Impairment of endogenous melatonin rhythm is related to the degree of chronic kidney disease (CREAM study)

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Abstract

Background. The nocturnal endogenous melatonin rise, which is associated with the onset of sleep propensity, is absent in haemodialysis patients. Information on melatonin rhythms in chronic kidney disease (CKD) is limited. Clear relationships exist between melatonin, core body temperature and cortisol in healthy subjects. In CKD, no data are available on these relationships. The objective of the study


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was to characterize the rhythms of melatonin, cortisol and temperature in relation to renal function in patients with CKD.

Methods. From 28 patients (mean age 71 years) with various degrees of renal function, over a 24-h period, blood samples were collected every 2 h. An intestinal telemetric sensor was used to measure core temperature. The presence of diurnal rhythms was examined for melatonin, temperature and cortisol. Correlation analysis was performed between Cockcroft–Gault GFR (GFR), melatonin, cortisol and temperature parameters.

Results. The mean GFR was 57 ± 30 ml/min. The subjects exhibited melatonin (n = 24) and cortisol (n = 22) rhythms. GFR was significantly correlated to melatonin amplitude (r = 0.59, P = 0.003) and total melatonin production (r = 0.51, P = 0.01), but not to temperature or cortisol rhythms. Interestingly, no associations were found between the rhythms of temperature, melatonin and cortisol.

Conclusions. As melatonin amplitude and melatonin rhythm decreased with advancing renal dysfunction, follow-up research into circadian rhythms in patients with CKD is warranted.

Keywords: chronic kidney disease; circadian rhythm; core body temperature; cortisol; melatonin

Introduction

Chronic kidney disease (CKD) is associated with circadian rhythm disturbances, such as sleep-wake problems, that have a major influence on quality of life and morbidity [1,2].

Circadian rhythms are fluctuations in body functions within a period of ~24 h. They are driven by the biological ‘clock’ located in the hypothalamic suprachiasmatic nucleus. Circadian rhythms are described as having a ‘mean’, a ‘period’, an ‘amplitude’ (the difference between the maximum and the mean of the curve of the rhythm), an ‘acrophase’ (time of the peak level) and a ‘bathyphase’ (time of the trough level) [3]. Individual peripheral tissue-specific oscillators, which are under the influence of the master circadian pacemaker in the suprachiasmatic nucleus, might be disturbed in CKD due to impaired partial oxygen pressure and blood flow in the kidney [3–5]. Indeed, CKD patients often exhibit a deregulated circadian blood pressure rhythm, such as nocturnal non-dipping profile [6], which is a risk factor for cardiovascular disease.

The pineal hormone melatonin, which is normally only secreted during the night, is an important marker of the circadian timing system [7]. Melatonin levels are usually nearly undetectable during daytime. The onset of the evening rise of endogenous melatonin is called the dim light melatonin onset (DLMO) and can be calculated as the first interpolated point > 10 pg/ml after which the serum concentration continues to rise [8]. This rise is associated with the onset of sleep propensity in healthy subjects [9]. In addition, endogenous melatonin reinforces the nocturnal decrease of central temperature, facilitated by increases in skin blood flow [10], an event that facilitates sleep propensity [11]. Furthermore, it has been suggested that melatonin can affect the production of cortisol in primates [12]. The nocturnal endogenous melatonin rise is absent in haemodialysis patients [13,14]. Information on melatonin rhythm in CKD patients is limited.

The main objective of this study was to investigate the relationship between melatonin rhythm and renal function in CKD patients. If indeed melatonin rhythm is impaired in these patients, follow-up research on exogenous melatonin might be warranted to restore the melatonin rhythm and improve sleep, as was demonstrated in haemodialysis patients [15]. The secondary objective was to investigate the circadian rhythms of cortisol and core body temperature and their synchronization with melatonin rhythm in CKD patients.

Subjects and methods

The study population consisted of patients with various degrees of renal function (n = 32) admitted to our hospital. The patients were stable, not acutely ill and were awaiting a procedure, such as an elective surgical procedure. The inclusion and exclusion criteria are outlined in Table 1.

The medical ethical committee approved the protocol of the study (ClinicalTrials.gov: NCT00698360), and informed consent was obtained from all subjects.

Study protocol

All subjects followed a semi-constant routine protocol and stayed in a dimly lit room. Normal sleep and wake times were observed. The patients were admitted to our hospital and were free to move during the study. From 6 pm till 8 am the intensity of the ambient light was < 20 lux and from 8 am till 6 pm the intensity of the ambient light was < 200 lux [16]. Over a 24-h period, blood samples were collected every 2–3 h (access via a permanent peripheral intravenous cannula) in 6-ml serum tubes and allowed to clot for 10 min at room temperature. Thereafter, samples were immediately centrifuged and separated in 1-ml aliquots and stored at −70°C until assay. Core body temperature was continuously measured using telemetry.

Analysis of laboratory parameters

Melatonin levels in serum were measured by the commercially available RIA kit (Bühlmann Laboratories, Allschwill, Switzerland). Aliquots of 400 microlitre of a serum sample were added directly in assay tubes having an inter-assay CV of 17.8% at 20.1 ng/l. The detection limit was 0.5 pg/ml. All samples originating from one subject were analysed in the same run.

Cortisol levels were measured by a competitive chemiluminescent enzyme immunoassay on an IMMULITE 2000 platform (Siemens

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**Table 1. Inclusion and exclusion criteria of the study**

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<thead>
<tr>
<th>Inclusion criteria</th>
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<tr>
<td>Age ≥ 18 years; &lt;85 years</td>
<td>Acute renal failure (ΔCockcroft–Gault eGFR &gt; 10 ml/min in two proceeding weeks)</td>
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<td>Cockcroft–Gault eGFR &gt; 10 ml/min</td>
<td>Instable angina pectoris</td>
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<td>Heart failure NYHA class IV</td>
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<td>Hypoxia (SO2 &lt; 95%)</td>
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<td>Treatment with erythropoietin, melatonin or hypnotics</td>
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<td>Deficiency of iron, folate and/or vitamin B12</td>
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<td>Haemoglobinopathies, bleeding or haemolysis as a cause of anaemia</td>
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<td>Chronic inflammatory disease or clinically significant infection</td>
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<td>Alcohol and/or drug abuse</td>
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<td>eGFR, estimated glomerular filtration rate; NYHA, New York heart association; SO2, oxygen saturation.</td>
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Healthcare Diagnostics, Breda, the Netherlands) having an inter-assay CV of 9.6% at 0.43 µmol/l. Aliquots of 200 microlitre of a serum sample were added directly in assay tubes. The detection limit was 0.03 µmol/l.

Furthermore, standard laboratory parameters were measured. Because we included patients with a glomerular filtration rate (GFR) >60 ml/min, we estimated GFR by means of the Cockcroft–Gault method, as MDRD estimated GFR is not validated for GFR > 60 ml/min.

**Core body temperature**

Core body temperature was continuously measured by means of a Jonah capsule (Respironics, Bend, OR, USA). This capsule is a disposable ingestible core body temperature sensor that telemetrically transmits information to a monitor, which has to stay <0.5 m from the ingested capsule. Transmissions begin ∼1 min after activation and are repeated approximately every 15 s thereafter. Due to individual variation of gastrointestinal motility, the first 2 h of data collection were deleted, as only intestinal positioning of the capsule provides valid data [17].

**Statistical analysis**

The presence of a 24-h rhythm time series was set to be examined by fitting of a dual harmonic cosine function for temperature, a baseline cosine function for melatonin and a skewed cosine function for cortisol [18–21]. The functions that parsimoniously and robustly capture the specific 24-h profiles of melatonin and cortisol cannot be linearized [18] and therefore we applied non-linearizable functions.

The characteristics of the individual curves were obtained from individual fits for each subject, resulting in a number of parameters for each subject. Group differences on these parameters were evaluated with ordinary statistical methods. In the case of non-symmetrically distributed parameters, the data were log-transformed before correlations were calculated. This was the case for total melatonin production. Pearson’s correlation analysis was performed to quantify the associations between the descriptive parameters for the whole range of renal function and the rhythms in melatonin cortisol and core body temperature. P-values < 0.05 were considered to represent statistical significance. The Statistical Package for Social Sciences (SPSS, Chicago, IL, USA) version 17 was employed for all statistical analyses.

**Results**

The 24-h study period was completed by only 28 patients, as 4 patients were excluded due to hospital discharge (n = 3) and problems with blood flow (n = 1) through the cannula. The general characteristics of the patients are displayed in Table 2. The mean age was 71, and 8 female patients were included. The causes of CKD are described in Table 2. Diabetes mellitus was reported in 29% of the population, which is comparable to the prevalence of diabetes mellitus in the general Dutch CKD population. Furthermore, Table 2 reveals that antihypertensive medication was frequently used.

**Melatonin**

Figure 1 shows the discrete mean melatonin concentrations and the standard deviations of the raw data at different time points in different renal function groups of all patients (n = 28). The patients with the worst renal function (GFR < 30 ml/min) had the lowest mean melatonin concentrations and patients with the best renal function (GFR > 80 ml/min) had the highest melatonin concentrations. In the group with the worst renal function, the DLMO concentration of 10 pg/ml was reached. However, the normal rise above this value was not observed in this patient group, as can be seen in Figure 1.

The melatonin rhythm parameters are presented in Table 3. The goodness of fit for the melatonin curve ($R^2$) was 0.95 ± 0.02 (mean ± SEM). In our patient group, 24 patients expressed a melatonin rhythm. The individual eGFRs of the patients without a melatonin rhythm varied considerably (eGFR range 17–79 ml/min). In the patients with a melatonin rhythm (n = 24), the amplitude of the melatonin rhythm was correlated with GFR ($r = 0.59, P = 0.003$), as shown in Figure 2. Correction for age and gender did not change the outcomes.

The log-transformed total 24-h melatonin production (area under the curve, AUC) was also correlated with GFR ($r = 0.51, P = 0.01$).

As the use of betablockers was equally divided across the different levels of renal function, it is unlikely that the
The main finding of our study is that renal function is associated with melatonin amplitude as well as total melatonin production. Renal function was not associated with cortisol or body temperature rhythm parameters. Interestingly, no association between the phases of the rhythms of melatonin, cortisol and core body temperature could be detected.

**Melatonin**

To our knowledge, this is the first study that shows a direct association between a decrease in renal function and decreases in melatonin rhythm and melatonin production. Patients with the worst renal function, shown in Figure 1, still expressed a DLMO concentration of 10 pg/ml. However, a normal rise above this value was not to be established. Recently, we have found an abolished nocturnal melatonin rise in daytime haemodialysis patients [15]. After administration of exogenous melatonin in haemodialysis patients, the absent endogenous melatonin rise was recovered and an improvement in objective as well as subjective sleep was measured. In the present study, we found the presence of a melatonin rhythm in the majority of patients (n = 24). We demonstrated, however, a decrease in melatonin amplitude and total melatonin production with advancing renal dysfunction. Sleep measurements were not performed in our study, which need to be incorporated in follow-up research on circadian rhythm and CKD. In addition to a role in sleep–wake rhythm, other effects of endogenous melatonin in CKD need further investigation. For example, endogenous melatonin was found to have a stimulating and protective effect on activity and quantity of anti-oxidative stress enzymes [22,23]. This property can be of interesting value in this patient group as oxidative stress is often seen in CKD patients. A primed state of polymorphonuclear cells, responsible for oxidative stress in haemodialysis patients, was associated with lower nocturnal plasma melatonin levels [24]. In addition, melatonin has also been associated with the immune response, impairing the mounting of an inflammatory response, while melatonin produced at the site of the injury by immunocompetent cells exerts anti-inflammatory effects [25]. Exogenous melatonin has also been useful in restoring the dipping profile in male patients with essential hypertension [26]. More research on endogenous and exogenous melatonin is needed to further establish the role of melatonin in CKD patients.

**Core body temperature**

Due to instabilities of the temperature curves and missing data during daytime (unfortunately often observed in these temperature studies [16]), the temperature amplitude could not be determined and only the timing and the level of the nocturnal minimum were established after smoothing (centered rectangular 15 min moving average). The temperature data are shown in Table 3.

Correlations between temperature minimum (P = 0.26) and GFR or temperature bathyphase (P = 0.07) and GFR were not to be established. Furthermore, no correlations, in contrast to normal observations, were found between the temperature bathyphase and the cortisol bathyphase (P = 0.201) or between the temperature bathyphase and melatonin acrophase (P = 0.24).

**Discussion**

The main finding of our study is that renal function is associated with melatonin amplitude as well as total melatonin production. Renal function was not associated with cortisol or body temperature rhythm parameters. Interestingly, no association between the phases of the rhythms of melatonin, cortisol and core body temperature could be detected.
CKD stage 4 patients and haemodialysis (HD) patients, the concentration at night was found to be even lower in HD patients, which suggests an additional effect of the dialysis process on the melatonin rhythm [14], which is—as we here demonstrate—already compromised in CKD patients.

Several factors affect the production of melatonin in CKD. Firstly, a decline in melatonin levels has been reported to be the result of impairment in beta-adrenergic receptor-mediated responsiveness in renal insufficiency [27]. The adrenergic system plays an important role in the synthesis of serotonin N-acetyltransferase (NAT), the key enzyme in melatonin biosynthesis [28]. Although suppression of NAT was observed in rats rendered uraemic by partial nephrectomy [29], research on NAT concentrations has not been performed in humans with CKD. Secondly, metabolic acidosis and reduction of airway muscle tone due to accumulation of uraemic toxins result in an increased prevalence of sleep apnoea in CKD, which is associated with increased melatonin levels during the afternoon [30]. However, as we did not investigate the prevalence of sleep apnoea in our population, we cannot confirm this. Considering the characteristics, medical history and BMI of the included patients, it seems unlikely that the prevalence of sleep apnoea would be increased in these patients.

Thirdly, uraemia has been associated with daytime sleepiness [31]. Daytime sleepiness may impair the regulation of the sleep–wake rhythm. This dysregulation might negatively affect the melatonin rhythm.

Melatonin can also be deregulated due to erythropoietin-deficiency anaemia as often found in CKD patients [5]. The fact that exogenous erythropoietin treatment can restore the circadian rhythm of melatonin in CKD [32, 5] could suggest that a relationship exists between melatonin rhythm and endogenous erythropoietin levels. We investigated this relationship in the other part of the submitted CREAM protocol.

Finally, medication can impair the melatonin production. Betablockers and benzodiazepines, often used in the CKD population, can flatten the normal nocturnal rise [28]. Benzodiazepines were not used during our study, but betablockers were still taken (n = 23). As the use of betablockers was equally divided across the different levels of renal function, it is unlikely that the association of renal dysfunction with melatonin levels results from betablocker use. Overall, antihypertensive treatment was frequently used, which could be the reason for the adequate blood pressure control.

The duration of our study period was 24 h. As we know from other studies diurnal melatonin measurements are a fingerprint for the melatonin rhythm of a subject, and therefore, longer study periods are not needed [28]. The mathematical model for the description of melatonin rhythms was developed by means of 24-h measurements [18]. As we used the same constant routine measurements in all patients, and excluded other influences, 24 h is a normal observation period. Other possible influences on melatonin production are corrected (betablockers), excluded (bright light, hypnotics) or minimized using a standard protocol.

As all female patients were post-menopausal, a difference in gender was not suspected. Correction for gender was performed to exclude a possible gender effect. The distribution of male and female patients was not equal (Table 2). Research with more patients might be needed to confirm the absence of a gender effect.

Core body temperature

In healthy persons, core body temperature variations over time follow an asymmetric sinusoidal rhythm with the temperature minimum normally occurring between 4 am and 6 am. Sleep propensity is closely linked to core body temperature, probably most specifically to the temperature of the skin [34]. Abnormalities in the rhythms in core and skin temperature have been reported in some patients with insomnia [35]. Dialysis patients show impaired nerve conduction, possibly due to uraemia [36]. Patients with proteinuria exhibit impaired vascular endothelial function, which can result in impairment in blood flow response to heating in the skin [37], which leads to less thermal conductivity [38]. These observations led us to investigate whether CKD patients have an abnormal core body temperature. We failed to find an association between CKD severity and core body temperature, however. Additional studies are needed to confirm the absence of such a relationship, as well as to investigate possible alterations in skin temperature regulation, which are associated with sleep problems in dialysis patients [39].

Melatonin has been proposed to be an endogenous synchronizer, able to stabilize circadian rhythms under normal circumstances [28]. The effect of melatonin on the temperature rhythms, under normal circumstances, meets this hypothesis [10, 11]. In our patient group, the timing of the core body temperature minimum was not associated with the timing of the melatonin peak. Autonomic deregulation, which might dissociate circadian rhythms, may be involved [40]. It is also possible that the normal synchronization aspects of endogenous disappear when melatonin rhythm is impaired, which might result in a dissociation of melatonin and temperature rhythm. Further research on this theory is needed.

Cortisol

Cortisol is synthesized in the adrenal gland under influence of ACTH. Under normal circumstances it exhibits a circadian rhythm. Normal levels of cortisol can be found in the range of 0.15–0.70 μmol/l during daytime and < 0.20 μmol/l at nighttime. As the kidney contributes to the excretion of cortisol, the serum half-life of cortisol becomes prolonged in advanced CKD [41]. Both normal and elevated levels of serum cortisol have been reported in CKD [42, 43]. We found cortisol levels in CKD not to be markedly different to reported normal values. The cortisol levels were also not related to renal function.

Furthermore, we did not find any association between cortisol rhythm parameters and renal function. Most patients exhibited a normal cortisol rhythm, which has also been shown in children with CKD [44]. Besides this paper from 1975, there is a paucity of research on cortisol and circadian rhythm in CKD. Due to frequently observed sleep-wake disturbances in CKD patients, affected cortisol rhythms might have been expected [45]. For example,
Knutson et al. reported that partial sleep deprivation resulted in changes in cortisol [46]. As patients in our study were admitted to the hospital, it should be noted that the environment in which cortisol is assessed affects its diurnal profile; for example, humans measured in a hospital setting showed elevated levels respective to their home-assessed levels, especially in the evening [47].

Experimental studies have suggested melatonin to affect cortisol and sleep-wake rhythms [7,13]. However, in patients with abnormal melatonin profiles, ACTH and cortisol levels were not changed [48]. Normal nocturnal melatonin levels have also been found in patients with steroid production disorders [49]. In our study, we also did not find a relationship between melatonin and cortisol.

In conclusion, we have demonstrated that melatonin production is increasingly compromised with advancing renal dysfunction and that CKD patients may have compromised coupling of melatonin, cortisol, and temperature rhythms. Our findings suggest the need for placebo-controlled studies into the application of exogenous melatonin to mimic the endogenous melatonin rhythm aiming to reinforce the coherence between rhythms and improving sleep disturbances in patients with CKD.

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Conflict of interest statement. None declared.

References

34. Van Someren EJW. Mechanisms and functions of coupling between sleep and temperature rhythms. Prog Brain Res 2006; 153: 309–324
47. Scheer FA, Van Paassen B, Van Montfrans GA et al. Human basal cortisol levels are increased in hospital compared to home setting. *Neurosci Lett* 2002; 333: 79–82

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