The studies described in this thesis were motivated by the increasing recognition that patients with End-Stage Renal Disease (ESRD) experience various sleep disturbances, which have been underexposed in clinical research. The quality of life of ESRD patients, which is considered to be impaired caused by increased mortality and morbidity, can be further deteriorated by sleep disturbances. For that reason, research on these disturbances in ESRD is worthwhile to conduct.

The main objectives of the thesis were:

• Is the circadian sleep-wake rhythm in patients with ESRD impaired?
• Does the rhythm of endogenous melatonin, as a synchronizer of the sleep-wake rhythm, decrease with advancing renal dysfunction?
• What external or internal factors or approaches can affect the sleep-wake pattern in the ESRD population?

On the following pages the summary and the general discussion of this thesis are outlined. The last part concerns directions for future research.

Chapter 1 Introduction to circadian sleep-wake rhythm disturbances in ESRD

Before conducting the studies of the thesis, the general opinion was that ESRD patients suffer from sleep problems. However, the focus of previous studies has been on sleep apnea syndrome, restless legs and periodic limb movement disorder (PLMD) [1]. In recent years, more patients have been treated with nocturnal dialysis. Yet, nocturnal dialysis studies focused on reducing fluid overload and improved biochemical parameters. When sleep studies were performed, the focus has been on reducing sleep apnea when changing from daytime to nocturnal hemodialysis [2,3].

The focus of research on circadian rhythm in kidney disease has been on the impaired diurnal blood pressure pattern. This impaired pattern is characterized by an absence in nocturnal dipping of blood pressure. This non-dipper profile is a risk factor for the development of cardiovascular disease [4,5].

In this chapter we elucidated the possible external and internal negative factors involved in sleep-wake rhythmicity in ESRD patients. The evidence of these factors is poor in most cases. Literature on the external factors, dialysis and medication, often addresses theories or other patient groups. The same critics apply, in general, to the internal factors: melatonin and biochemical parameters. Endogenous melatonin profiles in renal disease have become a field of interest recently, although literature is still scarce and contradicting. All authors found an absence of the nocturnal melatonin rise in serum [6,8], but the melatonin levels in serum during daytime were increased [6] or decreased [8]. Causes for increased melatonin levels have not been explained. A possible explanation is that in the studies that found increased daytime melatonin levels, the majority of the patients experienced sleep apnea. Sleep apnea is often seen in ESRD patients [9] and has been related to increased daytime melatonin levels [10]. These increased levels may be caused by pineal dysfunction in obstructive sleep apnea syndrome [10].
Decreased melatonin concentrations during daytime are more likely to be found in patients with renal failure, because of increased levels of blood urea nitrogen. These increased levels are associated with adrenoreceptor downregulation [11]. Adrenoreceptors are important in the pathway of melatonin synthesis [12].

When expounding all these somewhat theoretical factors, the contribution of the individual factors to sleep-wake rhythm is still questioned. This restriction also applies for the approaches sought to resynchronize the rhythm: bright light, exogenous erythropoietin, exogenous melatonin, cool dialysate, exercise and nocturnal dialysis. Most approaches have been researched in other patient groups. Studies on the effects of dialysate temperature [13] and exogenous erythropoietin [7] have shown positive results on sleep in the ESRD population.

Chapter 2 Prevalence

**Subjective sleep efficiency, hypnotics and potentially sleep-disturbing medication in hemodialysis patients in comparison to the general population**

Before starting intervention studies, it is important to understand and determine the problem in the study population. We found that subjective sleep efficiency in our daytime hemodialysis patient group was significantly impaired in comparison to the control group [14]. In addition, the classical hypnotics were not successfully used. This lack of efficacy of hypnotics has been reported earlier in the general older population [15]. All these findings justified the intervention studies, described in chapter 4.

Surprisingly, no association was found between dialysis adequacy and sleep efficiency. We expected to find this association, because of the described relationships between urea, melatonin and sleep apnea [2,3,11]. An explanation could be that standard dialysis adequacy focuses on the clearance of the small molecule urea, and that sleep might be related to the clearance of middle-sized or larger molecules.

As we did not find the standard age or gender effect in the prevalence of sleep disturbances, we hypothesized that the pathophysiology of sleep disturbances may differ in the medically ill in comparison to the general population. This hypothesis has been questioned earlier in literature [16].

Our hemodialysis population used significantly more benzodiazepines and beta-blockers, in comparison to the matched general population [17]. Due to the fact that beta-blockers disturb melatonin rhythm and increase nightmares, clinicians should be aware of existent sleep disturbances when prescribing lipophilic beta-blockers in this patient group. Other dosing regimens might be appropriate.

**Actigraphy measures in hemodialysis patients**

Actigraphy has become a valid alternative in case polysomnography is not feasible in insomnia patients [18]. We found that actigraphy can be valuable in evaluating sleep-wake rhythm disturbances and timing of sleep in the ESRD population [19]. Comparisons between sleep measures of polysomnography and sleep measures of actigraphy resulted in promising correlations, of which can be concluded that actigraphy can be assessed in hemodialysis patients. Hemodialysis expressed more impaired sleep parameters in comparison to insomnia patients. We concluded that in hemodialysis patients in whom sleep apneas and PLMD are not suspected, actigraphy can be used as a useful tool to objectify sleep disturbances, in addition to subjective sleep questionnaires. Furthermore, in sleep intervention studies, regarding timing of sleep or sleep-wake pattern, actigraphy can be assessed as an objective and easy method to evaluate the success of the intervention. However, if information on sleep architecture is warranted, polysomnography is the standard. More sophisticated methods of actigraphy measurements, such as combinations with oxygen measurement, need further investigation in the ESRD population. These novel methods can identify sleep disturbances together with the underlying causes, e.g. sleep apnea, and can amplify, therefore, the use of actigraphy in the ESRD population.

**Sleep-wake rhythms and endogenous melatonin rhythm in nocturnal hemodialysis, daytime hemodialysis and automated peritoneal dialysis patients**

Using actigraphy, we found that daytime hemodialysis patients experienced the worst sleep, in comparison to nocturnal hemodialysis and automated peritoneal dialysis patients [20]. With respect to sleep-wake rhythm, nocturnal hemodialysis has advantages, compared to daytime dialysis. The sleep promoting effects of dialysis coincide with the appropriate and conventional time of day. In this way, the shift to nocturnal dialysis could restore the normal temporal relationship between the sleep period and the other rhythms of the circadian system. The increased clearance contributes to this advantage of nocturnal hemodialysis.

Automated peritoneal dialysis, which is performed during night time, resulted in more disturbed melatonin rhythm than nocturnal hemodialysis [20]. The timing of dialysis seemed not to affect the melatonin rhythm. This observation, probably due to more autonomic deregulation in peritoneal dialysis [21] or the higher toxin clearance in nocturnal hemodialysis [3,22], can indicate the superior effect of elevated clearance above timing of day in nocturnal dialysis with regards to melatonin rhythm. Peritoneal dialysis patients did not sleep worse than nocturnal hemodialysis patients. This suggests that the melatonin rhythm might have a less significant role in sleep-wake rhythm of peritoneal dialysis patients, compared to hemodialysis patients.

Chapter 3 Circadian rhythm in Chronic Kidney Disease

Although this thesis concerns mostly ESRD, circadian rhythm was researched also in chronic kidney disease, with a special focus on sleep-wake rhythm. The objectives...
were, primary, to research if circadian rhythm is already disturbed prior to dialysis treatment. Second, when comparing the melatonin data of dialysis patients and patients with chronic kidney disease, the additional negative contribution of dialysis could be estimated.

Circadian rhythms of melatonin, cortisol and core body temperature in chronic kidney disease

The main result of our study is that renal function is associated with melatonin rhythm, but not with cortisol or core body temperature rhythm [23]. As endogenous melatonin rhythm was more impaired with advanced renal dysfunction, a future role for exogenous melatonin in improving endogenous melatonin rhythm, and subsequently sleep of patients with chronic kidney disease can be exerted. Lowered endogenous melatonin levels have been associated with increased oxidative stress [24,25] and impaired immune response [26]. Exogenous melatonin can be evaluated in studies on reduction of oxidative stress or immune processes of chronic kidney disease. In addition, exogenous melatonin has been useful in restoring the nocturnal dipping profile in male patients with essential hypertension [27]. As patients with chronic kidney disease often exhibit a non-dipper profile [4,5], restoring circadian blood pressure rhythm in this population by means of exogenous melatonin may be a future research topic.

While melatonin has been proposed to be an endogenous synchronizer [28], able to stabilize other circadian rhythms under normal circumstances, we failed to find an association between melatonin and core body temperature in patients with chronic kidney disease [23]. Due to the impaired melatonin rhythm, melatonin might not have the role of endogenous synchronizer, and cannot affect core body temperature, which rather does occur under normal circumstances [29]. There is a paucity of research on cortisol and circadian rhythm in chronic kidney disease. Due to frequently-observed sleep-wake disturbances in patients with chronic kidney disease and the linkage of sleep and metabolism [30], affected cortisol rhythms might have been expected. Nevertheless, in most patients we found a normal cortisol rhythm, not dependent on renal function.

Even though melatonin rhythm was impaired in patients with chronic kidney disease, dialysis patients still experienced the most deteriorated melatonin production [20,23], suggesting an additional contribution of the dialysis process, besides renal dysfunction, to melatonin rhythm.

Circadian rhythms of erythropoietin and insulin growth factor in chronic kidney disease

Conflicting data are found in literature, on whether a circadian rhythm of erythropoietin (EPO) and Insulin Growth Factor (IGF-1) exists. Nevertheless, appreciating the physiological rhythm can have clinical consequences for the treatment of anemia. Although we found a rhythm for EPO, the peak times varied inversely to mean levels of EPO [31]. Subjects with the greatest decrease in renal function presented an EPO rhythm. A possible cause could be that in the subjects with an EPO rhythm, more fluctuations in blood pressure are present [32]. Another cause can be that subjects have obstructive sleep apnea syndrome (OSAS) that had not been diagnosed. In OSAS, diurnal variation in EPO levels is higher than in healthy persons, due to nocturnal hypoxemia [33].

Furthermore, we were unable to demonstrate a circadian rhythm for IGF-1 in subjects with a decreased renal function, but we also did not find a IGF-1 rhythm in subjects with a normal renal function. A possible explanation (for the fact that we found no evidence for a circadian rhythm in IGF-1 in any of the subjects) is that IGF-1 rhythmicity indeed is absent in adults both with and without CKD, but present in children [34]. Another explanation for the absence of an IGF-1 rhythm in our study could be the relatively high prevalence of Diabetes Mellitus (DM) amongst the subjects, as DM is associated with lower levels of IGF-1 [33].

An interesting finding of this study is that mean levels of IGF-1 were correlated inversely to mean levels of EPO [31]. Subjects with the greatest decrease in renal function and a relatively low haemoglobin had the highest IGF-1 levels. This result suggests that EPO and IGF-1 both have a role in erythropoiesis in chronic kidney disease: when concentrations of EPO are not sufficient in resolving anaemia, levels of IGF-1 increase. All together, IGF-1 may constitute a potent pro-erythropoietic agent in chronic kidney disease. However, until now its therapeutic application is limited by the difficulty of assessing pituitary functional status in patients with chronic kidney disease, and the interaction with IGF-binding proteins that determine its bioavailability [36].

Chapter 4 Intervention studies

Two different approaches for strengthening the circadian sleep-wake rhythm, as described in chapter 1, are researched in this thesis: changing from daytime to nocturnal hemodialysis and exogenous melatonin.

Nocturnal dialysis

Nocturnal hemodialysis resulted in significant improvements of sleep parameters, measured with polysomnography in comparison to daytime hemodialysis [37]. Subjective measured sleep was also less impaired with nocturnal hemodialysis, compared to daytime hemodialysis. Sleep apnea tended to decrease. This decrease has also been found, with even more impressive results, in patients who received nocturnal hemodialysis at home [2]. Nocturnal hemodialysis at home is performed 7 nights a week, while nocturnal in-hospital hemodialysis is carried out only 4 nights a week. The difference in sleep apnea reduction between inhospital or at home nocturnal hemodialysis may, therefore, be found in the more...
The nocturnal melatonin surge was partially recovered after the intervention of nocturnal hemodialysis, probably due to both increased urea clearance and time of dialysis treatment [37]. Research on long daytime dialysis [22] has not been focused on circadian rhythm and sleep. When research on this theme is performed in the future, it can discriminate if longer dialysis or the time of day is the most prominent factor in melatonin rhythm return and improved sleep in nocturnal hemodialysis.

Exogenous melatonin

Exogenous melatonin led to an improvement of sleep parameters, when compared to placebo, in daytime hemodialysis patients [38]. Sleep onset even normalized [38]. In addition, the nocturnal melatonin rise was recovered. The recovery was more distinct when compared to nocturnal hemodialysis. This result suggests that exogenous melatonin might have a larger role than time of day or toxin clearance in improving melatonin and sleep-wake rhythm. The promising results of exogenous melatonin can indicate that the biological clock is a more prominent factor, compared to dialysis clearance, than suspected in advance.

Under normal circumstances, exogenous melatonin causes phase-resetting of the melatonin rhythm [39]. In this study, however, with absent endogenous melatonin rhythm, exogenous melatonin might exert another role, constituting a masking factor that selectively affects the onset of the melatonin curve. After a period of melatonin administration, the activity of the enzymes, involved in melatonin synthesis, may have been sensitized. That would facilitate the triggering of the synthesis and release of endogenous melatonin [40].

Most melatonin studies are performed in small patient groups during short investigation periods. Due to this flaw in research, a study on MELODY and quality of life (MELODY study) was recently started. This is a long-term (1 year) placebo-controlled study on the effects of melatonin 3 mg on sleep, quality of life and cardiovascular outcomes, to confirm the role of exogenous melatonin in ESRD.

The last patient (n=70) has just been included.

In summary, when returning to the questions presented in the introduction of this thesis, the main objective: circadian sleep-wake rhythm in patients with ESRD is indeed impaired. Although the internal factors have a prominent impact on sleep disturbances, the external factors contribute to these disturbances: - Endogenous melatonin rhythm decreased with advancing renal dysfunction. The dialysis process has an additional negative contribution to the impaired rhythm in hemodialysis patients, when comparing melatonin rhythms of hemodialysis and pre-dialysis patients. Under normal circumstances, endogenous melatonin exerts its role as endogenous synchronizer, thereby phase-shifting circadian rhythm of core body temperature. Caused by the abolished melatonin rhythm, melatonin might not exert this role in ESRD. The presence of the circadian rhythms of temperature, erythropoietin and cortisol is not associated with renal function. Interesting findings on exogenous melatonin are presented. In future, more research is warranted on the other approaches, described in chapter 1, to establish which intervention has to be followed in the dialysis patient with sleep disturbances.

Directions for future research

Future research should be carried out to get more insight in circadian rhythm and renal disease and to seek the most successful approach in resynchronizing rhythm. These studies should be subdivided in four categories of patients: hemodialysis patients, transplantation patients, patients with other forms of renal replacement therapy and patients with chronic kidney disease.

In hemodialysis patients, future catches up with the results of a long-term placebo-controlled study on the effects of exogenous melatonin in daytime hemodialysis patients (the MELODY study) coming up. This study might confirm the beneficiary role of exogenous melatonin on sleep and quality of life. As described in the discussion, melatonin has a role in oxidative stress and circadian blood pressure profile. Therefore, the cardiovascular outcomes will be researched also in this study. Related to this topic, more research on endogenous melatonin, oxidative stress, the immune system and blood pressure in hemodialysis patients is warranted.

In exogenous melatonin studies melatonin levels are measured after a single day of stopping study medication. It would be of additional knowledge on melatonin rhythm to perform day-to-day kinetic research to evaluate the effect of melatonin, even after stopping melatonin treatment during a longer period. If melatonin rhythm remains improved after stopping study medication, the circadian rhythm may be resynchronized, and the patient does not need to take the medication chronically.

With regards to transplantation patients, it would be of great interest to investigate the circadian rhythm before and after transplantation. Renal function significantly improves after transplantation, which may result in an improvement of circadian rhythm. In addition, the role of the donor might be important, as the donor may induce other diurnal rhythms in the acceptor of the graft after transplantation, compared to before transplantation. Studies on circadian rhythm of both donor and acceptor of grafts are worthwhile addressing in future years. This recommendation also applies for the chronopharmacologic research on transplant drugs, as the effects of circadian rhythm on these drugs might affect the outcomes of transplantation. In general, more attention must be given to the relationships between the time of dosing of drugs and their pharmacological or chronobiological.
effects. This ‘chronopharmacology’ is underexposed in many clinical trials and
could lead to the better effectiveness of several drug treatments.

We have learned from the CREAM study that renal dysfunction affects the levels
of melatonin in patients with chronic kidney disease. A wide range of research
possibilities on the influence of renal function on other circadian rhythms is
worthwhile pursuing.

Even fewer studies have been performed on sleep and circadian rhythm in
peritoneal dialysis. This type of dialysis is quite different from hemodialysis and
research on this therapy can establish the impact of dialysis technique on circadian
sleep-wake rhythm.

The last comment goes to acute renal failure. Intensive Care and Continuous
Vena-venous Haemofiltration (CVVH). This area of research in relationship with
circadian rhythm is fascinating to research, considering the impaired day-night
rhythm of patients, the prevalence of delirium and the frequent use of hypnotics
on the Intensive Care ward.

These are only some of the issues that might be addressed in the next couple of
years.

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