On the Origin and the Consequences of Circadian Abnormalities in Patients with Cirrhosis

Circadian and sleep profiles in cirrhosis

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List of Abbreviations

aMT6s: 6-sulphatoxymelatonin; AUC: area under the curve; CIU: clinical investigations unit; CV: coefficient of variation; EEG: electroencephalogram; PHES: psychometric hepatic encephalopathy score; RIA: radioimmunoassay; SCN: suprachiasmatic nuclei

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OBJECTIVES: Plasma melatonin profile abnormalities have been described in patients with cirrhosis and generally attributed to impaired hepatic melatonin metabolism. The possibility that they might reflect circadian clock dysfunction has not been explored. In addition, the relationship between plasma melatonin profiles and the sleep disturbances observed in these patients remains unclear. The aims of this study were: *i*) to evaluate circadian clock function and hepatic melatonin metabolism in cirrhotic patients; *ii*) to study the relationship between plasma melatonin profiles and sleep-wake behaviour.

METHODS: The study population comprised 20 cirrhotic patients (59 [39-77] yr) and nine healthy volunteers (60 [38-84]). Plasma melatonin/cortisol concentrations were measured hourly, for 24 hours, in light-/posture-controlled conditions. Urinary 6-sulphatoxymelatonin, the main melatonin metabolite, was measured simultaneously to determine clearance. The ability of light to suppress nocturnal melatonin synthesis was assessed. Habitual sleep quality/timing were evaluated using a questionnaire, actigraphy and sleep diaries.

RESULTS: There was evidence of central circadian disruption in the patients compared to the healthy controls: peak plasma melatonin/cortisol times were delayed (04:48±02:36 vs. 02:48±00:54, $p=0.01$; 10:18±02:54 vs. 08:54±01:24, $p=0.06$) and the plasma melatonin response to light reduced (12±19 vs. 24±15%, $p=0.09$). However, the mean 24-hour plasma melatonin clearance did not differ significantly between the patients and healthy volunteers (0.22±0.10 vs. 0.28±0.17 L/Kg/hr, $p=0.36$). Finally, although patients showed a degree of misalignment between sleep and circadian timings, there was no association between circadian abnormalities and impaired sleep quality.

CONCLUSIONS: Plasma melatonin profile abnormalities, predominantly central in origin, are observed in patients with mild to moderately decompensated cirrhosis. However, they are substantially unrelated to the sleep disturbances prevalent in these patients.

INTRODUCTION

The master circadian clock, which is located in the suprachiasmatic nuclei (SCN) of the hypothalamus, regulates sleep timing and other 24-hour, or *circadian*, behavioural, physiological and biochemical rhythms. The average, endogenous circadian period is approximately 24.18 hr (1) and must be synchronized constantly to the 24-hour day by external influences. Light is the major external time cue and reaches the SCN by afferent projections from the retina. The SCN projects, in turn, to the pineal gland, regulating the production of melatonin, which can be thought of as the ‘neuroendocrine transducer’ of the light-dark cycle. Thus, in healthy individuals, plasma melatonin levels are high during night sleep and low during the waking day. Night-time exposure to bright light causes an acute suppression of pineal melatonin synthesis (2); this effect is used to assess the integrity of the retinal-SCN-pineal pathway (3). Melatonin is hydroxylated/sulphated to 6-sulphatoxymelatonin (aMT6s), primarily in the liver (4); aMT6s is subsequently excreted in the urine, where its profile mirrors that of plasma melatonin (5).

Sleep-wake disturbances are common in patients with cirrhosis (6-8) but the pathophysiology remains unknown. As melatonin is metabolized in the liver, it has been hypothesized that circadian sleep regulation might be implicated. Indeed, certain reported abnormalities, including high day-time plasma melatonin concentrations (9,10), low urinary aMT6s concentrations (11) and a reduction in the clearance of exogenously administered melatonin (12), support this hypothesis. However, other reported abnormalities, for example delays in the nocturnal rise of plasma melatonin and in its time to peak (9, 13), can not be explained by impairment of hepatic metabolism but might indicate dysfunction of the central circadian clock, and hence circadian timing.

The primary aim of this study was to determine the aetiology of the abnormalities of the plasma melatonin profile observed in patients with cirrhosis and, specifically, to assess the

role of:

1. Central circadian output, by: *i)* simultaneous assessment of the 24-hour rhythm of two reliable markers of the hypothalamic clock phase, *i.e.* plasma melatonin/cortisol, and *ii)* measurement of the plasma melatonin response to nocturnal administration of bright light;
2. Hepatic melatonin metabolism, by: *i)* comparison of the plasma melatonin and urinary aMT6s 24-hour profiles; *ii)* measurement of plasma melatonin clearance.

The secondary aim was to assess the contribution of circadian regulation to the sleep-wake abnormalities observed in this patient population.

PATIENTS AND METHODS

Study subjects

The patient population comprised 20 consecutively enrolled individuals of mean (range) age 59 (39-77) yr, with biopsy-proven cirrhosis, which was alcohol-related in the majority. The functional severity of the liver injury was assessed using Pugh's modification of the Child grading system (14). Patients were further classified as having *compensated* (Pugh's score ≤ 7 ; n=15) or *decompensated* (Pugh's score > 7 ; n=5) disease.

Patients were excluded from the study if they had misused alcohol in the preceding 6 months, had an estimated glomerular filtration rate of < 80 ml/min, a history of insulin-dependent diabetes mellitus, significant head injury, cardio- or cerebro-vascular disease, significant neurological/psychiatric co-morbidity, corrected visual acuity of less than 6/6; if they were taking neuroactive medication/medication known to affect sleep and/or melatonin production, had travelled across more than two time zones in the preceding three months or undertaken shift work in the preceding five years or were not sufficiently robust to undergo the study procedures.

The reference population comprised nine healthy volunteers of mean age 60 (38-84) yr. None drank alcohol in excess of 20 g/day, had corrected visual acuity of less than 6/6, were taking prescription medication, or had travelled or undertaken shift work as defined above.

Study design

Individual studies were conducted over one 15-day period, in three separate locations *viz*: the Royal Free Hospital, London (clinical evaluation, sleep questionnaire), the patients' homes (sleep diaries, actigraphy and screening urinary aMT6s profile) and the Clinical Investigations Unit (CIU) of the University of Surrey (plasma melatonin/cortisol profiles; urinary aMT6s profile; plasma melatonin response to light) (Table 1).

Subjects were asked to refrain from taking caffeinated/alcohol-containing beverages and to avoid strenuous exercise and exposure to bright natural light from day 12 onwards. Controls were exercised for both light and posture during the time spent in the CIU (Table 2). A maximum of three subjects were studied at any one time, to allow close surveillance and to ensure compliance.

Sleep wake profile (Days 1-12)

- ***Subjective sleep quality (Day 1).*** Subjects were asked to complete the Pittsburgh Sleep Quality Index (PSQI) (15). This is a self-administered questionnaire and is used to assess subjective sleep quality over the preceding month; total PSQI scores of >5 (range 0-21) identify ‘poor’ sleepers (15).
- ***Sleep diaries.*** Subjects kept individual sleep diaries for 12 consecutive days, in which they recorded their *bed time*, *the time they started trying to sleep*, their *sleep onset*, *wake-up* and *get-up times* and the *number of naps* they took during the following day (16).
- ***Actigraphy.*** An actigraph is a device that records movement by means of an accelerometer. Its use in sleep studies is based on the simple assumption that lack of movement equates to sleep. The accuracy of this assumption is increased substantially if actigraphy is used in conjunction with sleep diaries. In this study, an Actiwatch® (Cambridge Neurotechnology Ltd., Cambridge, UK), which is the size of a wrist-watch, was worn on the non-dominant wrist throughout the 12 days, except when showering or bathing. The monitors were set to record for 60-second epochs and were downloaded at the end of the complete recording period. Quantitative information on the variables *trying to start to sleep* and *wake-up time* was obtained from the corresponding sleep diary and entered into the manufacturer’s program for calculation of the following indices: *actual sleep time* (duration in hours); *sleep latency* (time to fall asleep); *sleep efficiency* ($100 \times [\text{actual sleep time} / \{\text{wake-up time} - \text{trying to start to sleep time}\}]$); *immobile time*

(time spent immobile between trying to start to sleep and wake-up time, as a percentage); *night activity* (total number of activity counts between trying to start sleep and wake-up times); *fragmentation index*, an indicator of restlessness (the sum of percentage time spent moving and the percentage immobility phases of 1-min length) (17).

Urinary aMT6s profile (Days 1-3; 13-14)

Two separate urine collections were undertaken:

- Days 1-3: to determine the timing for light exposure (*vide infra*);
- Days 13-14: to determine plasma melatonin clearance.

Sequential urine samples were collected at 4-hour intervals during the day and 8-hour intervals overnight. Aliquots of 3 ml were extracted from each individual collection and kept at -20 °C until assayed for aMT6s, using a radioimmunoassay (RIA) (18). The intra-assay coefficients of variation (CV) ranged from 10-11%; the inter-assay CV ranged from 6-12%; the limit of detection was 0.3 ng/mL. The total 24-hour urinary aMT6s output was obtained. The urinary aMT6s profile was evaluated using cosinor analysis, which is based on the least square approximation of the time series using a cosine function with a period of 24 hr (19). If the function fits the time series, the latter is deemed to have a sinusoidal 24-hour rhythm and a number of so-called 'cosinor' parameters can be reliably obtained. Data were accepted if the probability of the cosinor fit (p) was <0.05 (19). The circadian parameter *acrophase time* - maximum of the fitted cosinor function, or time of the peak urinary aMT6s concentration - was obtained and classed as *delayed* if it occurred after 06:00 (20), regardless of sleep-wake timing.

Plasma melatonin and cortisol profiles (Days 13-14)

Venous blood samples for measurement of plasma melatonin/cortisol concentrations were obtained hourly between 18:00 and 10:00 and two-hourly between 10:00 and 18:00, between days 13-14.

Assays

Plasma melatonin was measured using a RIA (21); the intra-assay CV ranged from 7-14%; the inter-assay CV ranged from 9-11%; the limit of detection was 2.6 pg/mL. Plasma cortisol was also measured using a RIA (22); the intra-assay CV ranged from 5-12%; the inter-assay CV ranged from 8-15%; the limit of detection was 10.5 nmol/L.

Cosinor analysis

Plasma melatonin/cortisol profiles were examined by cosinor analysis (19); the data were accepted if the probability of the cosinor fit (p) was < 0.05 for melatonin and < 0.2 for cortisol, because of the more erratic nature of its rhythm (23). The following parameters were obtained: *acrophase time*: maximum of the fitted cosinor function, or time of the peak plasma melatonin/cortisol concentration; *mesor*: average plasma melatonin/cortisol concentration over the 24 hr and *amplitude*: difference between the mesor and the acrophase plasma melatonin/cortisol concentrations.

Onset/Offset analysis

The *onset* and *offset* of the plasma melatonin and cortisol rhythms were defined as the time points, on the rise and fall of the concentration-time curve, when the plasma concentrations equalled 25% of the peak concentration (24); these parameters could only be calculated in subjects with a discernable peak on inspection. The time interval between the rhythm *onset* and *offset* was defined as the peak *duration*.

Melatonin clearance

The area under the curve (AUC) of the plasma melatonin profile was calculated by integrating

point plasma concentrations over the time interval between consecutive samples. Estimates of plasma melatonin clearance (L/Kg/hr) were obtained, based on the assumption that aMT6s was the only metabolite:

$$\frac{k [24\text{-hour urinary aMT6s output (ng)]}{24 [\text{average plasma melatonin (pg/ml)}][\text{body weight (kg)}]}$$

where $k = \text{aMT6s molecular weight / melatonin molecular weight}$

The overnight (23:00-08:00) clearance was also calculated, using matched timed plasma/urine samples.

Sleep-circadian phase

The *phase relationship* between circadian and sleep rhythms was assessed by subtracting the average *wake-up time* over the 12 monitored days from the plasma melatonin rhythm *onset time* (reference range: 12.5-17.1 hr [25]).

Light suppression test (Day 15)

Light administration time was standardized so that individuals were exposed to light during the rising phase of their plasma melatonin rhythm; this was assumed to occur 2.5-4.5 hr prior to the urinary aMT6s acrophase, estimated using the urine collections performed on days 1-3 (26).

Pupils were dilated using 0.5% topical tropicamide. Subjects sat in front of a light sphere (45 cm diameter, Apollo Lighting) for 30 min, with their head on a chin rest; they kept their eyes open and looked straight ahead (27). White light (4.1×10^{14} photons/cm²/second) was generated by a metal halide arc lamp source (Enlightened Technology Associates, Inc., Fairfax, VA, USA), and delivered via a fibre-optic bundle (27). Blood was taken every 15 min for 90 min from the beginning of light administration.

Maximal melatonin suppression was defined as the percentage decrease in plasma melatonin concentration after 30 min of light exposure (27). An average of the point

suppression values was also calculated; individuals with negative averages were classed as showing no response.

Statistical analysis

Data are presented as mean $\pm$ 1SD or SEM (range). The distribution of variables was tested for normality using the Shapiro-Wilk's W test. Differences between normally/non-normally distributed variables were examined using the Student's t/Mann-Whitney U test. Categorized indices were compared by the Maximum Likelihood χ^2 or Fisher's exact test, as appropriate. Correlations between indices were tested using the Spearman's R.

Ethics

The study was conducted according to the Declaration of Helsinki (Hong Kong Amendment) and Good Clinical Practice (European Guidelines). The protocol was approved by the Royal Free Hampstead NHS Trust Ethics Committee and the University of Surrey Ethics Committee. All participating subjects provided written, informed consent.

RESULTS

Subject characteristics and sleep behaviour

Fourteen (70%) of the 20 patients enrolled were classed as Child's grade A, four (20%) as grade B and two (10%) as grade C; of these, 15 (75%) were further classified as having compensated and 5 (25%) as having decompensated disease. The patients and the healthy volunteers had similar age and gender distributions (Table 3).

Sleep quality was generally impaired in the patients compared to the healthy volunteers (PSQI abnormal: 70% vs. 33%, $p=0.07$; actigraphy night activity: 11380 ± 12089 vs. 5509 ± 2025 counts, $p=0.07$; actigraphy fragmentation index: 41 ± 19 vs. 32 ± 7 , $p=0.08$); the mean actigraphy immobile time was significantly decreased ($79 \pm 11\%$ vs. $86 \pm 3\%$, $p=0.04$) (Table 3). Sleep timing was generally delayed in the patients; the mean wake-up time was significantly delayed ($07:42 \pm 02:00$ vs. $06:36 \pm 00:54$ hr; $p=0.03$) (Table 3).

Circadian profiles in healthy volunteers and patients with cirrhosis

Urinary aMT6s

Cosinor analysis showed an acceptable fit in all subjects. The mean urinary aMT6s acrophase was delayed by approximately 1.5 hr in the patients compared to the healthy volunteers, but the difference was not significant ($05:12 \pm 02:24$ vs. $03:48 \pm 01:30$; $p=0.13$). The urinary aMT6s acrophase was abnormally delayed (after 06:00) in eight (40%) patients but in none of the healthy volunteers ($p=0.007$).

Plasma melatonin

Cosinor analysis showed a significant fit in seven (78%) healthy volunteers and in 19 (95%) patients; onset/offset analysis was possible in seven (78%) healthy volunteers and in 18 (90%) patients. There were no significant/trend differences in plasma melatonin *mesor* or *amplitude* between patients and healthy volunteers (Table 4). The plasma melatonin rhythm was

delayed in the patients compared with the healthy volunteers, the delay affecting the rhythm *onset* ($p=0.09$), its *acrophase* ($p=0.01$) and its *offset* ($p=0.08$) (Table 4, Figure 1). No significant/trend differences were observed in the peak *duration*.

Patients with decompensated cirrhosis showed trend delays in plasma melatonin rhythm *onset* ($24:21\pm02:12$ vs. $22:29\pm01:14$, $p=0.08$) and in urinary aMT6s *acrophase time* ($06:45\pm01:49$ vs. $04:45\pm02:26$, $p=0.07$) compared with their compensated counterparts; in addition, they had a significantly higher plasma melatonin *mesor* (47.5 ± 15.3 vs. 31.2 ± 20.6 pg/ml, $p=0.05$).

Significant correlations were observed between plasma melatonin rhythm parameters and stand-alone/composite indices of hepatic dysfunction (Table 5).

Plasma cortisol

Cosinor analysis showed an acceptable fit in eight (89%) healthy volunteers and in 18 (90%) patients. Onset/offset analysis could be performed in all subjects; however, in two (22%) healthy volunteers and seven (35%) patients the plasma cortisol rhythm *offset* occurred after 18:00, beyond sampling time, thus the parameters *offset* and *duration* could not be obtained. There were no significant/trend differences in plasma cortisol *mesor* or *amplitude* between patients and healthy volunteers (Table 4). The plasma cortisol rhythm was delayed in the patients compared to the healthy volunteers, the delay affecting the rhythm *onset* ($p=0.02$) and its *acrophase* ($p=0.06$) (Table 4). No significant difference was observed in the timing of the rhythm *offset* between patients and healthy volunteers (Table 4); the *duration* of the peak was significantly shorter in the 13 patients compared to the seven healthy volunteers in whom the parameter was obtained (Table 4).

No significant/trend differences in plasma cortisol rhythm parameters were observed between patients with compensated and decompensated cirrhosis. However, a significant correlation was observed between plasma cortisol *acrophase time* and the Pugh's score

($R=0.49$, $p<0.05$).

Light suppression

The time course of the mean plasma melatonin suppression profile of patients and healthy volunteers was similar, with minimal plasma melatonin levels (maximal suppression) 30 min after the beginning of light administration. However, patients showed a blunted though not significantly different maximal plasma melatonin response ($12\pm 19\%$ vs. $24\pm 15\%$, $p=0.09$; Figure 2); four patients showed no light response at all (23% vs. 0%, $p=0.03$).

Relationships between circadian variables

No significant differences were observed between patients and healthy volunteers in the time interval between the plasma melatonin and the urinary aMT6s *acrophase* (1.3 ± 1.8 vs. 1.6 ± 0.8 hr, $p>0.05$), the AUC of the plasma melatonin profile or the 24-hour plasma melatonin clearance. However, patients showed a significantly reduced overnight plasma melatonin clearance (0.11 ± 0.06 vs. 0.21 ± 0.13 , $p=0.02$).

A significant correlation was observed between the plasma melatonin and plasma cortisol *acrophase times* ($R=0.46$, $p<0.05$). Finally, significant, negative correlations were observed between the plasma melatonin/cortisol *acrophase times* and the *maximal suppression* at 30 min from the beginning of light administration ($R=-0.46$; $R=-0.43$, $p<0.05$; Figure 3).

Circadian profiles and sleep behaviour

No significant differences in plasma melatonin profile indices were observed between subjects classified as ‘good’ or ‘poor sleepers’ on the basis of the PSQI. Similarly, no significant correlations were observed between plasma melatonin profile and actigraphic indices.

A significant correlation was observed between the plasma melatonin rhythm *onset time* and *habitual wake-up time* in the healthy volunteers, but not in the patients with

cirrhosis, in whom these parameters showed greater variability (Figure 4). Four (22%) patients with cirrhosis had a *phase relationship* which was either shorter ($n = 1$) or longer ($n = 3$) than the reference range, a feature not observed in the healthy volunteers (Figure 4).

DISCUSSION

Patients with cirrhosis exhibited delays in both plasma melatonin and cortisol 24-hour rhythms and a reduction in their plasma melatonin response to light. These changes indicate the presence of central circadian disruption. In contrast, there was limited evidence of impaired hepatic melatonin metabolism. Patients with cirrhosis also showed a degree of misalignment between sleep and circadian timing but there was no obvious association between circadian abnormalities and impaired sleep quality.

Melatonin metabolism

The duration of the plasma melatonin peak and the time interval between the plasma melatonin and the urinary aMT6s peaks were comparable in patients and healthy volunteers and no differences were observed in the average AUC of the plasma melatonin profile or in the 24-hour melatonin clearance. Interestingly, overnight plasma melatonin clearance was reduced, probably indicating that some degree of impairment in hepatic disposition does exist in these patients but is only apparent when the plasma melatonin concentrations are high. This hypothesis is supported by the significant positive correlations observed between indices of increasing hepatic dysfunction and increases in the plasma melatonin mesor - the average concentration over the 24 hr - and delays in the offset of the plasma melatonin rhythm.

Thus, these data, which were obtained in patients with mild to moderately decompensated disease, would seem to indicate that the overall contribution of impaired hepatic metabolism to the disruption in the plasma melatonin profile in patients with cirrhosis is small. However, the effect may be more significant, and hence more evident, in patients with more severe decompensation. The demands of this study were such that individuals with end-stage liver disease could not be included.

There are few, if any, published studies with which to compare these data. Steindl and

co-workers (11) reported a reduction in urinary aMT6s output in 21 patients with cirrhosis, which they interpreted as showing impairment in hepatic melatonin catabolism yet were unable to show any relationship between the urinary MT6s concentrations and various indices of hepatic function. Their observations were based, however, on measurement of urinary aMT6s in a single, 8-hour overnight urine collection which, as such, will not have provided representative data.

Circadian clock output

Significant delays were observed in the rising phase of the plasma melatonin profile in the patients with cirrhosis, resulting in an overall shift of the 24-hour rhythm towards a later hour. The shift was more pronounced in patients with decompensated disease. These findings are substantially in line with those of Steindl and co-workers (9), who originally hypothesised that the clock output might be abnormal in this patient population. The attenuated melatonin response to light observed in the present study provides further and more robust evidence of central circadian disruption.

Significant delays were also observed in the rising phase the plasma cortisol profile in the patients with cirrhosis. These findings contrast with those of Bernardi and colleagues (28) and Velissaris and colleagues (10). However, these studies can be criticised for two reasons: *i*) the number of plasma samples obtained for cortisol measurement was smaller than required for accurate estimates of rhythm timing (29); *ii*) controls were not exercised for light exposure, which is important, as even ordinary room light has been shown to have phase-resetting effects on the cortisol rhythm, mediated via the hypothalamic clock (30). The reduced duration of the plasma cortisol peak observed in patients with cirrhosis in this study is difficult to interpret. Shortening of plasma cortisol peaks has been reported in healthy volunteers in response to chronic exposure to short nights (31). It is possible that chronically interrupted night rest might produce a similar result.

The observed delays in plasma melatonin and cortisol rhythms occurred in parallel, providing further evidence for dysfunctional clock output. Piscaglia and colleagues (13) obtained plasma melatonin and plasma fibrinolysis function profiles in patients with cirrhosis and healthy volunteers. Patients showed a delay in the plasma melatonin acrophase but not in that of fibrinolysis indices, leading to authors to conclude that circadian clock function is preserved in this patient population (13). The choice of plasma fibrinolysis as a circadian marker is questionable because the nature of plasma fibrinolysis rhythmicity and, more importantly, its relation to the SCN output remain unknown. In contrast, plasma melatonin and plasma cortisol are recognised, direct markers of clock phase (32).

Pathophysiology

The cause of the retinal-SCN-pineal pathway dysfunction, in patients with cirrhosis, remains unknown, but hypotheses can be formulated. Low-grade swelling has been observed in the retinal Müller cells in patients with cirrhosis and associated with changes in the electroretinogram and in colour discrimination (33). It is not known whether the photoreceptors or the retinal ganglion cells are also affected but, if so, this might impinge on the process of light entrainment. In this study, delays in the plasma melatonin acrophase were associated with blunted light-induced suppression responses, suggesting that the former might be a consequence of a reduced sensitivity to light. Similar abnormalities have been reported in blind subjects (34). Central GABAergic/glutamatergic neurotransmission is also disrupted in patients with cirrhosis (35). GABA and glutamate are involved in the transmission of the photic stimulus from the retina to the SCN (36). Thus, increased GABAergic tone might disrupt the retinal-SCN pathway and, in consequence, the output of the clock.

Clinical relevance

In this study, patients with cirrhosis showed a degree of misalignment between their sleep and

their circadian timing. The abnormal phasing of internal rhythms relative to one another, especially if long-standing, has been shown to have serious adverse effects on safety, performance and health. Shift workers, for example, have increased incidences of the metabolic syndrome, heart disease and even cancer (37, 38). Therefore, the long term effects of circadian delays and circadian-sleep misalignment in patients with cirrhosis are certainly worthy of further investigation.

However, there was no obvious association between circadian abnormalities and impaired sleep quality, indicating that the sleep disturbances observed in these patients are unlikely to depend on circadian derangement (39). Alternative pathophysiological pathways, in particular homeostatic sleep regulation, need to be explored.

In conclusion, the plasma melatonin profile abnormalities exhibited by patients with mild to moderately decompensated cirrhosis are predominantly central in origin, and are substantially unrelated to the sleep disturbances observed in this patient population.

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What is current knowledge

- Sleep-wake disturbances are common in patients with cirrhosis.
- Plasma melatonin profile abnormalities have also been described in these patients, and generally attributed to impaired hepatic melatonin metabolism.
- It has been hypothesised that plasma melatonin profile abnormalities might play an important role in the pathogenesis of sleep-wake disturbances in patients with cirrhosis.

What is new here

- The plasma melatonin profile abnormalities observed in patients with cirrhosis reflect, in the main, central circadian clock dysfunction.
- The contribution of impaired hepatic melatonin metabolism to the changes in plasma melatonin profiles in patients with mild to moderately decompensated cirrhosis is small.
- Melatonin profile abnormalities do not offer a comprehensive explanation to the sleep-wake disturbances exhibited by these patients and alternative pathophysiological pathways should be sought.

Figure Legends

Figure 1. Average ($\pm$ SEM) plasma melatonin profiles with accepted cosinor fit in the healthy volunteers and the patients with cirrhosis. The average, calculated *rhythm onset*, *acrophase times* and *rhythm offset* are indicated by labelled arrows. Patients showed an overall delay in the plasma melatonin rhythm, affecting both the rising and the declining phase.

Figure 2. Mean ($\pm$ SEM) plasma melatonin suppression, 15 to 90 min after the beginning of light administration in the healthy volunteers and the patients with cirrhosis. The response profile was similar in the two groups, with minimal plasma melatonin levels (maximal suppression) at 30 min from the beginning of light administration; however, patients showed an attenuated plasma melatonin response to light at all times. * $p=0.09$

Figure 3. Correlation between plasma melatonin suppression at 30 min from the beginning of light administration and plasma melatonin acrophase time in the healthy volunteers and the patients with cirrhosis. The grey shade highlights a substantially 'normal' area, where suppression is $>15\%$ and the acrophase occurs before 05:00. In general, patients with delayed plasma melatonin acrophase time also had poor plasma melatonin responses to light (red squares outside the gray-shaded area).

Figure 4. Relationship between the plasma melatonin rhythm onset time and habitual wake-up times in the healthy volunteers (*left panel*) and in the patients with cirrhosis (*right panel*). The correlation between the two parameters was significant in the healthy volunteers but not in the patients. Four patients had a *phase relationship* (plasma melatonin rhythm onset time - habitual wake-up time) which was either shorter (square) or longer (circles) than the reference 12.5-17.1 hr range (24).

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