

MINI REVIEW

Circadian rhythms and the liver

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Abstract

This narrative review briefly describes the mammalian circadian timing system, the specific features of the liver clock, also by comparison with other peripheral clocks, the role of the liver clock in the preparation of food intake, and its relationship with energy metabolism. It then goes on to provide a chronobiological perspective of the pathophysiology and management of several types of liver disease, with a particular focus on metabolic-associated fatty liver disease (MAFLD), decompensated cirrhosis and liver transplantation. Finally, it provides some insight into the potential contribution of circadian principles and circadian hygiene practices in preventing MAFLD, improving the prognosis of advanced liver disease and modulating liver transplantation outcomes.

KEYWORDS

decompensated cirrhosis, liver clock, liver transplantation, MAFLD, mammalian circadian timing system, metabolism

1 | INTRODUCTION

Translational and clinical chronobiological studies are flourishing thanks to the enormous amount of knowledge that has accumulated over the past decades in molecular chronobiology and its physiological correlates. This has been such that in 2017 the Nobel prize for Physiology or Medicine was awarded to Jeff Hall, Michael Rosbash and Michael Young “for their discoveries of molecular mechanisms controlling the circadian rhythm”.¹ The circadian timing system encompasses a master clock in the suprachiasmatic nuclei (SCN) of the hypothalamus and peripheral timekeepers in every organ of the body, and most tissues and cells.² In addition, clocks are also present

in areas of the brain other than the SCN.³ Circadian clocks control our physiology over the 24 h affecting, for example, the sleep–wake cycle.⁴ Several studies have linked persistently disturbed circadian rhythmicity, for example due to shift work, to an increased incidence of cardiovascular accidents,⁵ higher rates of substance misuse⁶ and certain types of cancer.⁷ Remarkable, isolated pieces of clinical research have linked circadian rhythmicity to other major health outcomes, for example, documenting an increase in the incidence of myocardial infarction after the switch to daylight saving time,⁸ better outcomes of aortic valve replacement in the afternoon compared to morning surgery⁹ and increased intravasation/metastatic proficiency of circulating tumour cells during the rest phase

Abbreviations: ETS, Erythroblast Transformation Specific; HCC, Hepatocellular Carcinoma; HE, Hepatic Encephalopathy; MAFLD, Metabolic-Associated Fatty Liver Disease; NAD⁺, Nicotinamide Adenine Dinucleotide; NAMPT, Nicotinamide Phosphoribosyltransferase; OSA, Obstructive Sleep Apnea; PPARs, Peroxisome Proliferator-Activated Receptors; PPAR α , Peroxisome Proliferator Activated-Receptor alpha; PPAR γ , Peroxisome Proliferator Activated-Receptor gamma; SCN, Suprachiasmatic Nuclei; SIRT1, Sirtuin 1 Deacetylase; TTFL, Transcription-Translation-Feedback Loop.

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in both patients and mouse models of breast cancer.¹⁰ This narrative review describes the mammalian circadian timing system and the peculiarities of the liver clock and examines liver disease and its management—with a particular focus on cirrhosis—from a chronobiological perspective.

2 | THE MAMMALIAN CIRCADIAN TIMING SYSTEM

The molecular model of the mammalian circadian timing system encompasses a set of so-called clock genes, which encode proteins involved in the mechanism generating the circadian oscillation. This is self-generated in each of the approximately 20000 neurons clusterings within the SCN. The system consists of interlocking transcription-translation-feedback-loops (TTFLs), the main one being organized in “positive elements” (Figure 1)—the transcription factors CLOCK and BMAL1—which form heterodimers and trigger the transcription of a number of genes containing, in their promoters, the so-called E/E' box motifs.¹¹ Amongst these genes, some encode the “negative elements” (Figure 1), the clock proteins PER1, PER2, PER3, CRY1 and CRY2. These also form heterodimers (PER/CRY) which, after a delayed accumulation process in the cytoplasm, are transported into the nucleus, where they inhibit the activity of CLOCK and BMAL1, and, therefore, temporarily block the transcription of their own genes.² This auto-repression is the core of the negative feedback loop. An overlapping loop in which CLOCK/BMAL1 heterodimers trigger transcription of the genes *Rev-erbs* and *Rors*, the products of which modulate the expression of BMAL1 in antiphase to the first loop, makes the circadian oscillation more robust (Figure 1).² It is now known that tens of additional proteins are also involved in the finer regulation of the TTFL, modulating chromatin structure,^{12,13} and miRNAs also play a role in TTFL regulation.¹⁴ Further, an additional core component of the TTFL has been identified and named *Chrono*, which seems to function as a core clock repressor (by inhibiting the activity of CLOCK/BMAL1) and is also under epigenetic control.¹⁵

The circadian clock oscillation is qualified as *endogenous* as it is self-sustained even in the absence of any external stimuli, signals or cues. The term *phase* (or internal time) refers to the time when a feature of the circadian rhythm occurs (i.e. the peak or the nadir of the expression of a clock gene or protein), in relation to an external or otherwise fixed time reference such as solar time, civil time etc. In humans, reliable markers of the phase of the SCN clock are the hormones melatonin and cortisol and core body temperature.¹⁶ Environmental signals (known as time givers or *Zeitgebers*), the most important being natural light, are capable of synchronizing the master clock with the day-night cycle generated by the rotation of the Earth around its axis (Figure 1). This process, which also encompasses cues such as fasting/feeding and physical or social activity patterns, is called *entrainment*.

Key points

- The circadian timing system encompasses a master circadian clock in the hypothalamus and peripheral time-keepers in most organs.
- The liver clock is a robust oscillator and is at the centre of the cross-talk between the circadian timing system and metabolism. Abnormalities in such cross-talk play a role in the pathogenesis of fatty liver and the vicious circle underpinning its progression.
- Cirrhosis is associated with functional abnormalities of the liver and master clocks, and the master-peripheral clocks communication system.
- A better understanding of such abnormalities may help improve the management of decompensated cirrhosis and the outcomes of liver transplantation.

Major recent discoveries have further added to the understanding of the complexity and plasticity of the master SCN clock. First, it is now accepted that SCN astrocytes also play an important role in generating mammalian circadian rhythmicity. Ablation of the clock gene *Bmal1* in mice SCN astrocytes lengthens the period of the SCN clock and that of rest-activity behaviour.¹⁷ Further, SCN astrocytes express clock genes in a rhythmic fashion, exhibit circadian Ca^{2+} oscillations with a broad night peak, in *quasi* antiphase with the sharper daytime peak observed in SCN neurons, and show circadian oscillations in glutamate release in phase with the Ca^{2+} peak.¹⁸ Thus, SCN astrocytes function as a *bona fide* circadian clock, and the SCN overall output oscillation is the result of the interaction between the *quasi*-antiphasic oscillations of clock neurons and astrocytes. Secondly, neighbouring clock neurons have been shown to have synchronous but not necessarily simultaneous electrical and transcriptional/translational activity within different SCN areas (e.g. ventral and dorsal). There is also data to suggest that the phase difference between such activity may reflect the length of the *photoperiod*, that is, the light hours within the 24-h day.¹⁹ This anatomical and functional SCN organization may provide organisms with information relevant to their adaptation to seasonal changes, suggesting that the master circadian clock may also have a calendar-type function.

Peripheral clocks in addition to the SCN have been located in virtually every organ and tissue of the body (Figure 1), including other regions of the brain.³ When isolated, these maintain circadian rhythms of gene expression for days, indicating that the master SCN clock is not indispensable for short-term rhythmicity.²⁰ However, the SCN master clock is the only one to be directly regulated by the light-dark cycle and plays a hierarchical role, affecting the phase and synchrony of clock gene expression in peripheral clocks. This process occurs via the autonomic nervous system and humoral

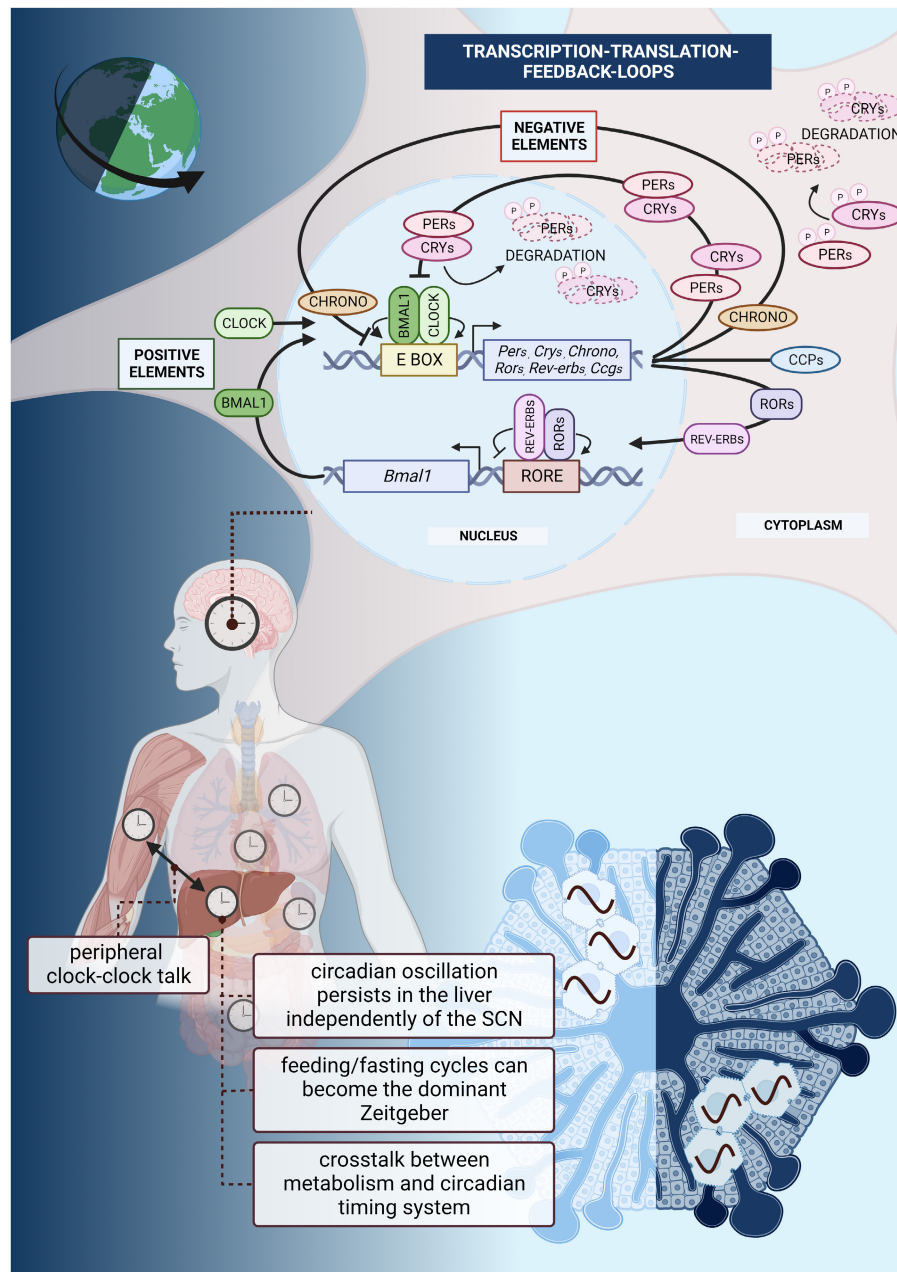


FIGURE 1 Schematic representation of the circadian timing system, including its hierarchy (bottom left: master and peripheral clocks), a hint at its being an adaptation to the Earth's rotation and therefore the light-dark cycle (top left), the core of the molecular mechanism generating the oscillation in the mammalian clock neurons (top right) and the specific features of the liver clock (bottom). With reference to the top right panel, the system consists of interlocking transcription-translation-feedback-loops. In the main one, the transcription factors CLOCK and BMAL1 form heterodimers and trigger the transcription of a number of genes containing, in their promoters, the so-called E box. Amongst these genes, some encode the clock proteins PER1, PER2, PER3, CRY1 and CRY2, which also form heterodimers (PER/CRY). After delayed accumulation in the cytoplasm, these enter the nucleus, where they inhibit the activity of CLOCK and BMAL1 and therefore temporarily block the transcription of their own genes. A synergic contribution to the inhibition of the CLOCK/BMAL1 transcription factors is provided by CHRONO. An overlapping loop in which CLOCK/BMAL1 heterodimers modulate the expression of the genes *Rev-erbs* and *Rors*, the products of which regulate the expression of BMAL1, acts in antiphase to the first loop.

signals.¹¹ Nonetheless, cues such as fasting/feeding, temperature and activity patterns can reset/phase-shift peripheral oscillators²¹ (Figure 1). Finally, and despite an obvious hierarchical relationship, signals from peripheral clocks have been shown to generate SCN feedback in specific circumstances.^{22,23}

3 | THE LIVER CLOCK

A large number of gastrointestinal processes, especially in the liver,^{24,25} are under circadian control. These include, for example, nutrient uptake and processing, which align gastrointestinal function

to the cycling of food availability and supply.²⁶ This way the circadian timing system allows the organism to anticipate and prepare for food intake,²⁷ a still poorly characterized yet crucial function in gastrointestinal physiology.

An endogenous hepatic circadian oscillation was first identified in 1998,²⁸ when the concept of a peripheral clock was extremely innovative. The liver clock has since been shown to generate a very robust oscillation and, by comparison with the clock of other organs, to function for a relatively long time once isolated from the master SCN clock.²⁰ The molecular machinery that generates the circadian oscillation in peripheral clocks, and, therefore, also the liver clock, is almost identical to that of the SCN. However, the liver clock has a set of interesting features (Figure 1).

- Feeding cycles can become the dominant *Zeitgeber* for the liver clock, so much so that extreme changes in meal frequency (i.e., meals that are either very distant or very close to one another in time) can result in the acquisition of a remarkable degree of independence, if not complete uncoupling from the SCN control.²⁹ It has been speculated that this may have an adaptive value.
- The relevance of feeding cycles to the liver clock and their effect on its phase³⁰ underpin a crosstalk between metabolism and the circadian timing system.³¹ This is essential because feeding/fasting patterns can modify some of the molecular characteristics of clock proteins, synchronizing the local clock to their periods. For example, the synthesis of Nicotinamide Adenine Dinucleotide (NAD⁺) involves the gene encoding phosphoribosyltransferase (NAMPT), which is regulated by the CLOCK/BMAL1 heterodimer.³² Thus, the synthesis of NAD⁺ is rhythmic, which results in the modulation of the activity of several proteins in the temporal domain.³³ Amongst these, sirtuin 1 deacetylase (SIRT1) affects, in turn, the transcriptional efficiency of the CLOCK/BMAL1 heterodimer and the stability of the PER2 clock protein, which both feedback to the circadian clock and contribute to the molecular oscillation affecting, for example, its phase and its amplitude.³³ Thus, the control of metabolite sensors like NAD⁺ allows the circadian clock to be informed of the energy status of cells, and to reset oscillations based on metabolic signals.²¹
- Sinturel et al. have recently shown that circadian oscillations persist in the liver of freely moving mice without a functioning SCN or functioning oscillators in cells other than hepatocytes.³⁴ They have also shown that when these SCN-lesioned animals are maintained in constant conditions, the amplitude of their overall body rhythmicity is considerably reduced, suggesting that the SCN is still important to maintain synchrony amongst non-SCN clocks. The authors propose³⁴ that signals—as yet unidentified—from extra-SCN clocks are required for phase coherence of peripheral clock rhythms. This hypothesis is supported also by recent evidence that rhythmic liver gene expression depends also on intercellular communication between different liver cell types.³⁵ In line with what discussed above, this last study also demonstrates that hepatocyte clock-driven changes in the rhythmicity of the transcriptome and metabolome of other liver cells are modulated

by food availability.³⁵ Disease-related alterations in liver structure would be expected to compromise intrahepatic intercellular communication and synchrony, and also the response of the liver to signalling from the SCN and/or other oscillators.

- Currently, the interaction between peripheral clocks is a hot topic in chronobiology. Whilst data are sparse, the most convincing ones come, yet again, from studying the liver clock. Greco et al.³⁶ have recently demonstrated that the liver clock and feeding patterns are sufficient for temporal carbohydrate homeostasis. However, liver rhythms tied to redox and lipid metabolism require the integrity of the skeletal muscle clock, suggesting peripheral clock-clock talk (Figure 1). This observation has implications for the relationship between cirrhosis and sarcopenia (*vide infra*). Along similar lines, it is expected that improved methods to study circadian rhythmicity in the human gut microbiome³⁷ will allow to perform studies on the interaction between the liver and gut/microbiota clocks, possibly offering more complete explanations for the therapeutic effects of microbiota manipulation in liver diseases and their complications.³⁸
- Strikingly and based on a single set of in vivo experiments in mice, it has even been hypothesized that a small portion of the autonomous hepatic oscillation (i.e., in the absence of the SCN) may directly depend on the light–dark cycle.²³ Ray et al.³⁹ have also reported that in animals lacking the core clock component BMAL1, liver slices (previously synchronized with dexamethasone) retained a 24-h rhythm of gene expression, protein abundance and protein phosphorylation for 2–3 days in the absence of any light or temperature cues. The authors suggest that such oscillations might result from the interplay between Erythroblast Transformation Specific (ETS) family transcription factors and redox oscillations for transcriptional and non-transcriptional regulation, respectively. Both the above observations need to be confirmed but remain intriguing.
- Information on hepatic temporal regulation has recently been coupled with the much better-known information on hepatic spatial organization, or zonation (i.e., the complex microarchitecture that allows both compartmentalisation and interplay between anabolic and catabolic activities within the liver), which has traditionally been analysed as a static phenomenon. By use of single-cell RNA sequencing, it has been demonstrated that the expression of many genes in the liver is both zoned and circadian, in most instances with multiplicative effects of the space–time interplay.⁴⁰ Such genes are implicated in lipid, carbohydrate and amino acid metabolism, and also in processes involving protein chaperones.⁴⁰ The effects of diseases affecting liver histology/anatomy to varying degrees on such a complex spatio-temporal organization are difficult to predict but obviously important.

Finally, whilst *ultradian rhythmicity*, i.e. intrinsic biological rhythmicity with a period shorter than 24 h, is considerably less explored than circadian rhythmicity and also beyond the scope of this review, it should be highlighted that evidence is emerging on the role of both 8- and 12-h rhythms—which cycle at the second and third harmonics of circadian frequency—in controlling liver function (for a review, please refer to Ref. [41]).

4 | MISALIGNMENT AND FATTY LIVER—A TOXIC RELATIONSHIP

The term *misalignment* refers to situations in which entrainment (i.e., the process through which the endogenous circadian timing system synchronizes with the environment, *vide supra*) is compromised.⁴² This is generally because of weak or inefficient *Zeitgebers*, often in relation to a set of time constraints imposed by the society we live in, which are collectively known as the *social clock*.⁴³ These include the times around which schools, firms, offices, commercial activities and transport are organized, but also the presence of time zones and, for part of the year, daylight saving time.⁴³ The availability of artificial light over the 24 h, together with the widespread use of devices such as televisions, computers and smartphones in the evening and night hours has also profoundly modified the timing of exposure to the natural light–dark cycle, resulting in poor light–dark hygiene.^{44,45} Misalignment of varying degrees and different origins results in shorter and worse sleep, and it has also been associated with an increased risk of systemic hypertension,⁴⁶ overweight/obesity,⁴⁷ cardiovascular events,⁵ reduced fertility, the likelihood of misusing cigarettes and alcohol,⁶ and certain types of cancer.⁷ These risks are likely to be mediated, at least in part, by shortened/disturbed sleep itself and have been more consistently associated with the most severe and prolonged forms of misalignment, such as night shift work.^{5,7} However, other and generally milder forms of misalignment (i.e., those related to extreme positions within a time zone, daylight saving time, a society functioning mostly in the morning and thus penalizing individuals who are genetically inclined to delayed sleep–wake habits, i.e., late *chronotypes*) are very prevalent in the Western world, and it has been hypothesized that they may contribute to the current metabolic syndrome epidemic⁶ and, in turn, that of metabolic (dysfunction) associated fatty liver disease (MAFLD). It should be highlighted how isolating any contribution of misalignment to an increased risk of MAFLD or alcohol-related fatty liver/steatohepatitis is extremely difficult phenotypically because of the confounding effects of sleep–wake abnormalities of other origins, the most obvious example being obstructive sleep apnea (OSA). OSA is associated with the development and evolution of MAFLD, independently of obesity or other common risk factors.⁴⁸ The OSA–MAFLD association is thought to relate to the degree of nocturnal hypoxemia, which leads to tissue hypoxia.⁴⁹ In turn, tissue hypoxia has been associated with insulin resistance, atherosclerosis, dysfunction in hepatic lipid metabolism and hepatic steatosis.⁴⁸ Despite significant confounding, a number of human studies have started to highlight some circadian-type abnormalities within the sleep–wake profile of MAFLD patients⁵⁰ and an increased prevalence of MAFLD in misaligned (albeit widely qualified as exhibiting “mistimed sleep, late sleep or irregular chronotype”) compared to non-misaligned individuals has also been reported.⁵¹ Some of the pathophysiological mechanisms potentially underpinning these epidemiological observations are also starting to emerge. Several human studies using lipidomics have documented that hundreds of plasma lipids are regulated in the circadian domain, including fatty acids, triglycerides, glycerophospholipids, sterol lipids and sphingolipids.⁵² A high-fat diet leads to significant remodelling of

the liver clock output, disrupting normal circadian cycles and, somewhat unexpectedly, also producing *de novo* oscillating transcripts.⁵³ The mechanisms underlying this remodelling involve impairment in CLOCK/BMAL1 chromatin recruitment and the rhythmic activation of alternative pathways through the transcriptional regulator peroxisome proliferator-activated receptor gamma (PPAR γ , also known as the glitazone reverse insulin resistance receptor).⁵³ The experiment also demonstrated how a short, three-day exposure to the high-fat diet was sufficient to start molecular reprogramming and how switching to a normal diet after a longer period of high-fat diet lead to the resolution of the diet-induced perturbations.^{53,54} This may have implications, for example, for the relationships between binge eating and MAFLD.⁵⁵ Another clinically relevant hypothesis is the possibility that glitazones, which are pharmacological PPAR γ ligands used in the management of diabetes, might be able to mimic the PPAR γ effects described above.⁵⁴ Another study documented how diet-induced obesity caused remodelling of circadian enhancer activity in the mouse liver, triggering detectable oscillations in fatty acid synthesis and oxidation.⁵⁶ The same diet caused a high amplitude circadian rhythm in PPAR α , which was also required for fatty acid oxidation.⁵⁶ Of note, peroxisome proliferator-activated receptors (PPARs) are under study as drug targets for steatohepatitis.⁵⁷ One human study has shown that a significant proportion of circulating metabolites implicated in food metabolism uncouple from the phase of the SCN after simulated shift work schedules, losing rhythmicity or acquiring rhythmicity that appears to be driven by the behavioural schedule (i.e., that of shift work, sleep/wake and feeding/fasting patterns).⁵⁸ Further, by use of unbiased high-throughput proteomics, acetylomics and circadian transcriptomics, Gaucher and co-workers⁵⁹ have shown how acute and chronic alcohol consumption differentially reprogram circadian gene expression in the mouse liver. In turn, genetic disruption of the liver clock worsens both abnormalities in lipid metabolism and steatosis in mice fed with alcohol.⁶⁰ Randomized clinical trials on the effects of modulating meal timing (*chrononutrition*) on weight, adiposity, energy expenditure and hormones/metabolites circadian profiles are sparse but promising,⁶¹ and suggest that circadian hygiene, especially in relation to meal timing, may come to play an important role in preventing MAFLD, and in breaking the vicious circle through which abnormalities in the cross-talk between metabolism and the circadian timing system sustain the onset and progression of fatty liver and steatohepatitis (Figure 2). Finally, and as for the association between MAFLD and hepatocellular carcinoma (HCC) [also in the absence of significant liver fibrosis/cirrhosis⁶²], a single but large and apparently solid US study has documented that an increase in East to West longitude within a given time zone is associated with a statistically significant increase in HCC risk in individuals under 65 years of age,⁶³ adding HCC to the list of neoplasms for which misalignment seems to represent a risk factor.⁶⁴ Of note, the impact on hepatocarcinogenesis depended on misalignment from residing to the West of a time zone, in agreement with the concept that jet-lag East, and thus the attempt to move endogenous timing forward, is generally more difficult and more unpleasant (more pronounced and more prolonged jet-lag symptoms) than the opposite process.⁶⁵

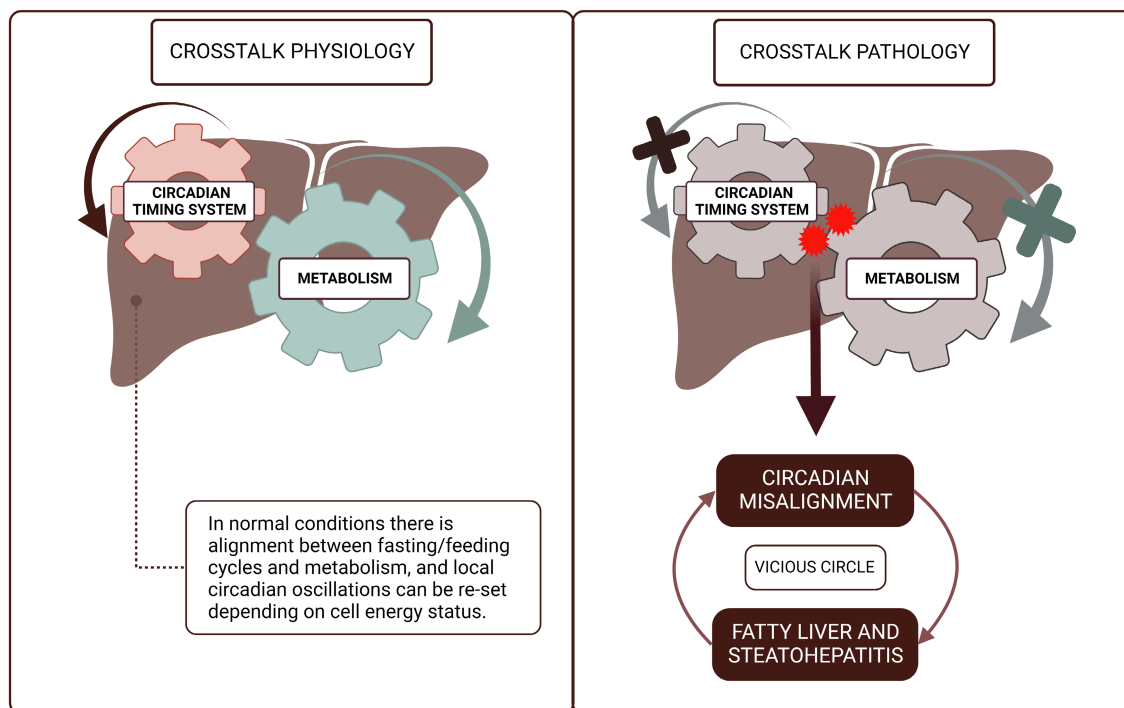


FIGURE 2 Physiology (left) and pathology (right) of the crosstalk between the circadian timing system and metabolism, which occurs in the liver and may contribute to the pathophysiology of fatty liver and steatohepatitis.

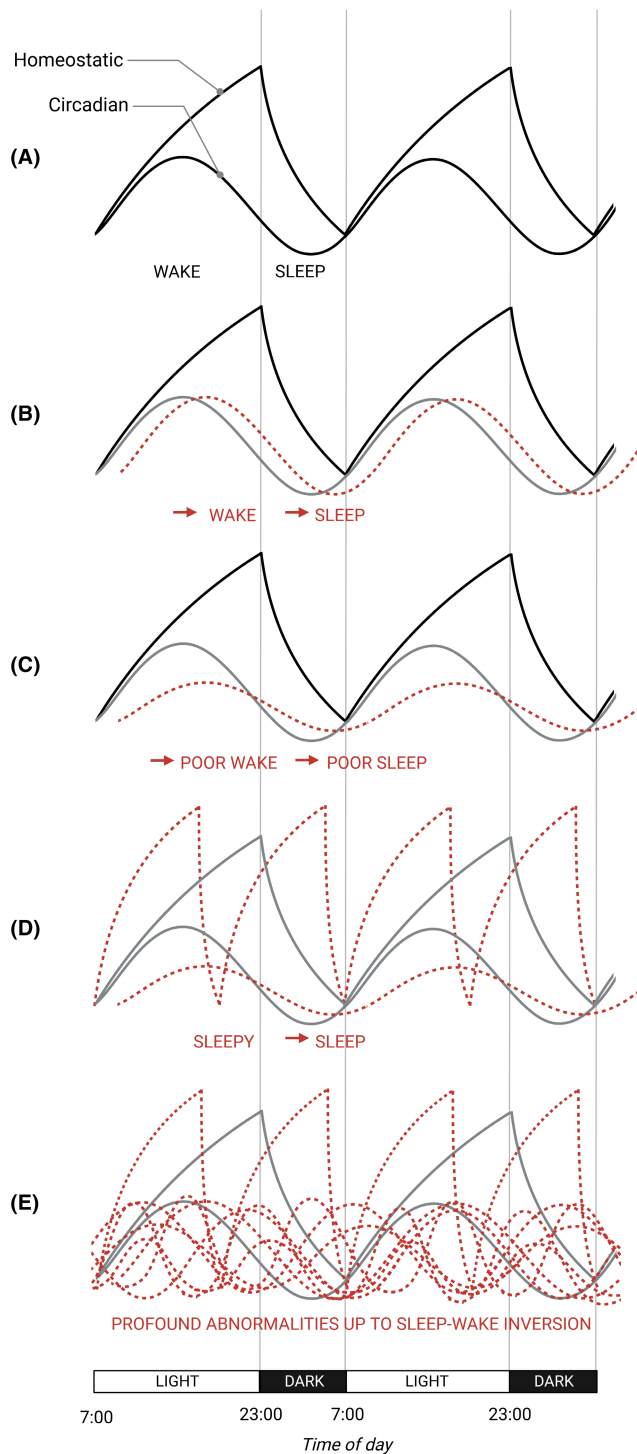
5 | CIRCADIAN DISRUPTION IN CIRRHOSIS

Patients with cirrhosis exhibit several circadian abnormalities. These were originally characterized with a view to identify and hopefully correct any circadian impairment that may contribute to the sleep–wake disturbances these patients commonly report, i.e. difficulties falling asleep, frequent night awakenings, delayed sleep–wake habits and excessive daytime sleepiness up to sleep–wake inversion.^{66–68} Such sleep–wake disturbances are difficult to manage with standard hypnotics both because of delays in hepatic metabolism and enhanced sensitivity of patients with cirrhosis to psychoactive medication.^{69,70} Further, whilst all sleep–wake abnormalities exhibited by patients with cirrhosis were traditionally considered to be part of the hepatic encephalopathy (HE) syndrome, in more recent years it has become clear that only excessive daytime sleepiness and the striking phenomenon of sleep–wake inversion is associated with HE of varying severity.^{71,72} By contrast, insomnia seems to be more of a feature of cirrhosis itself, also by comparison with other chronic diseases of similar severity^{73,74} and even in the absence of any cognitive impairment. It is however obvious that as sleep–wake disturbance impinges on cognitive performance, the relationship between sleep–wake disturbance and HE will always be difficult to study.⁷⁵ The circadian phenotype of patients with cirrhosis can be summarized as follows:

- Delayed sleep–wake habits (60–90 min), which tend to associate with worse sleep quality.^{73,76} Delayed sleep–wake habits seem to be independent of the presence/degree of HE⁷³ and most likely also of the

aetiology of cirrhosis^{76,77} but solid data on the latter issue are lacking.

- Reduced SCN sensitivity to dark/light cues plus minor impairment in hepatic clearance of melatonin, mostly at night, when the levels of the hormone are high.^{78–80} The combination of the above determines an overall delay in the melatonin peak and abnormally high daytime melatonin levels. Similar abnormalities are observed in the 24-h rhythm of cortisol,⁸¹ and they parallel the delays in the sleep–wake patterns.⁷⁶ The beneficial effect of the administration of strong morning light (*chronotherapy*) on sleep timing, sleep quality and urinary melatonin rhythmicity^{82–84} also supports the concept that central circadian disruption may underpin part of the sleep–wake abnormalities exhibited by patients with cirrhosis. Since it is now accepted that the SCN overall output oscillation is the result of the *quasi* antiphasic oscillation of clock neurons and astrocytes¹⁸ and it is also well established that hyperammonaemia causes both functional and morphological astrocyte abnormalities,⁸⁵ we have recently hypothesized and demonstrated that hyperammonaemia acts directly on the SCN to disrupt its output oscillation and, in turn, daily rhythms in physiology and behaviour.⁸⁶ Critically, these effects were reversed by ammonia removal or by glutamate receptor antagonists.⁸⁶
- Abnormal skin temperature, with high average values and limited variation over the 24 h,⁸⁷ most likely in relation to the peripheral vasodilation/abnormalities in blood distribution which characterize the disease. This translates into an inability to prepare for sleep with the physiological mechanisms of vasodilation and warming of the extremities and has been shown to be associated with increased sleep latency.⁸⁷ Whilst skin temperature is not a circadian parameter and cannot be used as an equivalent of core body temperature, when measured at multiple sites it provides a



reliable estimate of heat loss, which plays an important part in the circadian variation of core body temperature. We were able to explore and confirm the relationship between skin and core body temperature changes in bile duct-ligated rats.⁸⁸

As our understanding of the circadian timing system and its relationship with *homeostatic sleep regulation* (i.e., the phenomenon mediated by the neurotransmitter adenosine whereby our propensity to sleep increases over the waking hours, regardless of the time of day^{89,90}) evolves, it becomes more and more difficult to attribute

FIGURE 3 (A) Normal interaction between the circadian oscillation in sleep propensity over the 24 h and the increase in homeostatic sleep pressure (i.e., the increase in sleep propensity that accumulates during the waking hours) in a healthy person: the greater the distance between the two curves (23:00), the higher the sleep propensity/likelihood of falling asleep (adapted from Ref. [89]). (B) Abnormal interaction between the homeostatic regulation (black line) and the delayed circadian rhythm (red broken line) in a patient with cirrhosis with or without HE; grey line: reference circadian oscillation in the healthy population. The lack of synchrony between the two processes leads to a delayed sleep phase-type sleep disorder, which could contribute to the observed difficulties in commencing and maintaining sleep (adapted from Ref. [95]). (C) Abnormal interaction between the homeostatic regulation (black line) and the delayed circadian rhythm (red broken line) in a patient with cirrhosis with HE, in whom daytime somnolence, inactivity, reduced muscle mass etc. reduce the amplitude of the delayed circadian oscillation; grey line: reference circadian oscillation in the healthy population. (D) Abnormal interaction between homeostatic fluctuations (red broken line) and delayed plus blunted circadian rhythmicity (red broken line) in a patient with cirrhosis and HE; grey lines: reference circadian oscillation and homeostatic build-up in the healthy population. Hyperammonaemia/HE results in magnified and short-lived adenosine responses to the build-up of sleep pressure during the waking hours. This may translate into an inability to generate slow-wave, restorative sleep and a less efficient recovery from sleep deprivation (adapted from Ref. [93]). (E) Abnormal interaction between homeostatic fluctuations (red broken line) and profoundly desynchronised circadian rhythm (multiple red broken lines) in a patient with cirrhosis and a bout of severe HE; grey lines: reference circadian oscillation and homeostatic build-up in the healthy population. Hyperammonaemia/HE results in magnified and short-lived adenosine responses and completely desynchronises the output oscillation from the master clock. This results in profound sleep-wake abnormalities, up to sleep-wake inversion.

any specific circadian/sleep-wake feature observed in a patient with cirrhosis to cirrhosis *per se*, HE or their combination (Figure 3). It would seem reasonable to attribute mostly to HE excessive daytime sleepiness,^{72,91} which also results in inactivity and thus in weakened *Zeitgebers* (Figures 3 and 4), impaired light perception and consequent melatonin/cortisol rhythm abnormalities,^{80,81} desynchronisation of the master circadian clock output,⁸⁶ abnormalities in the homeostatic response to sleep deprivation⁹² and in the ability to produce deep, restorative sleep⁷² (Figure 3). It should also be highlighted that patients with cirrhosis - and those with HE in particular - are extremely sensitive to psychoactive medication,⁶⁹ including that used in attempts to treat their sleep-wake abnormalities, which often result in prolonged sedation and paradoxical reactions⁹³ or bouts of HE,⁷⁰ sustaining sleep-wake and possibly also circadian abnormalities in a vicious circle. Finally, alcohol itself and drugs that are commonly used in patients with cirrhosis, for example beta-blockers, are known to affect melatonin synthesis,⁹⁴ potentially worsening any HE-related abnormalities in the rhythm of this crucial circadian hormone.

There are further, mostly unexplored mechanisms through which cirrhosis and especially decompensated cirrhosis might affect the circadian system (Figure 4). For example, abnormal liver architecture/

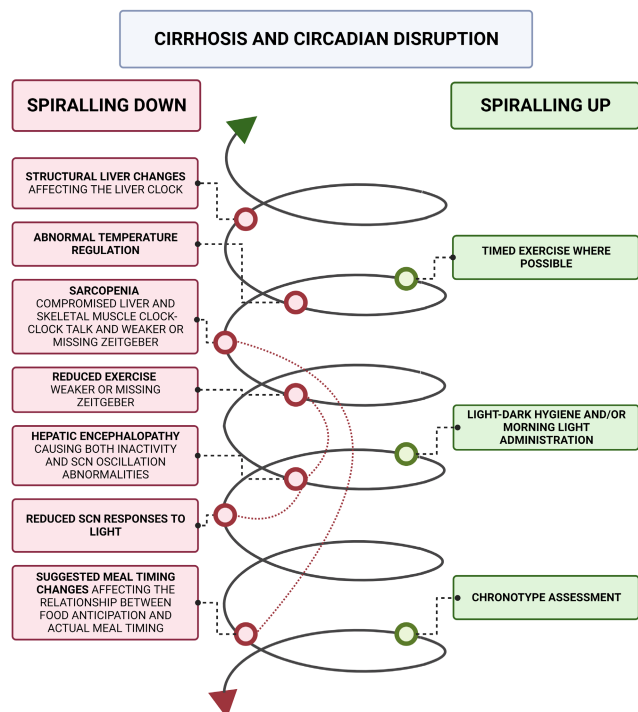


FIGURE 4 Schematic representation of the circadian abnormalities (proven or hypothetical) which characterize and then self-sustain in patients with cirrhosis (red, left and spiralling down) and a set of simple tests or hygienic measures that may help manage them (green, right and spiralling up). The dashed red connections between red circles highlight proven or hypothetical pathophysiological relationships.

vascularization may lead to liver clock dysfunction. Compromised glycogen storage and protein synthesis may affect the physiological, rhythmic oscillations in glucose/protein metabolism which anticipate and follow meals. Sympathetic/parasympathetic imbalance may compromise the autonomic transmission of signals from the SCN to the liver clock. In addition, malnutrition and sarcopenia lead to a reduced ability for physical activity and exercise,⁷⁵ which in healthy individuals act as accessory *Zeitgebers* for the circadian timing system. Similarly, HE reduces daytime alertness, weakening the wake-sleep cycle. The combination of all such mechanisms⁹⁵ may lead to an abnormal SCN-liver clock interaction, and possibly also to the uncoupling of the liver clock from the SCN. There is also evidence that stressors such as sleep fragmentation and sleep deprivation, which are extremely common in patients with cirrhosis, impinge on the 24-h rhythm of the healthy human metabolome⁵⁸ and that the liver clock also responds to signals from the microbiome,⁹⁶ which is heavily modified both by cirrhosis itself⁹⁷ and by the treatment of its complications.⁹⁸ Hepatic xenobiotic detoxification is also under temporal control, which provides some bases for the dosing time-dependence of drug efficacy and toxicity in otherwise healthy individuals.⁹⁹ This is likely to be relevant to the management of patients with cirrhosis and acute liver failure, who are on multiple drugs with stand-alone and combined pharmacokinetics/pharmacodynamics which become very difficult to predict.¹⁰⁰

Finally, the relationship between malnutrition/sarcopenia which is frequently associated with decompensated cirrhosis and circadian disruption deserves special attention (Figure 4). The onset of malnutrition/sarcopenia marks a defining point in the evolution of cirrhosis, with all its major complications (HE, uncontrolled ascites, spontaneous bacterial peritonitis and hepatorenal syndrome) being more common in malnourished patients.¹⁰¹ Malnutrition is also associated with the severity of cirrhosis, so much so that the original version¹⁰² of the Child-Pugh score¹⁰³ included a nutritional status index, which was subsequently dropped because its assessment was perceived to be too operator-dependent. Ultimately, malnutrition/sarcopenia seems to be more of a feature than a complication of cirrhosis, and its aetiology has been generally attributed to combined abnormalities in oral intake, absorption and metabolism of nutrients.¹⁰⁴ The possibility that it may also relate to liver clock dysfunction and central-peripheral clock desynchrony has remained substantially unexplored. However, given the features of the liver clock we have already discussed, and the structural abnormalities that characterize a cirrhotic liver, it seems a plausible hypothesis. Whichever its exact origin, a premature switch from satiety to fasting state metabolism, and thus from carbohydrate to amino acid utilization for energy production, ultimately sustains the hypercatabolic state which characterizes cirrhosis. Accordingly, commonly utilized treatment strategies have been focused on increasing caloric intake and/or shortening the interval between meals.¹⁰⁵ The main emphasis has been on late-evening snacks, which are meant to cover the longest inter-meal interval (evening meal to breakfast), and thus avoid the overnight utilization of amino acids as energy substrates.^{106,107} Accordingly, malnourished patients with cirrhosis are encouraged to avoid long periods of fasting and to split their food intake into small, frequent meals. This is difficult operatively, as these patients struggle to cope with three main meals and three snacks (frequent hyporexia, dysgeusia, early satiety and prescription of unpalatable diets¹⁰⁴), and has not been consistently shown to be beneficial, with the exception of iatrogenic malnutrition deriving from inappropriate or excessively strict dietary prescriptions.¹⁰⁸ A circadian perspective of fasting/feeding patterns may help to explain the difficulties in managing malnutrition in advanced liver disease. Firstly, and as already discussed, the circadian timing system allows to anticipate food intake and the timing of such anticipatory/preparatory behaviour is not easily modified. Further, it has been demonstrated that in healthy individuals a 5-h delay in meal timing resulted in a comparable delay in the phase of the rhythm of glucose in laboratory conditions. Such abnormal glucose rhythms were not accompanied by changes in markers of the phase of the master clock, or in the rhythms of insulin and triglycerides.¹⁰⁹ Therefore meal timing seems to affect glucose more than lipid metabolism, and to dissociate their rhythmicity. The study also concluded that the absence of changes in SCN phase markers in relation to changes in meal timing suggests that feeding patterns can synchronize peripheral clocks in humans.¹⁰⁹ In another human experiment studying the temporal response of lipids and hepatic proteins to combined light and food cues, greater shifts were observed for lipids and hepatic

proteins compared to melatonin, with albumin and triglyceride exhibiting peak times close to that of melatonin, and cholesterol and high-density lipoprotein being offset by approximately 12 h.¹¹⁰ It is therefore reasonable to hypothesise that in patients with cirrhosis changes in meal timing, and especially the introduction of the evening snack, may have rather unpredictable and possibly even damaging effects on an already very deranged carbohydrate and protein metabolism. Finally, the recent observation³⁶ that liver rhythms tied to redox and lipid metabolism require the integrity of the skeletal muscle clock offers a novel, potential explanation for the vicious circle between the onset of sarcopenia and the worsening in liver function that is so commonly observed in decompensated cirrhosis.

6 | LIVER TRANSPLANTATION WITHIN A CIRCADIAN PERSPECTIVE

To date, limited attention has been devoted to liver transplantation from a circadian perspective. However, the liver clock is an extremely robust autonomous oscillator,³⁹ thus the timing of transplantation may modulate transplant outcomes. Further, the phase of a transplanted liver may be more or less close to that of the recipient's, most likely impinging on the time it will take for the transplanted liver to be "captured" by the recipient's SCN. This process may be slowed or impaired also by the fact that the transplanted liver is denervated,

and thus receives SCN signals only via humoral pathways, and not via both humoral and neural pathways as in healthy individuals. In addition, this process generally takes place in an intensive care environment, where *Zeitgebers* are profoundly abnormal (i.e., continuous light, continuous feeding etc.). It is more than likely that all the above will impinge, at least to some extent, on immediate and mid-term transplant prognosis. Also, it is well known to chronobiologists that dexamethasone synchronizes the phase of circadian liver gene expression across the 24 h,¹¹¹ thus the use of steroids prior to, during or immediately after transplantation may have effects other than the anti-inflammatory and immunosuppressant expected ones, and could probably be timed in a constructive fashion. All these issues are difficult but not impossible to study in the clinical setting, and even retrospective, collaborative studies might be useful.

7 | CONCLUSIONS AND FUTURE WORK

In conclusion, it would appear that both a circadian perspective in analysing the pathophysiology of liver disease and the application of chronobiological principles to its treatment may contribute to the understanding and management of MAFLD,¹¹² the complications of cirrhosis and those of liver transplantation (Table 1). Another interesting application, which has not been covered in this review, may be the treatment of pruritus and fatigue in primary biliary cholangitis,

TABLE 1 Future research agenda

Review section	Topics for future studies
The liver clock	How disease-related structural changes in liver architecture affect intercellular communication and synchrony within the liver How disease-related structural changes in liver architecture affect interactions with the master clock and other peripheral clocks Liver and gut/microbiota clock interaction, also in relation to gut/microbiota clock modulation as part of liver disease management
Misalignment and fatty liver	Baseline circadian profile of individuals who go on to develop MAFLD/NASH over time Long-term effects on MAFLD/its evolution of circadian hygiene measures, particularly in relation to food and exercise timing Time-course of the metabolomic, lipidomic and acetyloomic profiles of MAFLD patients Role of misalignment in the development of HCC in MAFLD/NASH
Circadian disruption in cirrhosis	Relationship between delayed sleep phase syndrome features and the aetiology of cirrhosis Effects of timed melatonin administration (with or without timed light administration) on delayed sleep phase syndrome Effects of proximal temperature modulation on delayed sleep onset Effects of timed food administration on nutritional status and sarcopenia Circadian and 24-h metabolomic effects of the prescribed evening snack in malnourished patients with cirrhosis
Circadian disruption in hepatic encephalopathy	Exact relationship between circadian and cognitive impairment by interventional, controlled studies Effects of timed melatonin administration (with or without timed light administration) on sleepiness and cognitive performance Relative role of the neuronal and astrocytic components of the master clock in the response to hyperammonaemia Relative role of circadian and homeostatic sleep regulation in the pathogenesis of sleep-wake inversion Role of the impairment in liver and muscle clock-clock talk in the relationship between sarcopenia and cognitive performance
Circadian perspective of liver transplantation	Transplantation outcomes in relation to: <ul style="list-style-type: none"> • timing of transplantation • chronotype of donor and recipient • circadian-friendly intensive care/general wards versus standard of care • timed steroid utilization prior to and after transplantation

which has been shown to exhibit diurnal variation¹¹³ and also to benefit from light therapy.⁸⁴ Ultimately, the specific features of the liver clock and those of the sleep–wake profile of patients with cirrhosis have already made hepatology a well-established *niche* model for the study of disease-related circadian abnormalities. As knowledge continues to grow on the interactions between peripheral clocks, tools start to become available for an easier assessment of human internal circadian time¹¹⁴ and chrononutrition becomes a discipline with a clearer pathophysiological basis¹¹⁵ and better-established protocols, hepatology may also become an ideal field to test the value of circadian hygiene advice and that of chrononutrition in both disease prevention and disease management.¹¹⁶ Like all translational processes, this will require interaction between different disciplines and different professions, and a considerable amount of patience, goodwill and open-mindedness from all parts involved. Hepatologists will need to learn to factor time into their pathophysiological and clinical reasoning more and more, and chronobiologists will need to accept that clinical studies in any healthcare setting will always be less controlled and “noisier” than the ones they are used to.¹¹⁷ Still, this process has already started, and it only needs to be made smoother and faster, for the benefit of both liver patients and society at large.

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