Chapter 7

Summarizing discussion and future perspectives

The studies described in this thesis were inspired by the recognition that patients with end-stage renal disease (ESRD) experience various sleep disturbances at night as well as excessive sleepiness during the day. These have a negative effect on their quality of life.\(^1\,\text{,}\,^2\) In addition to a disturbed sleep/wake rhythm, ESRD patients suffer from other disrupted circadian rhythms as well, e.g. their blood pressure rhythm.\(^3\) The negative consequences of disturbed sleep and chronic disruption of circadian rhythms on health are emphasized in recent literature. They promote the development of diseases such as obesity, diabetes and hypertension.\(^4\,\text{–}\,^9\) However, only little is known on the causes and consequences of and remedies for circadian rhythm disturbances in ESRD patients.

Remarkably, both with regard to sleep disruption and loss of circadian rhythmicity, worsening of kidney function in chronic kidney disease (CKD) patients is associated with decreasing nocturnal melatonin concentrations.\(^10\) In many ESRD patients, nocturnal melatonin concentrations have even further decreased.\(^11\) Since a decline in kidney function is associated with lower nocturnal melatonin levels, we questioned whether an increase in kidney function could restore nocturnal melatonin levels.

Melatonin not only promotes sleep\(^12\), it is a robust parameter of the body’s circadian clock.\(^13\) It plays an important role in giving nighttime cues to the body. It stabilizes and reinforces circadian rhythms and maintains phase relationships between them.\(^12\)

Because of its roles in sleep regulation as well as biomarker of the circadian clock, we questioned whether the low nocturnal melatonin concentrations, represents an underlying broader dysfunction of the circadian clock in ESRD patients. Therefore we measured different circadian rhythms in hemodialysis patients and compared these to the same rhythms in healthy subjects.

Several explanations for disturbed night time sleep in ESRD patients can be put forward, one of which could be melatonin deficiency.

Indeed, previously it was shown that short-term daily supplementation with exogenous melatonin led to improved subjective and objective sleep in hemodialysis patients.\(^14\)

Here we studied whether the positive effect of melatonin supplementation would persist for a longer period of time.

In addition to melatonin as internal biomarker of circadian clock time, its external counterpart is light. Since melatonin treatment in hemodialysis patients has not been optimized yet, we aimed to strengthen the circadian rhythm by giving a strong daytime light pulse to hemodialysis patients.
In sum, the main objectives of this thesis were:
- Are circadian rhythms of hemodialysis patients disturbed compared to healthy age-matched control persons?
- Can the decrease in melatonin levels in ESRD patients, which is correlated to a decline in renal function, be reversed by improving renal function with kidney transplantation? Does this have effects on sleep quality?
- Can sleep and quality of life of hemodialysis patients be improved by long-term treatment with melatonin?
- Does light therapy improve melatonin concentrations and sleep in hemodialysis patients?

**Disturbed peripheral circadian rhythms in hemodialysis patients**

In chapter 3, the CLEXID study, we studied circadian rhythmicity in hemodialysis patients and compared these rhythms to age and gender matched control persons. We determined circadian rhythmicity on different levels in patients and healthy control persons.

On the behavioural level, we measured the sleep/wake rhythm. Previously found sleep disturbances in hemodialysis patients were confirmed by actigraphy results. Hemodialysis patients suffered from a longer sleep onset latency, a lower sleep efficiency and a higher number of awake minutes during the night after initial sleep onset compared with healthy control subjects. Furthermore, hemodialysis patients reported elevated daytime sleepiness.

On a biochemical level, we measured 24-hour profiles of plasma melatonin and cortisol concentrations. Both hormones are known to follow a stable 24-hour rhythm.(13) As expected, mean nocturnal melatonin peak levels were lower in hemodialysis patients than in control subjects. This is in line with previous results.(11,14,15) Also, cortisol rhythms differed between patients and controls. Hemodialysis patients seemed to have a lower amplitude in cortisol rhythm. Although not significantly different, our data pointed to higher evening cortisol levels than in control persons, which has been found earlier.(16) The significance of a circadian rhythm can be calculated. Significant circadian oscillations of cortisol were found in 2 out of 9 hemodialysis patients and in 6 out of 9 control subjects. Our data suggest that the cortisol and melatonin rhythms are affected in hemodialysis patients.

With regard to clock gene expression, 24 h expression rhythms of 4 robust clock genes, *Per1, Per3, Rev-erba* and *BMAL1* were measured. Of these 4 genes, *Rev-erba* turned out to be the most reliable circadian marker, since significant circadian *Rev-erba* mRNA rhythms
were observed in 6 out of 9 control subjects, whereas circadian Per1, Per3 and BMAL1 mRNA rhythms were detected in only 2, 3 and 2 of the 9 controls, respectively. We found less significant circadian mRNA profiles of these 4 genes in hemodialysis patients than the control persons. The difference between patients and controls was most pronounced for Rev-erbα, where none of the hemodialysis patients showed a circadian Rev-erbα rhythm. Moreover, mean clock gene mRNA levels were consistently lower in hemodialysis patients than in control persons. The meaning of this is unclear. Taken together, our data show that circadian clock gene expression is markedly affected in peripheral white blood cells of hemodialysis patients.

Peripheral clocks are synchronized by the SCN through the release of neuronal and humoral factors. We explored the hypothesis that the dampened amplitudes of peripheral clock gene expression in hemodialysis patients may (in part) be caused by increased levels of clock synchronizing factors in the blood as a result of reduced renal clearance. One could expect a stronger signal of biological clock time with higher levels of clock resetting factors. But continuously saturated levels of clock synchronizing factors above a certain threshold might in contrast interfere with adequate oscillating timekeeping. Indeed, serum of hemodialysis patients turned out to have a higher capacity to synchronize the circadian clocks of cells in vitro than serum of healthy controls. This could not be explained by frequently used medications of hemodialysis patients and did not differ between pre- and post-dialysis serum.

Our results on disturbed peripheral circadian timekeeping in hemodialysis patients are of importance. There is increasing knowledge on the negative consequences of clock disruption on health. From literature it is known that disturbances in peripheral circadian timekeeping have affected metabolic processes resulting in the development of obesity and type 2 diabetes (reviewed in (17)). Cardiovascular disease is also linked to disruption of the circadian clock.(18) In addition, our results in hemodialysis patients are not isolated results in chronic illness. In rheumatoid arthritis disconcerted circadian timekeeping of peripheral clock gene expression was also found.(19)

The results of the CLEXID study show that peripheral timekeeping in hemodialysis patients fails eventually. From our results, we cannot conclude at what level in the clock hierarchy the problem arises. Are there shortcomings in the signalling from the central clock in the SCN, maybe as a result of the uremic milieu? Or are circulating factors present that interfere with proper signalling from the SCN? The latter could be hypothesized based on the results of our cell culture experiments in which we found a higher synchronizing activity of patients’ serum compared with control serum. Apparently, the concentration of clock influencing factors is higher in patient serum.
Disturbances in circadian timekeeping have traditionally not been the centre of clinical and research attention in ESRD patients. But with growing evidence of their negative effects in otherwise healthy persons or people with other chronic illnesses, they should not be ignored in the future.

### Treatment targets

Three different treatment strategies for improving melatonin concentrations, sleep and quality of life were researched in this thesis: improved kidney function as a result of kidney transplantation, 12 months of daily intake of 3 mg exogenous melatonin and 3-week exposure to light therapy during dialysis treatment.

#### Kidney transplantation

Since decreased glomerular filtration rates are related to reduced melatonin levels \(^{(10)}\), in chapter 4, the CRIKT study, we investigated whether the opposite i.e. an improvement in renal function due to kidney transplantation would subsequently modify melatonin levels, sleep, circadian rhythmicity of blood pressure and core body temperature and quality of life in kidney transplant recipients (KTR). As all measurements had to be performed one month prior to and 3 months after transplantation, only transplants from living donors (LD) could be included.

In contrast to our hypothesis, nocturnal melatonin concentrations did not change with transplantation.\(^{(20)}\) Possible explanations are the following. The earlier work assessed the effects of a gradual, long-term change in renal function, whereas in the CRIKT study there was a relatively short time span between increase in renal function and melatonin measurements. Maybe not glomerular filtration per se, but other long-term renal function-related mechanisms may be the connection between kidney disease and disturbed melatonin synthesis. An example could be pineal calcification. A decrease in melatonin secretion is correlated with increased pineal calcification, at least in aging.\(^{(21)}\)

Secondly, for practical reasons we did not ask participants to take saliva samples at the time of highest melatonin levels (between 2 and 4 a.m.). Perhaps a difference in nocturnal melatonin concentrations pre- versus post-transplantation was present, but not found.

Thirdly, prior to transplantation the nocturnal melatonin concentrations and sleep of KTR in this study were not as disturbed as expected based on results in hemodialysis patients. Regarding sleep, the amount of night time awake minutes tended to be reduced in transplant recipients after transplantation. Nevertheless, although with actigraphy we
found only marginal improvements in night time sleep quality, KTR reported that daytime sleep propensity had improved. (20) Others have found high percentages of sleep problems in solid organ transplant patients after transplantation and point out that sleep problems are an undertreated problem in transplantation. (22)

Blood pressure dipping profile did not change, as well as dim-light melatonin onset and time of core body temperature minimum. So we did not find changes in circadian parameters, nevertheless quality of life (QoL) had improved. (20) Obviously, it is not possible to conclude that improvement of QoL is related to an improvement in circadian rhythmicity of melatonin, sleep, or blood pressure since we did not find any changes in these parameters.

Long-term exogenous melatonin use
Previously, short term daily supplementation of melatonin 3 mg resulted in significant improvements of objective and subjective sleep compared to placebo treatment in hemodialysis patients. (14) In chapter 5, the MELODY study, we investigated whether long-term melatonin supplementation also had a positive effect on sleep and quality of life. Hemodialysis patients suffering from subjective sleep problems were randomized to receive 3 mg melatonin per day or placebo during 12 months. With melatonin, no beneficial effect on the quality of life parameter vitality was seen. Other quality of life parameters showed both advantageous and disadvantageous effects of melatonin. Considering sleep, at 3 months sleep efficiency and actual sleep time had improved with melatonin compared with placebo on haemodialysis days, but at 12 months none of the sleep parameters differed significantly from placebo. Endogenous nocturnal melatonin salivary concentrations at 6 months had significantly increased in the melatonin group compared with the placebo group. (15) We conclude that in this long-term study the positive effects on sleep disappeared during follow up (6–12 months). This is the first clinical trial evaluating long-term use of melatonin in this patient group. We aimed to find an alternative to commonly prescribed hypnotics (such as benzodiazepines and other psychotropic drugs) to alleviate sleep problems and with less side effects. Although, some clinical benefit of exogenous melatonin has been found, efforts should be made to elucidate the mechanism responsible for the loss of effect with chronic use. It is worthwhile to try and optimize melatonin treatment in the future.

Bright light exposure
In chapter 6, the SHINE study, the effects of daytime bright light therapy on sleep, daytime sleep propensity, drowsiness, melatonin levels and depressive symptomatology...
in hemodialysis patients were explored. Bright light therapy was given during dialysis treatment for 3 weeks. Total sleep time tended to be longer with light exposure. In contrast to our current understanding of human circadian biology, light treatment in the (late) afternoon tended to advance the timing of sleep onset compared with morning light. If these findings can be confirmed in another study, then this might reduce the feelings of insomnia in hemodialysis patients. This was an unexpected result, since light exposure during the late afternoon was not expected to advance sleep onset. Early morning light generally leads to a phase advance, late evening light causes phase delays. Since in this study light was given in the least responsive part of the phase response curve, only little, if any, chronotherapeutic effect was expected. One explanation could be that not only Zeitgeber time, but also Zeitgeber strength, i.e. the difference between daytime and nighttime light intensity influences time of sleep onset. We hypothesize that the bright light exposure in the (late) afternoon to our HD patients constituted a large contrast to the low light exposure that followed directly in the evening at home. This could have provided a signal to the SCN that the period of darkness had already begun in the early evening at the moment of homecoming, resulting in earlier times of sleep onset for the patients with afternoon light exposure compared to morning exposure. In this pilot study, we concluded that there were no significant effects on nighttime sleep quality, evening melatonin concentrations and depressive symptomatology. As expected, light exposure immediately decreased feelings of drowsiness and probably reduced daytime sleep propensity.

**Conclusion**

In summary, peripheral circadian rhythms of hemodialysis patients are disturbed compared to healthy age-matched control persons. At least rhythms of sleep, melatonin, cortisol and peripheral clock gene expression differ from those in healthy persons. The exact mechanisms remain unclear. Our research suggests that blood borne clock resetting compounds accumulate in hemodialysis patients. The decrease in melatonin levels in ESRD patients which is correlated to a decline in renal function cannot be reversed by improving renal function with kidney transplantation within 3 months after transplantation. Sleep improves marginally. Other circadian rhythms of blood pressure and body temperature do not change. Long-term treatment of hemodialysis patients with exogenous melatonin improves sleep on the short-term. This effect does not last longer than 3 months. Quality of life measure-
ments showed both advantageous and disadvantageous effects of melatonin. We could not show an effect of daytime light therapy on night time melatonin levels. The finding that late afternoon light therapy might advance timing of sleep onset is interesting and needs further investigation.

Future perspectives

First of all, efforts should be made to understand the interaction between renal disease and circadian rhythm disturbances in ESRDD patients. This is important in order to further optimize current treatment strategies and to identify novel therapeutic targets. Questions that should be answered to understand the origin of the problem are: at what level in the clock hierarchy does the problem arise? Does accumulation of blood borne compounds interfere with otherwise normal signaling from the SCN? Or is circadian timekeeping by the SCN impaired due to the uremic milieu?

Studies should focus on the finding that serum of HD patients has a higher clock synchronizing capacity than serum of matched control subjects. This strongly suggests that circadian timekeeping in HD patients is impeded by accumulation of serum factors as a result of reduced renal clearance. In a first approach, efforts should be made to determine the nature of the accumulated clock resetting compounds. We already concluded that these are non-dialyzable substances. Therefore they must be of a certain size or perhaps they are largely protein bound. Physicochemical characteristics such as thermolability and pH sensitivity might give clues. It might even be possible to compare protein profiles of patient serum and healthy serum to find suspect molecules.

Secondly, more knowledge should be gained on how to interpret the low melatonin levels in ESRD patients. Which consequences does this have? The decrease in melatonin concentrations was not reversed by increased kidney function. Apparently, melatonin secretion is not related to improvement of kidney function in the short-term. It would be interesting to see if melatonin synthesis is restored with longer-term improvements in glomerular filtration rates or relates to secondary processes that are a consequence of kidney failure.

Thirdly, why did the short term beneficial effects of melatonin on sleep not last longer than 3 months? Optimization of melatonin treatment should be aimed for. From our data of the MELODY trial, it seems that after 6 months daily intake of melatonin 3 mg, melatonin has accumulated. Therefore, pharmacokinetics of melatonin in HD patients, especially after multiple daily ingestions, should be measured to answer the question of possible melatonin
accumulation. Perhaps the dose of 3 mg per day is too high, leading to a ‘spillover effect’ of melatonin in the following morning. Possible strategies that are worthwhile to investigate are lower melatonin doses and so called ‘drug holidays’, i.e. alternating periods of melatonin administration with periods without exogenous melatonin use.

We have only explored the effects of light therapy in in hemodialysis patients. In order to assess its potential beneficial effects, light treatment for hemodialysis patients should be optimized. Future research should focus on more frequent light exposure (e.g. home treatment) in larger groups of hemodialysis patients to study if an earlier sleep onset with afternoon light exposure and possible increased total sleep time can be confirmed. Since light reduces feelings of drowsiness, a nap analysis should be included to measure day- and nighttime sleeping behavior.

Finally, the effects of giving strong time cues to the internal circadian clock by combining melatonin and light treatment could be an interesting approach.
References


