ORIGINAL ARTICLE

The role of renal function loss on circadian misalignment of cytokines EPO, IGF-1, IL-6 and TNF-alfa in chronic renal disease

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Abstract

OBJECTIVE: Chronic inflammation plays a pivotal role in the development of renal disease. Circadian sleep-wake rhythm is disturbed in renal disease. Awareness of other disturbed rhythms, such as inflammation processes, can affect the treatment of patients with renal disease. Knowledge of possibly related circadian misalignment of the cytokines erythropoietin (EPO), Insulin Growth Factor-1 (IGF-1) and interleukins (IL) however is limited. We therefore performed an observational study. The objective of this study was to characterize levels of EPO, IGF-1 and inflammation markers IL-6 and TNF-α, related to renal function. **METHODS:** The study population consisted of patients with various degrees of renal function, admitted to our hospital. During 24 hours, blood of 28 subjects with various degrees of renal function was collected every 2 hours. The patients were stable, not acutely ill and they were waiting for a procedure, such as elective surgery. Circadian parameters of EPO, IGF-1, IL-6 and TNF-α were measured in serum and were correlated with glomerular filtration rate (GFR) and Hb, using Pearson correlations. **RESULTS:** Although diurnal variations in EPO level were found in 15 out of 28 patients, the curves did not show a consistent phase. The presence of an EPO rhythm was not related to GFR. No diurnal rhythm could be detected for IGF-1, IL-6 and TNF-α. Mean levels of IGF-1 were correlated inversely to mean levels of EPO (p=0.03). When divided based on GFR and Hb subjects with GFR 10–30 ml/min and lower Hb had the highest IGF-1 levels (p=0.02). A relationship between Il-6, TNF-α and EPO or GFR was not found. **CONCLUSION:** The existence of a circadian (mis)alignment of EPO, IGF-1, IL-6 and TNF-α was not found. The association between high IGF-1 and low Hb suggests that EPO and IGF-1 have an alternating role, dependent on GFR, in stimulating erythropoiesis. These results could have consequences for the treatment of anemia.

INTRODUCTION

Patients with chronic kidney disease exhibit markedly disrupted circadian body functions. For example, the diurnal blood pressure rhythm is disturbed in renal patients, showing a nocturnal non-dipping profile (Elung-Jensen *et al.* 2008), which is associated with increased cardiovascular mortality (Clement *et al.* 2003). The nocturnal endogenous melatonin rise, which is associated with the onset of nocturnal sleep propensity, is decreased in patients with reduced GFR and completely absent in hemodialysis patients (Koch *et al.* 2010; Karasek *et al.* 2005; Koch *et al.* 2009). Melatonin secretion is governed by the suprachiasmatic nucleus (SCN) in the hypothalamus. Importantly, in addition to the central endogenous timing system, in peripheral cells oscillators share a similar core clock based on transcriptional activators (such as *Clock*), and of feedback repressors (such as *Cry1, Cry2*). Thus, circadian oscillations of the core clock entrain circadian rhythms in expression of output genes in peripheral cells, which are, in turn, translating these transcriptional oscillations into tissue-specific functional rhythms (Firsov & Bonny 2010). Recently it was shown that *Clock* deficient mice and (*Cry1,Cry2*) double knockout mice had marked, "clinically important", disturbances in water or sodium homeostasis.

Renal disease is, in addition to loss of melatonin rhythm, associated with anemia, erythropoietin (EPO) deficiency, and with inflammation, endothelial dysfunction and EPO resistance. The endothelium is an important non-hematological target of EPO (Congote *et al.* 2010) and both anemia and EPO resistance are important predictors of cardiovascular complications and mortality in CKD. It is unknown whether changes in circadian rhythms and loss of melatonin secretion in CKD are associated with changes in EPO effect and/or rhythm. Associations between melatonin and erythropoietin rhythm have been published (Vaziri *et al.* 1996) and erythropoietin response in the treatment of anemia in chronic renal disease is influenced by the time of administration (Buemi *et al.* 1993). There are several additional reasons that support the existence of a circadian rhythm of EPO and other cytokines. The primary stimulus for EPO production is (lack of) oxygen availability. The fact that during the night the metabolic requirement for oxygen is lower, could lead to decreased levels of EPO during nighttime. To our knowledge, no information is available on whether IGF-1 exhibits a circadian rhythm. IGF-1 is produced mainly in the liver under the influence of growth hormone (GH), which is, however, secreted pulsatile in a circadian fashion (Stratakis *et al.* 1996). An increase in IL-6 and TNF-α has been associated with an increased need in exogenous EPO (Macdougall & Cooper 2005). In healthy volunteers the concentration of Il-6 is regulated in a circadian fashion with peak levels in the early morning and early evening and through levels later in the morning and at night. TNF-α has shown to have peak levels at night (Vgontzas *et al.* 2002).

We therefore asked the question whether changes in circadian rhythms and loss of melatonin secretion in CKD is associated with changes related to EPO and modulators of its erythropoietic effect. In the present study we examined the circadian (mis) alignment of the cytokines erythropoietin (EPO), Insulin-like Growth Factor-1 (IGF-1, co-factor in erythropoiesis (Brox *et al.* 1996) and inflammatory markers (related to EPO resistance (van der Putten *et al.* 2008)), in comparison to changes in melatonin rhythm, in chronic kidney disease.

MATERIAL AND METHODS

Subjects

The study population consisted of patients with various degrees of renal function (n = 32, age 71±7, 29% female), admitted to our hospital. The patients were stable, not acutely ill and they were waiting for a procedure, such as elective surgery. 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

Over a 24-hour period, blood samples for measurement of serum EPO, total IGF-1, TNF-α and IL-6 were collected every 2–3 hours (access via a permanent peripheral intravenous cannula) in 6-ml serum tubes and allowed to clot for 10 minutes at room temperature. Thereafter, samples were immediately centrifuged and separated in 1 ml-aliquots and stored at –70 °C until assay. In addition, blood was withdrawn in a 3-ml EDTA tube for measurement of hemoglobin (Hb) in supine position after 0, 12 and 24 hours.

Tab. 1. Inclusion and Exclusion Criteria.

Inclusion Criteria
Age >18 years, <85 years
GFR-Cockcroft-Gault >10 ml/min
Exclusion Criteria
Acute renal failure (Δ GFR-Cockroft >10 ml/min in 2 proceeding weeks)
Instable Angina Pectoris
Heart failure NYHA class IV
Hypoxia (SO_2 < 95%)
Treatment with erythropoietin, melatonin or hypnotics
Deficiency of iron, folate and/or vitamin B12
Hemoglobinopathies, bleeding or hemolysis as a cause of anemia
Chronic inflammatory disease or clinically significant infection
Alcohol and/or drug abuse

Measurements

Medical history and medication use were recorded and blood pressure was measured in all participants. Serum EPO levels were measured by a two-site sandwich chemiluminescent immunoassay on an IMMULITE 2000 platform (Siemens Healthcare Diagnostics, Breda, the Netherlands) having an inter-assay CV of 7.2% at 16 IU/l. Total IGF-1 levels were measured using an enzyme-labeled chemiluminescent immunometric assay on an IMMULITE 2000 platform (Siemens Healthcare Diagnostics, Breda, the Netherlands) with an inter-assay CV of 6.9% at 128 ng/ml. Levels of Cystatin-C were measured once at the start of the study, by means of the N-Latex Cystatin-C assay and a PROspec nephelometer (Siemens Healthcare Diagnostics, Breda, the Netherlands) having an inter-assay CV of 2.2% at 0.90 mg/l. All samples originating from one subject were analyzed in the same run. IL-6 and TNF-alpha are measured by means of an ELISA kit (enzyme immunoassay, Pelikine™. The sensitivity for IL-6 is 0.3 pg/ml, and no cross-reactivity is observed. The sensitivity for TNF-alpha is 1–3 pg/ml, and no cross-reactivity is observed. Furthermore, standard laboratory testing was performed. Glomerular Filtration Rate (GFR) was calculated according to the Cockcroft-Gault method.

Statistical analysis

The sample size was based on an analysis of statistical power using data of a pilot study on the relationship between circadian EPO rhythm and renal disease in pre-terminal uremia (Buemi *et al.* 1993). Given the prior variances and mean difference in EPO amplitude between healthy subjects and pre-dialysis patients, a sample of n=32 was chosen to obtain a power of 0.90 at an alpha of 0.05). For IGF-1 rhythm detection, previous studies that demonstrated circadian rhythms used a sample size of n=6 (Stratakis *et al.* 1996; Heuck *et al.* 1999).

The existence of a circadian rhythm was examined fitting of a cosine function to time series (Van Someren & Nagtegaal 2007). In case of non-symmetrically distributed parameters, the data were log-transformed before correlation calculations were performed. This was the case for Cystatin-C, mean EPO levels, EPO amplitude and mean IGF-1, IL-6 and TNF-α levels. Continuous variables were compared using unpaired t-tests and categorical variables were compared using Fishers' Exact Test. Data regarding EPO and IGF-1, IL-6 and TNF-alpha rhythm/production were correlated with GFR and Hb using Pearson correlations. 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 analysis.

Tab. 2. Baseline Characteristics (n=28)*

Variable	
Age (years)	71 ± 7
Gender (M:F)	71 : 29
BMI (kg/m^2)	
Median	25.8
Interquartile range	24–28
Diabetes (%)	29
Smoking (%)	14
Systolic blood pressure (mmHg)	125 ± 20
Diastolic blood pressure (mmHg)	73 ± 14
Hemoglobin (g/dL)	13.3 ± 1.4
GFR-Cockcroft (ml/min)	57 ± 30
Cystatin C (mg/l)	
Median	1.01
Interquartile range	0.79–1.98

*Plus-minus values are means ± SD.
Conversion factors for units: serum haemoglobin in g/dl to g/L x10; serum GFR in mL/min/1.73 m^2 to mL/s/1.73 m^2 × 0.01667.

Tab. 3. EPO rhythm characteristics (n=15).

Variable	Median [interquartile range]
EPO level (IU/l)	8.9 [7.8–15.7]
Peak EPO level (IU/l)	12.0 [9.4–20.4]
Acrophase (hours after midnight)	14.2 [10.3–22.1]
Amplitude (IU/l)	2.7 [1.5–3.3]

RESULTS

The 24-hour study period was completed by 28 subjects, as hospital discharge and problems with blood flow through the cannula resulted in missing a significant amount of data in 4 patients. The general characteristics of the subjects are displayed in Table 2.

EPO

In 15 out of the 28 subjects, a significant cosine rhythm was present for EPO (Table 3). However, the acrophase (time of peak concentration) varied widely among these subjects. No determinants for the existence of an EPO rhythm could be identified. The presence of an EPO rhythm was not related to GFR (p=0.46) and no correlation was found between amplitude of EPO rhythm and GFR (p=0.10). Furthermore, no relation was found between the presence of an EPO rhythm and several general characteristics of the study population (CKD cause, gender (p=0.69), age (p=0.54), BMI (p=0.85), diabetes mellitus (DM) (p=1.00), smoking (p=0.60)). Also, no relation was found between EPO rhythm and medication use (beta-blocking agents (p=1.00), ACE-inhibitors (p=0.70), acetylsalicylic acids

(p=0.71)). As expected, the mean EPO levels for all 28 subjects were correlated to GFR (r=0.52, p=0.005) and to levels of Cystatin-C (r=–0.52, p=0.005). Mean levels of EPO for all subjects were not correlated to Hb (p=0.29).

IGF-1

Cosine curve fitting of the IGF-1 time series did not reveal a clear rhythm for IGF-1 in any of the subjects. On an individual basis, there was some time-of-day variation in levels of IGF-1. However, no systematic peak time range was found. Mean levels of IGF-1 did not correlate to GFR (p=0.26) or to Cystatin-C (p=0.08), but interestingly, mean levels of IGF-1 did correlate inversely to mean levels of EPO (r=–0.41, p=0.03) (Figure 1). Levels of IGF-1 did not correlate to Hb (p=0.68). To determine the relation between IGF-1, Hb and GFR, participants were divided into six groups based on the degree of CKD (GFR 10–30, 31–60, > 60) and Hb lower/higher than 12.6 g/dL. Subjects with GFR 10–30 ml/min and a low Hb had the highest IGF-1 levels (p=0.02, ANOVA) (Figure 2).

IL-6 and TNF-α

Cosine curve fitting of the Il-6 and TNF-α time series did not reveal a clear circadian rhythm in any of the subjects. The mean level of IL-6 was 9.6 pg/ml (normal < 9.7 pg/ml) and the mean level of TNF-α was 0.8 pg/ml (normal < 2 pg/ml). On an individual basis, there was some time-of-day variation in levels of IL-6. However, no systematic peak time range was found. Mean levels of Il-6 and TNF-α did not correlate with EPO, IGF-1 and GFR (all p>0.1). There seemed not to be an association between mean levels of IL-6 and Hb (p=0.1, r=0.49). Furthermore, no other relationships were found.

DISCUSSION

The main finding of our study is that circadian (mis) alignment for cytokines EPO, IGF-1 and inflammation is not a consequence of the degree of chronic renal disease, in contrast to melatonin rhythm (Koch *et al.* 2010). Secondly, circadian rhythms of these parameters or associations between these parameters amongst themselves could not be identified. Interestingly, mean levels of IGF-1 correlated inversely to mean levels of EPO. Subjects with the greatest decrease in renal function and the lowest Hb levels had the highest IGF-1 levels.

EPO

In the present study we found no clinical significant circadian EPO rhythm. EPO is a glycoprotein that acts to prevent the programmed cell death of erythroid progenitor cells in the bone marrow, thereby stimulating these cells to proliferate and mature (Fisher *et al.* 1996). EPO is mainly produced in the kidney by peritubular cells (Fisher 2003) and the primary stimulus for its production is hypoxia (Scortegagna *et al.* 2005). In chronic renal disease, production of EPO is disrupted, which is one of the main reasons for anemia in chronic renal disease patients (van der Putten *et al.* 2008).

It is unclear whether EPO is regulated in a circadian manner in healthy persons (Roberts & Smith 1996; Klausen *et al.* 1993). With exception of one small series of 5 uremic patients (Buemi *et al.* 1993), no study has been performed on a circadian rhythm of EPO in CKD. In our study, we found diurnal variations for EPO in 15 of the 28 subjects. The peak times however varied, and therefore it is unlikely that EPO levels are regulated intrinsically in a circadian fashion. No relation was found between the presence of an EPO rhythm and the degree of renal disease. Our search for additional factors that could influence EPO levels did not reveal

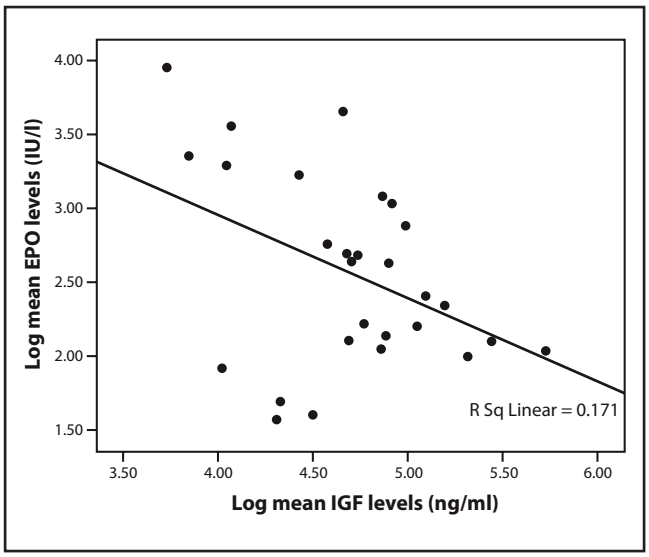

Fig. 1. Correlation between levels of IGF-1 and EPO.

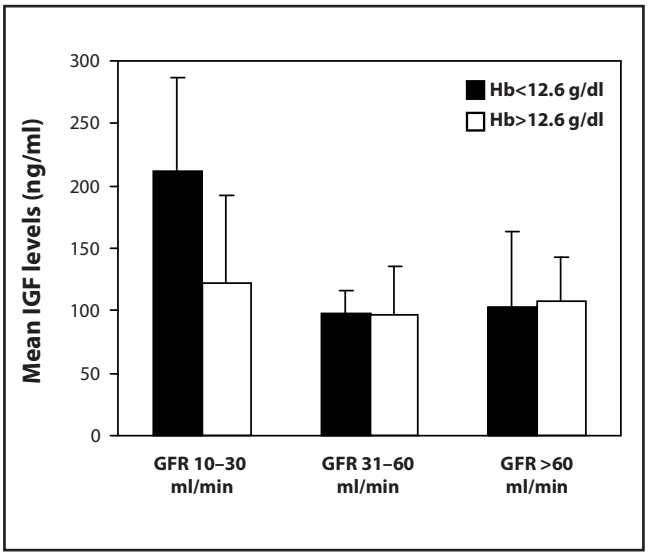

Fig. 2. Relations between IGF-1, Hb and GFR. Means ± SD are shown.

any other determinants for the existence of a rhythm for EPO. It thus remains unclear why the 15 subjects in our study did demonstrate an EPO rhythm, while the other 13 subjects did not. A possible explanation could be that in the subjects with an EPO rhythm, more fluctuations in blood pressure were present, as blood pressure is related to EPO levels (Schmieder *et al.* 1997). Unfortunately, the frequency of blood pressure measurements that we used was too low to detect such fluctuations. Another possible explanation could be the presence of unrecognized sleep apnea. In sleep apnea, the diurnal variation in EPO levels is higher than in healthy persons due to nocturnal hypoxemia (Cahan *et al.* 1992). In our study, we did not perform nocturnal oxygen measurements.

IGF-1

In the present study we have found no significant IGF-1 rhythm. IGF-1 has been shown to exhibit EPO-like erythropoietic activity (Correa & Axelrad 1991). IGF-1 is produced in the liver under the influence of GH. In chronic renal disease, the release of GH is increased and the metabolic clearance rate is decreased, leading to increased circulating levels of GH (Haffner *et al.* 1994). This would lead to higher levels of IGF-1, however in CKD, IGF-1 synthesis in the liver is impaired. Despite ensuing normal IGF-1 levels, the effectiveness of IGF-1 is reduced. This is due to decreased levels of free bioactive IGF-1, as levels of circulating inhibitory binding proteins are higher (Tonshoff *et al.* 1997).

To our knowledge, no information is available on whether IGF-1 exhibits a circadian rhythm in healthy adults and in patients with chronic renal disease. In our study, we were unable to demonstrate a circadian rhythm for IGF-1 in subjects with a decreased GFR. We also found no rhythm for IGF-1 in subjects with a normal GFR. Possibly the relatively old age of our study population (mean age 71.3 yrs) has influenced our results. At the age of 65 years, daily spontaneous GH secretion is reduced by 50–70%, leading to a decline in IGF-1 levels (Lombardi *et al.* 2005). Theoretically, this could lead to an increased difficulty in detecting a circadian rhythm. Another explanation for the absence of an IGF-1 rhythm in our study could be the relatively high prevalence (29%) of DM amongst the subjects, as DM is associated with lower levels of IGF-1 (Lissoni *et al.* 2004).

EPO and IGF-1

Mean levels of IGF-1 were correlated inversely to mean levels of EPO. When divided based on GFR and Hb, subjects with the lowest GFR 0–30 ml/min and lowest Hb levels had the highest IGF-1 levels.

Brox et al showed that EPO and IGF-1 act synergistically on erythropoiesis in a mouse model of CKD (Brox *et al.* 1996) in inducing a substantial rise in Hb. In addition, several possible feedback mechanisms between EPO production and IGF-1 secretion have been suggested (Lissoni *et al.* 2004; Sohmiya & Kato 2005). In our study, the fact that mean levels of IGF-1 correlated inversely to mean levels of EPO and that subjects with the greatest decrease in CKD and a relatively low Hb had the highest IGF-1 levels, suggests that EPO and IGF-1 both have a role on erythropoiesis in CKD. Taken together, it is possible to speculate that IGF-1 constitutes a synergistic role with EPO in erythropoiesis and that when EPO falls a compensatory increase of IGF-1 occurs. However, until now the therapeutic application of IGF-1 is limited by the difficulty of assessing pituitary functional status in CKD patients, and the interaction with IGF-binding proteins that determine its bioavailability.

IL-6 and TNF-α

In the present study we found no circadian IL-6 and TNF-α rhythm, Inflammation, in this study characterized by IL-6 and TNF-α, has been linked to EPO-resistance and renal disease. An increase in IL-6 and TNF-α has been associated with an increased need in exogenous EPO (Macdougall & Cooper 2005). In healthy volunteers the concentration of Il-6 is regulated in a circadian fashion with peak levels in the early morning and early evening and through levels later in the morning and at night (Vgontzas *et al.* 2002). TNF-α has shown to have peak levels at night. In a study with 60 patients with chronic renal disease IL-6 was significantly increased in comparison to the healthy control group. Information on times of blood collection was not available (Oberg *et al.* 2004). Knowledge on TNF-α levels in renal disease is absent. In our patient group IL-6 levels were raised in contrast to TNF-α levels. No circadian rhythm in IL-6 was found, and furthermore no relationship was found between inflammation and EPO levels or IGF-1 levels. This finding is in keeping with another study that showed no association between GFR and IL-6 (Oberg *et al.* 2004).

CONCLUSION

In conclusion, we failed to substantiate our hypothesis that the GFR related decrease in melatonin rhythm is associated with circadian changes in EPO levels, in the levels of IGF (a co stimulator of EPO) and/or in the levels of inflammatory markers (inhibitors of the action of EPO). Furthermore we could not identify the main driving factor for rhythmic fluctuations in endogenous EPO levels on the basis of the results of this study. Due to the varying peak times, it is unlikely that it concerns a circadian (mis)alignment, therefore providing no reason for specific timing of administration of exogenous EPO. Also for IGF-1, TNF-α and IL-6 levels, we were unable to identify a rhythm in any of the patients. Finally, the correlation between IGF-1 and EPO as well as the association between IGF-1, Hb and renal function warrants future research on the role of IGF-1 in CKD patients with persistent anemia.

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The work described in this article has been carried out in accordance with the code of ethics of the world medical association (declaration of Helsinki) for experiments involving humans.

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All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

REFERENCES

1 Brox AG, Zhang F, Guyda H, Gagnon RF (1996) Subtherapeutic erythropoietin and insulin-like growth factor-1 correct the anemia of chronic renal failure in the mouse. Kidney Int. **50**: 937–943.
2 Buemi M, Allegra A, Laganà A, Aloisi C, Privitera M, Morabito N, Frisina N (1993) Effects of the evening i.v. administration of erythropoietin in haemodialyzed patients. Riv Eur Sci Med Farmacol. **15**: 195–197.
3 Cahan C, Decker MJ, Arnold JL, Washington LH, Veldhuis JD, Goldwasser E, Strohl KP (1992) Diurnal variations in serum erythropoietin levels in healthy subjects and sleep apnea patients. J Appl Physiol. **72**: 2112–2117.
4 Clement DL, De Buyzere ML, De Bacquer DA, de Leeuw PW, Duprez DA, Fagard RH, Gheeraert PJ, Missault LH, Braun JJ, Six RO, Van Der Niepen P, O'Brien E (2003) Prognostic value of ambulatory blood-pressure recordings in patients with treated hypertension. N Engl J Med. **348**: 2407–2415.
5 Colao A, Di Somma C, Cascella T, Pivonello R, Vitale G, Grasso LF, Lombardi G, Savastano S (2008) Relationships between serum IGF1 levels, blood pressure, and glucose tolerance: an observational, exploratory study in 404 subjects. Eur J Endocrinol. **159**: 389–397.
6 Congote LF, Sadvakassova G, Dobocan MC, Difalco MR, Li Q (2010) Erythropoietin-dependent endothelial proteins: potential use against erythropoietin resistance. Cytokine. **51**: 113–118.
7 Correa PN, Axelrad AA (1991) Production of erythropoietic bursts by progenitor cells from adult human peripheral blood in an improved serum-free medium: role of insulin like growth factor 1. Blood. **78**: 2823–2833.
8 Elung-Jensen T, Strandgaard S, Kamper AL (2008) Longitudinal observations on circadian blood pressure variation in chronic kidney disease stages 3–5. Nephrol Dial Transplant. **23**: 2873–2878.
9 Firsov D, Bonny O (2010) Circadian regulation of renal function. Kidney Int. **78**: 640–645.
10 Fisher JW (2003) Erythropoietin: physiology and pharmacology update. Exp Biol Med. (Maywood). **228**: 1–14.
11 Fisher JW, Koury S, Ducey T, Mendel S (1996) Erythropoietin production by interstitial cells of hypoxic monkey kidneys. Br J Haematol. **95**: 27–32.
12 Haffner D, Schaefer F, Girard J, Ritz E, Mehls O (1994) Metabolic clearance of recombinant human growth hormone in health and chronic renal failure. J Clin Invest. **93**: 1163–1171.
13 Heuck C, Skjaerbaek C, Orskov H, Wolthers OD (1999) Circadian variation in serum free ultrafiltrable insulin-like growth factor I concentrations in healthy children. Pediatr Res. **45**: 733–736.
14 Karasek M, Szuflet A, Chrzanowski W, Zylinska K, Swietoslawski J (2005) Decreased melatonin nocturnal concentrations in hemodialyzed patients. Neuro Endocrinol Lett. **26**: 653–656.
15 Klausen T, Dela F, Hippe E, Galbo H (1993) Diurnal variations of serum erythropoietin in trained and untrained subjects. Eur J Appl Physiol Occup Physiol. **67**: 545–548.
16 Koch BC, Nagtegaal JE, Hagen EC, van der Westerlaken MM, Boringa JB, Kerkhof GA, Ter Wee PM (2009) The effects of melatonin on sleep-wake rhythm of daytime haemodialysis patients: a randomized, placebo-controlled, cross-over study (EMSCAP study). Br J Clin Pharmacol. **67**: 68–75.
17 Koch BC, van der Putten K, Van Someren EJ, Wielders JP, Ter Wee PM, Nagtegaal JE, Gaillard CA (2010) Impairment of endogenous melatonin rhythm is related to the degree of Chronic Kidney Disease (CREAM study). Nephrol Dial Transplant. **25**: 513–519.
18 Lissoni P, Perego M, Veronese E, Fumagalli G, Brivio F, Colciago M, Messina G, Rovelli F, Brivio R, Gardani G (2004) Endocrine effects of erythropoietin in cancer patients. Hematology. **9**: 363–367.
19 Lombardi G, Di Somma C, Rota F, Colao A (2005) Associated hormonal decline in aging: is there a role for GH therapy in aging men? J Endocrinol Invest. **28**: 99–108.
20 Macdougall IC, Cooper AC (2005) Hyporesponsiveness to erythropoietic therapy due to chronic inflammation. Eur J Clin Invest. **35** Suppl 3: 32–35.
21 Oberg BP, McMenamin E, Lucas FL, McMonagle E, Morrow J, Ikizler TA, Himmelfarb J (2004) Increased prevalence of oxidant stress and inflammation in patients with moderate to severe chronic kidney disease. Kidney Int. **65**: 1009–1016.
22 Roberts D, Smith DJ (1996) Erythropoietin does not demonstrate circadian rhythm in healthy men. J Appl Physiol. 80: 847–851.
23 Schmieder RE, Langenfeld MR, Hilgers KF (1997) Endogenous erythropoietin correlates with blood pressure in essential hypertension. Am J Kidney Dis. **29**: 376–382.
24 Scortegagna M, Ding K, Zhang Q, Oktay Y, Bennett MJ, Bennett M, Shelton JM, Richardson JA, Moe O, Garcia JA (2005) HIF-2alpha regulates murine hematopoietic development in an erythropoietin-dependent manner. Blood. **105**: 3133–3140.
25 Sohmiya M, Kato Y (2005) Human growth hormone and insulin-like growth factor-I inhibit erythropoietin secretion from the kidneys of adult rats. J Endocrinol. **184**: 199–207.
26 Stratakis CA, Mastorakos G, Magiakou MA, Papavasiliou EC, Panitsa-Faflia C, Georgiadis E, Batrinos ML (1996) 24-hour secretion of growth hormone (GH), insulin-like growth factors-I and -II (IGF-I, -II), prolactin (PRL) and thyrotropin (TSH) in young adults of normal and tall stature. Endocr Res. **22**: 261–276.
27 Tönshoff B, Powell DR, Zhao D, Durham SK, Coleman ME, Domené HM, Blum WF, Baxter RC, Moore LC, Kaskel FJ (1997) Decreased hepatic insulin-like growth factor (IGF)-I and increased IGF binding protein-1 and -2 gene expression in experimental uremia. Endocrinology. **138**: 938–946.
28 van der Putten K, Braam B, Jie KE, Gaillard CA (2008) Mechanisms of Disease: erythropoietin resistance in patients with both heart and kidney failure. Nat Clin Pract Nephrol. **4**: 47–57.
29 Van Someren EJ, Nagtegaal E (2007) Improving melatonin circadian phase estimates. Sleep Med. **8**: 590–601.
30 Vaziri ND, Oveisi F, Reyes GA, Zhou XJ (1996) Dysregulation of melatonin metabolism in chronic renal insufficiency: role of erythropoietin-deficiency anemia. Kidney Int. **50**: 653–656.
31 Vgontzas AN, Zoumakis M, Papanicolaou DA, Bixler EO, Prolo P, Lin HM, Vela-Bueno A, Kales A, Chrousos GP (2002) Chronic insomnia is associated with a shift of interleukin-6 and tumor necrosis factor secretion from nighttime to daytime. Metabolism. **51**: 887–92.