cholesterol and bile acid production, decreased lipogenesis, and a higher hepatic glucose output, while simultaneously reducing inflammation[156]. In contrast, whole-body knockdown of murine FGF21 promotes the development of jetlag or diet-induced NAFLD[154] and leads to higher carcinogenesis in an obesogenic setting[157]. Liver-resident neutrophils also secrete IL-6, which enhances liver inflammation in the early phase of liver disease and promotes the final development from cirrhosis to HCC[158]. However, IL-6 also has a protective function in the transition state of fibrogenesis and therefore plays a dual role in the development of liver disease[159].

The total number of immune cells in the circulation and the fraction of immune cells at target sites in tissues, both of which are dependent on the time of day, are important factors in the function of the

immune system. Blood immune cell counts as well as the turnover of immune cells are rhythmic[160,161]. Furthermore, the influx and egress of immune cells in target tissue cells are gated by the circadian clock via the diurnal expression of chemokines and their receptors and cellular adhesion molecules[162–164]. In HCC as well as in other cancers, a high level of macrophage infiltration is associated with immunosuppression and enhanced resistance to therapy[165]. Macrophages have a circadian clock that regulates the rhythmic secretion of TNF and IL-6 (ref. 166) and rhythmic bactericidal activity[167,168]. The core clock proteins NR1D1 and NR1D2 repress the expression of the chemokine C-C motif ligand 2 (CCL2) and signalling dependent on CCL2-activated mitogen-activated protein kinase 1 (MAPK1; also known as ERK) and MAPK14 (also known as P38), thereby blocking macrophage migration and cell adhesion[169]. The tight circadian regulation of the immune system, including many processes of the innate and adaptive immune system, might provide novel approaches for the treatment of HCC and other cancers[170]. Driving immune reactions at tumour sites towards the engulfment of cancer cells by macrophages and the creation of a non-permissive environment for tumour growth are therefore promising avenues for future cancer therapies that could be supported by clock-based immunomodulatory strategies.

Circadian clock-based preventive medicine

A popular and effective nutritional approach to prevent or reverse adverse metabolic effects is to generate overt eating rhythms that are aligned to a short interval within the active phase of an individual[171]. This approach is believed to generate an optimal alignment of food uptake with circadian physiological regulation[172] and is called TRE (formerly known as time-restricted feeding) (Fig. 4). TRE can also be practised alongside calorie restriction, and studies in rodents and humans provide evidence that supports the beneficial effects of TRE or TRE with calorie restriction[11,12,173]. Whereas it is generally believed that most dietary regimens improve the symptoms of metabolic syndrome via weight loss due to prolonged calorie restriction, TRE can ameliorate pathological metabolic changes without calorie restriction or weight loss[12,173], provided that meals are timed to the first half of the day (early TRE). In contrast, meal consumption in the afternoon (late TRE) results in neutral or worsened metabolic and cardiovascular endpoints as compared to ad libitum eating[174,175], which is in line with the detrimental metabolic effects of food uptake during resting time in rodents[11]. Even just consuming the majority of calories during the first half of the day, when glucose tolerance and insulin sensitivity are higher than in the second half[176], has been demonstrated to offer some protection against weight gain in prospective human studies[177,178]. A prolonged eating phase of 12 h or more, which is typical in western societies, does not seem to support physiological improvements or weight loss[179,180], and constant misalignment of eating times with the circadian clock, with or without sleep restriction, under controlled laboratory conditions, exposes the human body to a prediabetic state within a couple of days[181,182].

Equally important seems to be macronutrient quality and distribution, in particular the relative amount of fat per consumed calories and the ratio of fat to carbohydrates. A HFD, which is a typical 'poor' nutrition strategy in the western world, and the ketogenic diet are both very high in fat but differ in the content of carbohydrates (20–50% in HFD versus 5–10% in ketogenic diet)[183]. In contrast to a HFD, the metabolic effects of which are similar to eating during destined rest or sleep times and which leads to a misalignment of environmental and endogenous circadian time, a ketogenic diet simulates a starvation state and triggers

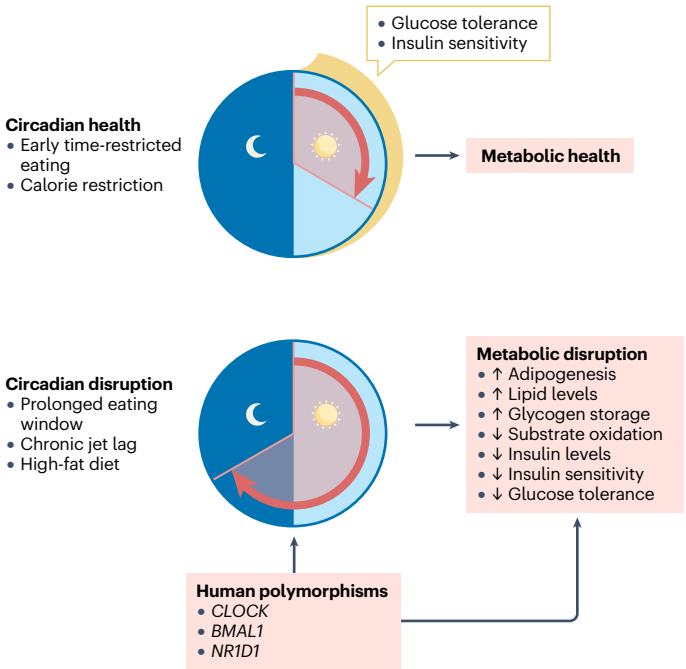

Fig. 4 | Time-restricted eating as a chronobiological health intervention. Metabolic health and successful weight management are supported by aligning the eating window to the phase of the circadian clock by taking up food in the early activity phase (light blue part of clocks) (early time-restricted eating), when high insulin sensitivity and glucose tolerance support the catabolism of macronutrients. Ideally, the total calorie intake is limited (calorie restriction) or at least well controlled at the same time. By contrast, extending the eating window into the rest phase (dark blue part of clocks) can lead to overweight and metabolic diseases, in particular when combined with poor nutrition in the form of a western or high-fat diet[171]. Both a prolonged eating window and a high-fat diet lead to chronodisruption, which can also be triggered by living against one's circadian clock, for example, during chronic jetlag[182]. Clock gene polymorphisms similarly lead to disruptions of the circadian system and to altered feeding behaviour and can thereby lay the foundation for metabolic disorders[88]. In the same way that a healthy lifestyle in accordance to the circadian clock can prevent or even revert metabolic disorders, living against the clock can provoke and amplify them[5].

many advantageous metabolic adaptations, including effects on the human immune system via the microbiome[184]. A ketogenic diet also induces distinct reprogramming and phase changes in the circadian clock in animal models[185,186]. HFD, on the other hand, results in loss of rhythmicity in mice[187], to which improper CLOCK–BMAL1 recruitment and de novo oscillations of PPARγ mediated by the microbiome (Box 1) can contribute[188–191]. These effects are independent of weight gain[121]. Notably, TRE can, at least in mice, not only prevent but partially reverse many adverse metabolic changes induced by HFD[11] (Fig. 4).

These chrononutrition interventions have become extremely popular lately and seem to carry metabolic health benefits both in animal models and in human clinical studies. The underlying mechanisms and the exact role of the circadian clock that pertain to these metabolic benefits are yet to be determined; hence, it is expected to be an exciting and valuable research area in the future.

The past decade has seen an increase in drug development strategies that explore the potential of circadian clock modulators as therapeutic modalities against metabolic diseases. These molecules

target core clock proteins, such as CRYs, REV-ERBs and RORs, as well as circadian clock-related proteins and metabolic regulators, including CK1s, PPARs, AMPK and SIRT, to manipulate circadian timing in various pathologies. For instance, KL001, a small molecule that specifically interacts with both CRY1 and CRY2 and prevents their ubiquitin-dependent degradation, was shown to stabilize CRY1 and CRY2 and inhibit glucagon-induced gluconeogenesis in liver cells[192] and glucose tolerance in diet-induced obesity in mice[193]. Hence, KL001 could potentially be used as a clock-based therapy for T2DM via the regulation of CRY-dependent metabolism.

Other attractive clock targets are REV-ERBs and RORs. Despite the similarities in the DNA-binding domains for REV-ERB and ROR, their interacting ligands are different: REV-ERB binds to haem whereas ROR binds to cholesterol and oxysterols. Both physiological (haem)[102] and synthetic (GSK4112)[194] REV-ERB agonists suppress the expression of hepatic gluconeogenic genes and reduce glucose output in animal models[102]. Furthermore, administration of SR9009 and SR9011, which are synthetic GSK4112-derived REV-ERB agonists, reduce fat mass and improve dyslipidaemia in diet-induced obesity in mice[195,196]. In addition, there are several synthetic[197] and naturally occurring (such as nobiletin and neoruscogenin)[198,199] clock-enhancing ROR agonists that have been shown to alter the expression of genes implicated in glucose and lipid metabolism and protect against metabolic syndromes in a clock-dependent manner in mice. Future studies in animal models and humans with CK1 inhibitors (C261, CKI-7), PPAR activators (fenofibrate, rosiglitazone), and AMPK (metformin, AICAR) and SIRT (SRT2183, SRTCD1023) modulators that specifically address their potential effect as metabolic modifiers in the context of the circadian clock are vital; these compounds can alter circadian clock properties and consequently modify metabolic outputs in the context of various metabolic diseases.

Last, and as previously mentioned, given the importance of circadian clocks being in synchrony with the environment and among different tissues throughout the body, it is conceivable that clock-resetting agents would be another attractive therapeutic intervention, in particular in the context of shift workers and jetlag. However, the intricate nature of circadian clock resetting, namely its time dependency[200,201], calls for comprehensive studies that identify and examine the effects of different compounds on the clock both in vitro and in vivo in animal models and humans.

In summary, compounds that target components of the circadian clock either directly or indirectly represent a promising therapeutic avenue for the alleviation of circadian clock-associated pathologies in general and in liver-related diseases specifically.

Conclusions

A multitude of studies across the past few decades supports the notion that the circadian clock controls rhythmic liver physiology and metabolism on a daily basis and has elucidated the molecular underpinnings. Moreover, many studies have addressed the potential involvement of clock components and the circadian clock in various pathologies both in animal models and humans. Mounting evidence suggests that chronobiology can be harnessed, at multiple levels, in preventive and therapeutic approaches for various conditions. This exciting field integrates the molecular knowledge of circadian clocks with clinical evidence to promote health benefits both in general and specifically for liver-related pathologies.

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