

1 **Circadian rhythms and the kidney**

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1 **Abstract**

2 Numerous physiological functions exhibit substantial circadian oscillations. In the
3 kidneys, renal plasma flow, the glomerular filtration rate as well as tubular reabsorption
4 and/or secretion processes have been shown to peak during the active phase and decline
5 during the inactive phase. These functional rhythms are driven, at least in part, by a self-
6 sustaining cellular mechanism termed the circadian clock. The circadian clock controls
7 different cellular functions, including transcription, translation and protein post-
8 translational modifications (phosphorylation, acetylation, ubiquitylation, and so on) and
9 degradation. Disruption of the circadian clock in animal models results in the loss of blood
10 pressure control and substantial changes in the circadian pattern of water and electrolyte
11 excretion in the urine. Kidney-specific suppression of the circadian clock in animals
12 implicates both the intrinsic renal and extra-renal circadian clocks in these pathologies.
13 Alterations in the circadian rhythm of renal functions are associated with the
14 development of hypertension, chronic kidney disease, renal fibrosis and kidney stones.
15 Furthermore, renal circadian clocks might interfere with the pharmacokinetics and/or
16 pharmacodynamics of various drugs and are therefore an important consideration in the
17 treatment of some renal diseases or disorders.

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1 **Introduction**

2 The primary function of the kidneys is to maintain the homeostasis of extracellular fluids.
3 The traditional concept of homeostasis is centered on the idea that water and solute
4 balance in the body is achieved through numerous self-adjusting renal mechanisms that
5 are based on negative feed-back control – that is, homeostatic reactions are activated in
6 response to a change that has already occurred. However, research indicates that
7 adaptation of the kidneys to dramatic homeostatic transitions that are induced by
8 circadian rhythms in activity and feeding rely, at least in part, on a self-sustaining
9 molecular mechanism that continuously adjusts both glomerular and tubular functions in
10 anticipation of these circadian changes. This molecular mechanism, termed the circadian
11 clock, has been found in most tissues, including the kidneys. Peripheral circadian clocks
12 are thought to coordinate peripheral physiological functions with the pattern of activity
13 and/or feeding, which is, in turn, synchronized with circadian oscillations in
14 environmental variables by the central circadian clock in the suprachiasmatic nucleus in
15 the brain (Figure 1). Here, we summarize current knowledge of renal circadian rhythms
16 and renal circadian clock mechanisms. We also review the implications of disrupting
17 circadian rhythms or the circadian clock mechanism for the development and progression
18 of kidney diseases in humans.

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21 **Circadian rhythms in the kidney**

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23 **Biological rhythms**

24 Most, if not all, vital functions in animals and plants show significant periodic fluctuations.
25 These functional fluctuations are the result of evolutionary adaptation to periodic
26 changes in environmental variables to which all life on Earth is exposed. Geophysical
27 cycles are by far the most important source of these environmental oscillations, including
28 the ~24 h light–dark cycle caused by the Earth’s rotation on its axis, the seasonal changes
29 imposed by the combined effect of the ~365 day orbit of the Earth around the Sun and the
30 tilting of the Earth on its axis and the well-known but still poorly understood effects of the
31 lunar cycle. Importantly, many of the functional rhythms are self-sustaining and continue

1 to oscillate even in the absence of external environmental stimuli, which suggests that the
2 ability to anticipate the geophysical rhythms probably offered a selective advantage.

3 All biological rhythms can be classified on the basis of their period length, that is,
4 circadian rhythms (~24 h), infradian rhythms (>24 h) and ultradian rhythms, (<24 h). In
5 this Review, we focus on circadian rhythms in the kidneys, whereas other types of renal
6 functional rhythms, such as infradian rhythms, are reviewed elsewhere (1).

7

8 **Functional rhythms in the kidney**

9 Functional circadian oscillations are probably most easily seen in the kidneys, as a marked
10 reduction occurs in the volume of urine excreted during the night compared to during the
11 day (Figure 2). The first scientific description of circadian rhythms in the kidney was
12 published in the mid- 19th century by Edward Smith, a British physician who detected
13 urinary urea and water excretion rhythms by analyzing urine samples that were collected
14 hourly from prisoners in London's Coldbath Fields prison (2). Since then, circadian
15 oscillations have been documented for most renal functions.

16 We now know that multiple renal processes show circadian rhythms, including
17 the glomerular filtration rate (GFR), renal plasma flow (RPF) and renal excretion of water
18 and major urinary solutes (reviewed in (3-8)). Most renal functional rhythms have similar
19 kinetics, reaching their peak value in the middle of the phase of maximal behavioural
20 activity and reaching a trough during the phase of minimal behavioural activity, which are
21 hereafter referred to as the active and inactive phases, respectively. Of note, the active
22 phase in humans and other diurnal animals is during the day, whereas it is during the
23 night in nocturnal animals, such as rats and mice. However, the amplitude of the
24 oscillations in different renal functional variables differs substantially. For example, the
25 amplitude of circadian oscillations in GFR and RPF is ~50% (9-11), whereas the rate of
26 water and major electrolyte excretion is several-fold higher during the active phase than
27 during the inactive phase (12-15). In mice, circadian oscillations in the inner medullary
28 concentrations of sodium, chloride and urea exist, resulting in a substantial increase in
29 the inner medullary osmolality during the active phase (16). These findings suggest that
30 the cortico-medullary gradient in the kidneys parallels the oscillations in GFR, thereby
31 facilitating water reabsorption when the GFR and, hence, the filtered load of water are
32 substantially increased. Experiments using oxygen-sensitive carbon paste electrodes
33 implanted into the rat kidney demonstrated that oxygen levels in the renal cortex and

1 renal medulla follow the circadian pattern of the RPF (17). As the oscillations in renal
2 oxygenation parallel those of nutrient delivery to the kidneys, these data strongly suggest
3 that renal energy production also follows a circadian pattern. Importantly, these results
4 might have clinical relevance, as they suggest that the kidney is more vulnerable to
5 hypoxic stress during the night.

6 Thus, a growing body of evidence suggests that the homeostatic control of
7 extracellular fluid volume and composition involves continuous adjustment of diverse
8 renal functions throughout the 24 h circadian cycle. Current research in renal circadian
9 physiology is focused on identifying the molecular mechanisms underlying circadian
10 rhythmicity of renal functions, determining whether renal functional rhythms are driven
11 by systemic circadian time cues (activity, food components, hormones, body temperature
12 and so on) or by local mechanisms and whether disruption of circadian rhythms causes
13 human kidney diseases.

14 Although it is almost impossible to isolate the kidneys from factors that might be
15 involved in the generation and/or maintenance of circadian oscillations, it is clear that the
16 GFR rhythm is independent of systemic blood pressure oscillations and of sympathetic
17 nervous system activity (18, 19). Furthermore, the circadian rhythm of potassium
18 excretion in the urine is not influenced by posture or feeding pattern (20). These studies
19 suggest the existence of a self-sustaining molecular mechanism that is involved in the
20 circadian control of renal functions. The discovery of a ubiquitous, highly conserved
21 molecular core of this mechanism, which was termed the circadian clock, was a significant
22 step forward in the field, which was recognized with the award of the 2017 Nobel Prize in
23 Physiology and Medicine (21).

24

25 **Transcriptional oscillations in the kidneys**

26 The circadian clock is a network of interconnected transcription feedback loops that
27 generate cell-autonomous, self-sustaining transcriptional circadian oscillations
28 (reviewed in (22, 23)). In mammals, the main feedback loop is activated by a
29 heterodimeric transcription activator comprising brain and muscle ARNT-like 1 (BMAL1)
30 and circadian locomotor output cycles protein kaput (CLOCK) (Figure 1). BMAL1–CLOCK
31 heterodimers trigger the transcription of a wide range of circadian clock-controlled genes
32 (CCGs), including the period (*PER1*, *PER2* and *PER3*) and cryptochrome (*CRY1* and *CRY2*)
33 family of genes. PER–CRY heterodimers are transcription repressors that inhibit the

1 activity of BMAL1–CLOCK, thereby forming the inhibitory limb of the feedback loop.
2 Another important feedback mechanism involves the nuclear receptors REV-ERB and
3 ROR, which control the transcription of *BMAL1*. This core clock circadian machinery is
4 ubiquitously expressed, including throughout the central nervous system and peripheral
5 tissues, as well as in the kidneys (24-26). All individual cellular clocks are synchronized
6 to each other and to geophysical time through the activity of a central oscillator termed
7 the ‘master clock’, which is located in the suprachiasmatic nucleus (SCN) of the
8 hypothalamus (reviewed in (27)). The master clock, in turn, is continuously synchronized
9 to geophysical time by photic (light–dark) cues that are perceived by the retina and are
10 transmitted to the SCN through the optic nerve (28). The major signals transmitted by the
11 master clock to reset the peripheral oscillators include circadian synthesis and/or release
12 of circulating factors (hormones, food components, food metabolites and so on), circadian
13 control of neuronal activity (including control of activity and feeding behaviour) and
14 circadian control of body temperature (reviewed in (29)). Collectively, the SCN and the
15 extra-SCN circadian oscillators constitute a hierarchically organized circadian timing
16 system that enables the coordination of most biological processes (at the cellular, tissue
17 and organ levels) with geophysical time.

18 Current estimates of the number of CCGs vary substantially (from 10 to 100% of
19 genes) depending on which algorithm is used to identify CCGs (reviewed in (30)). An
20 analysis of the transcriptomes of 12 adult mouse organs showed that the transcription of
21 ~43% of all protein-coding genes in the mouse genome showed circadian oscillations in
22 at least one of the organs tested (31). Importantly, only the liver exceeded the kidneys in
23 the total number of circadian transcripts (~16% in the liver versus ~13% in the kidney),
24 suggesting that robust circadian clock activity exists in renal cells. This and other studies
25 (32, 33) also showed that the circadian rhythmicity was organ-specific for most oscillating
26 transcripts, supporting the idea that the main role of the circadian clock is to make
27 temporal adjustments to cell-type-specific and tissue-specific functions. Marked circadian
28 oscillations in hundreds of mRNAs were identified in microdissected mouse renal
29 tubules(34, 35), including in the distal convoluted tubule (DCT), the connecting tubule
30 (CNT) and the cortical collecting duct (CCD), and genetic inactivation of *Clock* leads to
31 dramatic changes in the transcriptome of these tissues(34). These transcriptome-wide
32 studies and other targeted, gene-specific analyses of transcripts encoding renal
33 transporters or transport-related proteins have revealed rhythmic expression of renal

1 vasopressin V1a receptor (*Avpr1*) and *Avpr2*(16, 34), urea transporter 2 (*Ut2*; also known
2 as *Slc14a2*) (16), potassium-transporting ATPase alpha chain 2 (*Atp12a*)(36), the
3 aquaporins *Aqp1*, *Aqp2* and *Aqp3*(10, 16, 34), epithelial sodium channel subunit alpha
4 (α ENAC; encoded by *Scnn1a*)(10) and ubiquitin carboxyl-terminal hydrolase 2
5 (*Usp2*)(37).

6

7 **Post-transcriptional circadian rhythms**

8 Circadian oscillation in transcription is not the only cellular mechanism that couples renal
9 functions to circadian rhythms in environmental factors — translational and post-
10 translational regulations are also crucially important (Figure 3). For example, the
11 circadian clock imposes rhythmic transcription and translation on RNAs that are involved
12 in ribosome biogenesis in the mouse liver (38, 39). As the peak of ribosome biogenesis
13 and polysome formation occurs in the middle of the active phase, the circadian clock likely
14 coordinates the energy-consuming process of protein synthesis with the circadian pattern
15 of energy production in cells. Ribosome profiling in mouse kidneys to identify
16 rhythmically translated renal mRNAs found that 41% of circadian transcripts are
17 rhythmically translated and 55% of rhythmically translated transcripts had a circadian
18 expression pattern(40). This analysis revealed rhythmic transcription and translation of
19 several key genes or proteins that are involved in many renal homeostatic functions,
20 including podocin(*Nphs2*), the aquaporins (*Aqp2*, *Aqp4* and *-Aqp8*), claudin 1(*Cldn1*),
21 serum glucocorticoid-regulated kinase 1 (*Sgk1*), mitochondrial 1,25-dihydroxyvitamin D₃
22 24-hydroxylase (*Cyp24a1*), the urate transporter GLUT9(*Glut9*; also known as *Slc2a9*) and
23 many others. The category of rhythmically translated transcripts that do not cycle at the
24 transcription level (and thus are not detected by RNA profiling as genes that are relevant
25 for circadian physiology) is of special interest, and include the adenosine A1 receptor
26 (*Adora1*), the prostaglandin E2 receptors EP3 (*Ptger3*) and EP4 (*Ptger4*), prostaglandin
27 F₂ α receptor (*Ptgfr*), peroxisome proliferator receptor activator alpha (*Ppara*), claudin 19
28 (*Cldn19*), the b^{0,+} amino acid transporter (*Slc7a9*), the sodium-driven anion-exchanger
29 NDCBE (*Slc4A8*), the peptide transporter PEPT1 (*Pept1*) and the TASK2 (*Task2*)
30 potassium channel. Overall, this study showed that ~1,000 renal transcripts (of ~12,000
31 detected transcripts) are translated in a circadian pattern. However, these results must
32 be interpreted with caution, as protein levels in a cell might be strongly influenced by

1 other factors, such as protein degradation and/or protein secretion. Therefore, precise
2 analysis of the circadian proteome of the kidney awaits further investigation.

3 The wide range of post-translational modifications that might contribute
4 substantially to periodic changes in protein stability, subcellular localization, protein-
5 protein interactions and protein function, are another level of complexity to consider
6 when correlating circadian translation or proteome data with functional circadian
7 oscillations in renal functions. For example, targeted analysis of the WNK4-OSR1-SPAK-
8 sodium chloride co-transporter (NCC) signalling cascade, which controls sodium
9 reabsorption in the distal convoluted tubule, showed circadian oscillations in the levels of
10 phosphorylated OSR1, SPAK and the active form of NCC , but not in their total protein
11 levels(41, 42).

12 Although substantial progress has been made in defining the role of post-
13 translational modifications in regulation of core clock proteins(reviewed in (43), little is
14 known about circadian patterns of post-translational modifications in the entire
15 proteome. Global analyses of the circadian phosphorylome and the circadian acetylome
16 have been carried out in the mouse liver and identified ~20,000 phosphorylation sites (in
17 ~ 4,400 liver proteins), ~25% of which are regulated in a circadian manner(44). Of note,
18 the amplitude of phosphorylation cycles substantially exceeded the amplitudes of the
19 cycles in the circadian transcriptome and circadian proteome in the same tissue. Analysis
20 of the liver acetylome detected circadian oscillations in ~13% of ~1,000 acetylation sites
21 in liver proteins (45). Analysis of the molecular pathways affected by circadian acetylation
22 revealed an enrichment of proteins involved in the urea and tricarboxylic acid cycles and
23 in the metabolism of amino acids and lipids. Collectively, these studies open new avenues
24 of research in circadian physiology and provide technical solutions to study the role of
25 circadian post-translational modifications in other tissues, including the kidney.

26 27 **[H2] Intrinsic versus external regulation**

28 As discussed above, circadian oscillations in the kidney can be entrained by intrinsic renal
29 circadian clocks and/or by external circadian time cues. Circulating hormones are the
30 most obvious candidates for the external circadian entrainment, as most hormones show
31 substantial circadian fluctuations in their plasma levels. One of the best-known examples
32 is aldosterone, a mineralo-corticosteroid hormone that is secreted by the adrenal glands
33 and that has a crucial role in the maintenance of extracellular sodium homeostasis and in

1 blood pressure control. Aldosterone regulates apical sodium entry in principal cells of the
2 collecting duct and in the connecting tubule via the epithelial sodium channel ENaC
3 (reviewed in (46)). Plasma aldosterone levels peak in the first half of the active phase (47,
4 48), a pattern that parallels that of the GFR and, hence, of the filtered sodium load. Thus,
5 circadian oscillations in plasma aldosterone levels might be necessary for the continuous
6 adaptation of tubular sodium reabsorption capacity to circadian oscillations in the filtered
7 sodium load. Plasma aldosterone levels are dramatically increased and circadian
8 aldosterone oscillations are substantially reduced in mice deficient in CRY1 and CRY2
9 (*Cry1/Cry2*-null mice)(48). Analysis of the circadian transcriptome of the adrenal glands
10 in these mice detected a chronic increase in the expression of the aldosterone biosynthetic
11 enzyme type VI 3-hydroxyl-steroid dehydrogenase (HSD3B6). Functional defects in
12 *Cry1/Cry2*-null mice included salt-sensitive hypertension and a non-dipping pattern of
13 arterial blood pressure. This study was the first to show that an extra-renal circadian
14 clock controlled a vital renal function through the circadian synthesis and release of a
15 hormonal mediator. Another study in mice provided evidence that a circadian rhythm in
16 oxygen levels in the circulation and in kidney tissue might entrain the intrinsic renal
17 circadian clocks by inducing circadian oscillations in the levels of hypoxia-inducible factor
18 1α (HIF1 α)(49); the nuclear levels of HIF1 α in the kidney oscillated with a peak in the first
19 half of the active phase. In addition, exposure of cultured cells to circadian oscillations in
20 physiological oxygen resynchronized the individual cellular circadian clocks to follow the
21 oxygen rhythm, an effect that was abolished by knockdown of HIF1 α (49). Interestingly,
22 acid produced during hypoxia was recently shown to decrease the translation of clock
23 constituents by suppressing mTORC1 signaling, inducing a disruption of the circadian
24 clock (50)

25 Other studies have characterized the role of the core clock components PER1,
26 CLOCK and BMAL1 in renal function. PER1 positively regulates aldosterone synthesis in
27 an adrenal cell line and plasma aldosterone levels *in vivo*(51). Functional analysis of *Per1*-
28 null mice in conditions of high-salt and mineralocorticoid treatment revealed a non-
29 dipping hypertension resulting from PER1 deficiency(52) (53). Furthermore, PER1
30 controls the transcription of the genes encoding several key proteins involved in solute
31 reabsorption along the nephron, including α ENaC(54-56), NCC(57), the kinases with no
32 lysine 1 (WNK1) and WNK4(57), sodium/hydrogen exchanger 3 (NHE3)(58), sodium-

1 glucose cotransporter 1 (SGLT1)(58) and a group of genes that are involved in the
2 endothelin axis of sodium reabsorption in the kidney(59).

3 A detailed renal phenotyping of *Clock*-null mice(12, 34) demonstrated that CLOCK
4 deficiency results in the loss of circadian rhythmicity of urinary excretion of sodium,
5 potassium and water. In addition, the circadian rhythm in plasma aldosterone levels was
6 disrupted and blood pressure was substantially reduced in *Clock*-null mice.
7 Transcriptome analysis of the kidneys of *Clock*-null mice revealed substantial changes in
8 the expression levels and/or in the circadian expression patterns of *Avpr1*, *Avpr2*, *Aqp2*,
9 *Aqp4*, *Ppara*, *Slc9a3* (which encodes NHE3) and many other transcripts that are important
10 for diverse renal functions. Pathway enrichment analysis identified a group of renal
11 cytochrome p450 enzymes (*Cyp4a12a*, *Cyp4a12b*, *Cyp4a14*, *Cyp2c44* and *Cyp2j13*) that are
12 involved in the conversion of arachidonic acid to different active metabolites, including
13 20-hydroxyeicosatetraenoic acid (20-HETE). In the kidneys of *Clock*-null mice, the
14 circadian rhythm of 20-HETE exhibited a substantial shift in acrophase and a substantial
15 reduction in average 24 h levels. As 20-HETE is a potent modulator of glomerular
16 filtration and tubular reabsorption (60-62), these results suggest that regulation of 20-
17 HETE synthesis is one of the crucial mechanisms by which the circadian clock controls
18 renal function. Increased renal fibrosis and renal parenchymal damage in *Clock*-null mice
19 after unilateral ureteral obstruction (a model of renal fibrosis) suggests that the circadian
20 clock also has a role in renal fibrosis (63). At the molecular level, the circadian clock
21 inhibits the transforming growth factor- β (TGF β)-cyclooxygenase 2 (COX2; also known
22 as PTGS2) profibrotic axis in the kidney(63), leading these researchers to suggest that the
23 renal circadian clock might be a therapeutic target for treatment of chronic kidney disease
24 (CKD).

25 BMAL1 is thought to be the sole indispensable component of the core clock
26 machinery (64). *Bmal1*-null mice have a multitude of mild-severe dysfunctions, including
27 arrhythmic behaviour (64), impaired metabolism of glucose (65) and fatty acids (66),
28 early aging (67, 68) and fertility problems (69). Consequently, the general health
29 impairment in *Bmal1*-null mice complicates analysis of organ-specific phenotypes.
30 Circadian rhythmicity in the medullary concentration of sodium and urea and in the
31 corticomedullary osmotic gradient is lost in *Bmal1*-null mice(16), and molecular analyses
32 revealed blunted circadian expression of *Avpr1*, *Avpr2*, *Aqp2* and *Slc14a2* in the kidneys
33 of *Bmal1*-null mice. However, these data must be interpreted cautiously, as other factors

1 in addition to renal medullary circadian clocks might be involved in the circadian
2 rhythmicity of corticomedullary osmolality, including circadian oscillations in blood
3 pressure, GFR and RPF. Interestingly, *Bmal1*-null mice exhibited a non-dipping blood
4 pressure pattern and a substantial reduction in blood pressure (70), a result that was
5 confirmed and extended in mice with a smooth muscle-specific knockout of *Bmal1*, which
6 also had a similar blood pressure phenotype(71). However, a detailed analysis of renal
7 function was not carried out in either of these *Bmal1*-null mice strains.

8 The role of the kidney in circadian oscillations of the main circulating components
9 of the renin–angiotensin–aldosterone system (RAAS) and in blood pressure rhythms was
10 examined in two mouse strains with kidney cell-specific knockout of *Bmal1*. To
11 specifically disrupt the circadian clock in renin-secreting granular cells, mice that
12 constitutively express Cre recombinase from the promoter of the renin^d(*Ren1^d*)
13 gene(72) and carry a conditional allele of the *Bmal1* gene(73) (*Bmal1^{lox/lox}/Ren1^dCre*
14 mice) were generated (72)(72)(72)(72)(71)(10). In these mice, the circadian pattern of
15 renin protein expression in kidney tissue was disrupted and plasma aldosterone levels
16 were moderately reduced. Analysis of urine samples revealed a mild polyuria, moderate
17 changes in the circadian rhythm of urinary sodium excretion and low urinary pH. In
18 addition, the GFR was substantially increased and blood pressure was markedly
19 decreased, although the dipping pattern of blood pressure was maintained. These results
20 suggest that circadian oscillations in arterial blood pressure are driven mostly by
21 circadian clocks in extra-renal tissues (adrenal glands, smooth muscle cells and possibly
22 other tissues) rather than by intrinsic renal circadian clocks. Of note, the phenotypic
23 differences between different constitutive knockout strains might be masked by adaptive
24 mechanisms that are activated during mouse embryonic and postembryonic
25 development.

26 In a second study(74), the role of intrinsic circadian clocks in renal tubular cells
27 was examined using mice with a doxycycline-inducible, nephron-specific knockout of
28 *Bmal1* (*Bmal1^{lox/lox}/Pax8-rtTA/LC1* mice)(75). In these mice, aside from reduced kidney
29 size, disruption of clock activity in the nephron did not result in any overt renal
30 abnormalities. Circadian patterns of urinary sodium, potassium and water excretion did
31 not differ from those of control animals, thereby providing evidence for extrarenal
32 control of renal excretory rhythms. However, increased plasma creatinine and urea levels
33 despite a normal GFR indicated that tubular function was impaired, and kidney

1 transcriptome analysis detected enrichment of genes involved in fatty acid and amino acid
2 metabolism and in organic anion transport. As renal metabolism relies predominantly on
3 fatty acid oxidation, these results suggest that the intrinsic tubular circadian clocks have
4 a substantial role in the control of metabolic processes in renal tubular cells. In support
5 of this hypothesis, the NAD⁺/NADH ratio, which is a marker of the oxidative
6 phosphorylation/glycolysis ratio and/or mitochondrial function, was substantially
7 reduced in *Bmal1^{lox/lox}/Pax8-rtTA/LC1* mice. The increase in plasma creatinine levels in
8 these mice might be explained by a marked reduction in their expression of *Slc22a8* (also
9 known as *Oat3*), which encodes an organic anion transporter that contributes
10 substantially to the basolateral secretion of creatinine in the proximal tubule in mice(76).
11 Systolic, but not diastolic, blood pressure was modestly reduced in *Bmal1^{lox/lox}/Pax8-
12 rtTA/LC1* mice, whereas the blood pressure circadian rhythm was preserved.

13 In general, studies of these two mouse strains with kidney-specific disruption of
14 the circadian clock showed that intrinsic renal circadian clocks are important but not
15 crucial for kidney function (at least in the absence of additional factors, such as pathology,
16 stress and so on), and that most renal functional oscillations are entrained by external
17 circadian time-cues.

18

19 **Clinical relevance**

20 Although circadian rhythms in several renal functions have been described, their clinical
21 importance is not yet clear. Assigning renal or systemic circadian rhythms have important
22 clinical implications when (i) the molecular clock is genetically altered, (ii) the circadian
23 clock is misaligned with the biological clock (such as when time is shifted rapidly, for
24 example after a flight across longitudes) or (iii) when the balance between the efficacy
25 and the adverse effects of a given pharmacological agent needs to be finely tuned (such as
26 in chemotherapy). As a substantial number of drugs are eliminated in the kidney, the
27 plasma concentration or the bioavailability of these drugs might depend on the circadian
28 expression of renal transporters. For instance, inactivation of *Bmal1* in mouse renal
29 tubules markedly affects the efficacy of the diuretic furosemide owing to a strong
30 decrease in the levels of OAT3, the transporter that secretes furosemide in the tubular
31 lumen (74).

32 Genetic alterations of the molecular clock are present in some individuals with the
33 rare familial advanced sleep phase syndrome that is linked to mutations in *PER2* (77),

1 *CRY2* (78) and casein kinase 1 delta (*CSNK1D*) (79), and with familial delayed sleep phase
2 syndrome that is caused by mutations in *CRY1*, with a surprisingly high frequency of
3 variants in the population (80). However, to the best of our knowledge, no renal
4 phenotypes have been described in patients affected by these rare syndromes.

5 In genome-wide association studies (GWAS), common variants in the genes encoding the
6 core clock proteins *BMAL1*, *CLOCK* and *NR1D1* and the *ROR*, *PER* and *CRY* family proteins
7 have been associated with mental illnesses, central nervous system degeneration, sleep
8 disorders and metabolic diseases (reviewed in (81)). Of note, variants in *BMAL1* have
9 been associated with hypertension and/or diabetes, two major risk factors for chronic
10 kidney disease (82, 83). However, none of these genes or loci have been directly
11 associated with kidney diseases (source: GWAS catalog(84)). However, GWAS are limited
12 because only some specific SNPs in the core clock genes were analysed, and GWAS may
13 miss other possible genomic variants or translational and post-translational processes
14 that might trigger the development of renal diseases.

15 Rapid changes in time zone, such as during an overseas flight, or chronic disruption of
16 circadian rhythms, such as for night shift workers, might create conflicts between the
17 external time and the endogenous molecular time and result in insulin resistance and
18 hypertension (85). This outcome indicates that misalignment of external time cues and
19 endogenous core clocks can increase the cardiovascular risks, largely confirming
20 epidemiological data showing that night shift workers are at increased risk of obesity,
21 diabetes and cardiovascular diseases. However, whether the kidneys are also more prone
22 to disease in these circumstances is unknown.

23

24 **The circadian system in renal diseases**

25

26 **Fibrosis.** Alterations of the core clock can lead to tissue fibrosis (Table 1). For example,
27 a natural mutation in the gene encoding casein kinase 1 epsilon, an important regulator
28 of the core clock, causes severe cardiac and renal fibrosis that results in renal insufficiency
29 and premature death in hamsters (86). In addition, indirect evidence points to a role for
30 the molecular clock in exacerbating or enhancing fibrogenic repair processes after a first
31 'hit'. For example, unilateral ureteral obstruction results in more TGF β -dependent renal
32 fibrosis in *Clock*-deficient mice than in wild-type littermates(63). The increased fibrosis
33 in these mice was attributed to elevated expression of cyclooxygenase 2 (*Cox2*) and was

1 rescued by treatment with the COX2 inhibitor celecoxib (63). Thus, CLOCK might have a
2 protective effect in renal fibrosis by controlling *Cox2* expression. By contrast,
3 deoxycorticosterone acetate (DOCA)-salt treatment resulted in more renal inflammation
4 and fibrosis in wild-type animals than in mice with the hypomorphic *Clock* Δ 19 allele,
5 suggesting that CLOCK exacerbates renal fibrosis in this hypertension model (87). The
6 apparently contradictory observations about the role of the clock in renal fibrosis in these
7 two mouse models might be due to a direct effect of salt on the molecular clock, but
8 differences in the genetic background of the two mouse strains might also have a role.
9 Indeed, high-salt treatment alters the expression of several components of the core clock,
10 establishing a link between the renal expression of core clock genes and environmental
11 cues. In rats fed a high-salt diet, the time of peak *Bmal1* expression was delayed by several
12 hours and the expression of *Cry1* and *Per2* was completely lost in the inner medulla of the
13 kidney, but not in the renal cortex. This effect seems to be dependent on the endothelin
14 system and, in particular, the type B endothelin receptor(88). The absence of detectable
15 expression of some components of the core clock in the renal medulla in mice fed a high-
16 salt diet might be the result of the special role that this part of the kidney has in controlling
17 the corticomedullary osmotic gradient. Furthermore, this gradient has been shown to be
18 under the strict control of the circadian system(16). Thus, alterations of this osmotic
19 gradient by salt intake or other gradient modulators might contribute to changes in the
20 expression of some core clock genes and might modulate fibrosis.

21 Intriguingly, studies have shown that the nervous system might mediate the effects of
22 circadian clock disruption on renal injury and fibrosis. For example, exposure of mice to
23 blue light prior to ischaemia-reperfusion reduced renal injury by decreasing neutrophil
24 recruitment to the injured tissue. This effect was mediated, at least in part, by a
25 sympathetic (β 3 adrenergic receptor-dependent) pathway, independently of melatonin
26 or corticosterone levels (89). A role for the sympathetic nervous system in renal
27 inflammation is further strengthened by the finding that renal denervation alone reduced
28 cardiac fibrosis and rescued blood pressure dipping in a rat model of metabolic syndrome
29 (90).

30

31 **Kidney stones.** One of the most prevalent diseases of the urinary tract and one that
32 results in substantial morbidity, kidney stones form when urine becomes supersaturated
33 with salts, resulting in the generation of microcrystals. If the crystals aggregate and attach

1 to the urothelium, the stones grow and might eventually detach when they reach a critical
2 size, blocking the ureter and inducing renal colic. Supersaturation depends on the
3 concentration of solutes in urine, which changes during the day and follows a diurnal
4 rhythm (highest during the day and lowest at night) (91-96). The concentration of an ion
5 in the urine depends on the rate of its excretion and on urinary volume. Furthermore,
6 urinary pH also strongly affects lithogenic risk by modulating salt supersaturation, and
7 also displays a circadian rhythm(97, 98). Overall, the risk of forming kidney stones is
8 increased in the early morning, when the urine is more concentrated and more acidic.
9 Interventional measures, such as drinking a glass of water before going to sleep or
10 alkalizing urine during the night by providing a citrate supplement, might reduce the risk
11 of developing stones. However, these interventions have not been formally tested in
12 prospective trials.

13

14 **Glomerulonephritis.** Although glomerulonephritis is a common cause of kidney
15 diseases, little is known about how circadian rhythms affect the clinical outcome. The
16 proteinuria that occurs in several types of nephrotic syndrome, including membranous
17 nephropathy, follows a circadian rhythm(99, 100); peak protein excretion occurs at
18 around 4:00 pm and its nadir is at 3:00 am, and is independent of GFR(18). In addition,
19 the circadian rhythm in blood pressure is markedly disrupted in patients with nephrotic
20 syndrome, and the outcome of IgA nephropathy is dependent on the circadian rhythm of
21 blood pressure in adults and children (101-103). Furthermore, plasma sodium levels
22 correlate with average 24h ambulatory blood pressure in patients with nephrotic
23 syndrome (104) and thus its control might be crucial for the treatment of nephrotic
24 syndrome.

25 Consistent with this idea, a combination treatment with thiazides and angiotensin-
26 converting enzyme (ACE) inhibitors enhanced nocturnal dipping and decreased
27 proteinuria in patients with IgA nephropathy(105).

28 **Chronic kidney disease.** Circadian rhythms in renal functions are disrupted in patients
29 with chronic kidney disease as a result of perturbations such as nephrotic syndrome,
30 fibrosis, hypertension and so on. Although a potential correlation between altered
31 circadian rhythms in renal functions and progression of CKD has not been examined to
32 date, it is clear that hypertension is one of the main markers and/or risk factors of CKD
33 progression(106).

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The circadian system in renal dysfunction

Dialysis. In patients on haemodialysis or peritoneal dialysis, the circadian rhythm of several renal functions is disturbed, which might contribute to morbidity in these patients (107, 108). Dysregulation of melatonin secretion might partly explain the symptoms in these patients or might be, at minimum, a biomarker of the dysrhythmicity. In an uncontrolled trial, nocturnal haemodialysis improved melatonin levels and sleep efficiency and quality, which could enhance the quality of life of patients on dialysis (109).

Kidney transplantation. Renal transplantation is also associated with improvements in the quality of life for patients with end-stage renal disease (ESRD), but this effect seems to be independent of any changes in circadian rhythms or, at least, is not accompanied by substantial changes in melatonin secretion, blood pressure rhythmicity or sleep (110).

Blood pressure. Several hypotheses have been proposed to explain the circadian rhythmicity of blood pressure and, in particular, the nocturnal dipping of blood pressure and its absence in hypertensive patients. A prevalent hypothesis is that the high nocturnal blood pressure in hypertensive patients maintains natriuresis and eliminates sodium that was not excreted during the day. Further support for this blood pressure–natriuresis hypothesis was provided by studies of uninephrectomy in rats and humans. Although uninephrectomy in rats did not alter the circadian rhythm of sodium, potassium and water excretion(111), uninephrectomy in humans resulted in a change in blood pressure rhythms(112), suggesting that acutely reducing the capacity of the kidney to eliminate sodium affects blood pressure rhythms.

As discussed above for fibrosis, sodium might disrupt circadian rhythms, as observed in hypertensive Dahl rats fed a high-salt diet (113). Inhibition of the sympathetic nervous system with bisoprolol in these salt-sensitive, hypertensive rats rescued the salt-induced hypertension and disruption of the sleep–wake cycle(113). However, in hypertensive patients, blood pressure levels improved after renal denervation, despite no change in sodium content in their muscle and skin, although the circadian rhythm of blood pressure was not examined in this study(114). Of note, sodium might also be involved in regulating infradian rhythms in blood pressure(1), although additional factors might also contribute to this effect. Feeding time also affects blood pressure rhythms(115). For example, the circadian rhythms of the renin–angiotensin–aldosterone system and of blood pressure

1 were markedly shifted depending on the feeding time in dogs, suggesting that peripheral
2 clocks can be strongly entrained by nutrients and supersede other systemic cues.

3 In humans, studies of the effectiveness of treatments based on when they are
4 administered (that is, chronopharmacology) showed that blood pressure control is
5 affected by the timing of the treatment. Thus, in a seminal study, an anti-hypertensive
6 drug was more effective in controlling blood pressure when administered at night than
7 when administered in the morning (116). Several observational studies have confirmed
8 these results; for example, taking anti-hypertensive drugs at bedtime improved blood
9 pressure control (117), and valsartan taken at bedtime in non-dipper patients with CKD
10 was more effective at controlling blood pressure than when taken in the morning (118).
11 Furthermore, taking at least one anti-hypertensive drug at bedtime reduced blood
12 pressure and the risk of cardiovascular events in hypertensive patients with chronic
13 kidney disease (119). In the same population, CKD progression, albuminuria and glucose
14 and lipid levels were reduced by taking one or more anti-hypertensive medications at
15 bedtime (120).

16 Although the importance of chronopharmacology in the treatment of hypertension has
17 been studied in several observational trials, only a few of these trials were randomized
18 and controlled and most had major flaws in their design. Meta-analyses on the few
19 existing randomized controlled trials (RCTs) confirmed that blood pressure control is
20 improved by administering anti-hypertensive medications at bedtime in patients with
21 hypertension and/or CKD (121-124).

22 The precise mechanisms by which the timing of administration improves the efficacy
23 and/or reduces the adverse effects of a drug are not clear, but might be related to the
24 absorption, metabolism, and/or excretion of the drug and its metabolites. Although
25 chronopharmacology is certainly underexplored, numerous renal genes with circadian
26 expression have been identified(31) that might be targets of known pharmacological
27 agents, which should be validated in future studies.

28 Interestingly, exosomal levels of Na-Cl cotransporter (NCC; also known as SLC12A3),
29 which is the target of thiazides, showed a circadian rhythm (that is, lower levels in the
30 morning and higher levels in the afternoon and evening)(125). This result might partly
31 explain why, similar to furosemide (74) discussed above, thiazides have increased efficacy
32 when taken later during the day.

1 In general, the sodium excretion rate seems to be the main driver of the circadian
2 rhythmicity of blood pressure. Thus, sodium restriction can shift blood pressure from a
3 non-dipping to a dipping pattern in patients with essential hypertension (126), which the
4 researchers in this study suggest occurs because hypertensive patients have decreased
5 sodium excretion during the day; therefore their blood pressure and natriuresis increase
6 during the night to eliminate sodium and control blood pressure. Furthermore, the
7 endothelin pathway might also have a role in the circadian rhythm of blood pressure(127,
8 128).

9

10 **Conclusions**

11 The multiple functions of the kidneys, which maintain the homeostasis of the whole
12 organism, are subject to well-described circadian rhythms. The demonstration that
13 molecular clocks exist has provided new insights into single-cell regulation. However,
14 even as the links between renal functions and the molecular core clock are beginning to
15 be uncovered with the demonstration of multiple levels of regulation, our overall
16 understanding at the whole-organism level remains largely unclear. For example, the
17 number of variables that affect blood pressure and show circadian rhythmicity, from
18 heart function to aldosterone-sensitive tubular salt reabsorption, has complicated efforts
19 to identify the crucial factors that control the circadian rhythm in blood pressure.

20 Future work integrating cell-specific as well as transcriptional, translational and post-
21 translational rhythms will require the establishment of mathematical models that can
22 incorporate all of this complexity(129). Studies using tissue-specific and cell-type-specific
23 inactivation of the molecular clock will further improve our understanding of renal
24 circadian rhythms.

25 Thirty four years after the initial crucial discovery of a gene involved in the molecular
26 clock(130, 131), integrating the physiological and clinical relevance of circadian rhythms
27 in the kidneys remains a challenge and should drive future research.

28

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3 **Author contributions**

4 Both authors contributed to all aspects of the conception, content, revisions and editing
5 of this manuscript.

6 **Competing interests statement**

7 The authors declare no competing interests.

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1 **Table 1. The clinical relevance of circadian rhythms**

Renal condition	Putative mechanisms	References
Fibrosis	<ul style="list-style-type: none"> - Increased TGFβ signaling - Increased <i>COX2</i> expression - Altered endothelin pathway - Altered activity of the sympathetic nervous system 	(63, 87-90)
Kidney stones	<ul style="list-style-type: none"> - Increased excretion rate of urine salts - Decreased urinary volume - Reduced urinary pH 	(91-98)
Glomerulonephritis	<ul style="list-style-type: none"> - Membranous glomerulonephritis and nephrotic syndrome: <ul style="list-style-type: none"> -Proteinuria rhythm -Control of plasma sodium levels and blood pressure - IgA glomerulonephritis: <ul style="list-style-type: none"> -Control of blood pressure 	(99-105)
Blood pressure	<ul style="list-style-type: none"> - Rhythm of the sodium excretion rate - Reduced activity of the sympathetic nervous system - Reduced endothelin pathway - Change in feeding time 	(111-115, 127, 128)
Haemodialysis or peritoneal dialysis	<ul style="list-style-type: none"> - Melatonin rhythm 	(108, 109, 132)

2 COX2, cyclooxygenase 2; TGF β , transforming growth factor- β .

3

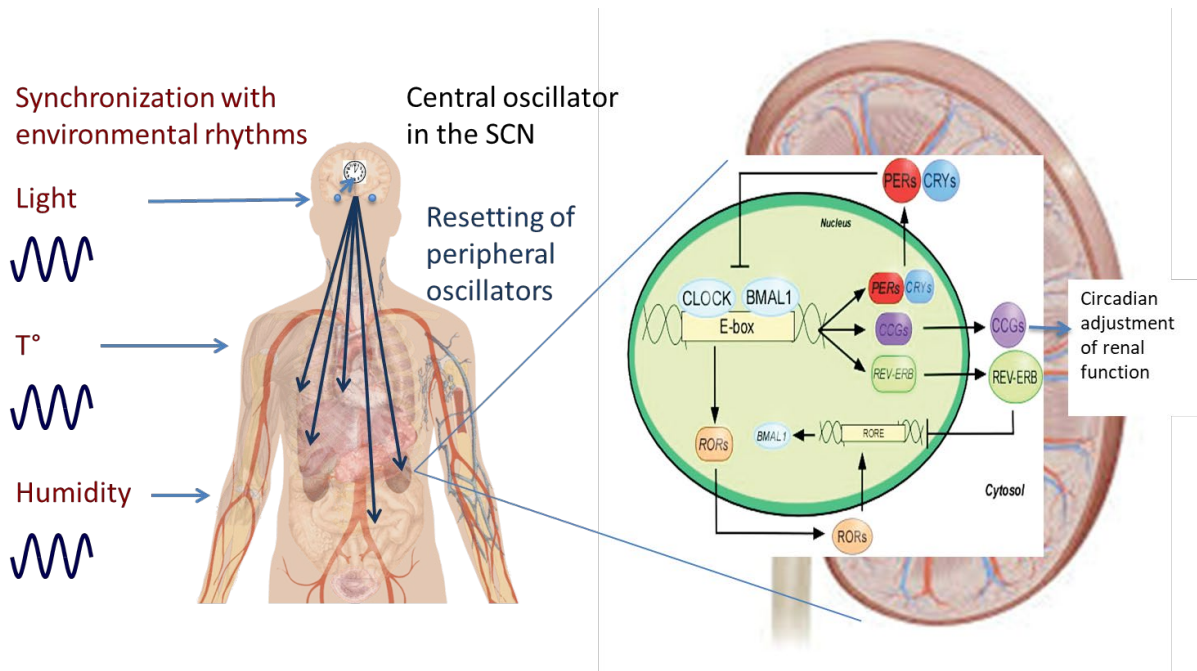
4

1 **Figure legends**

2

3 **Figure 1. Molecular clocks in humans.**

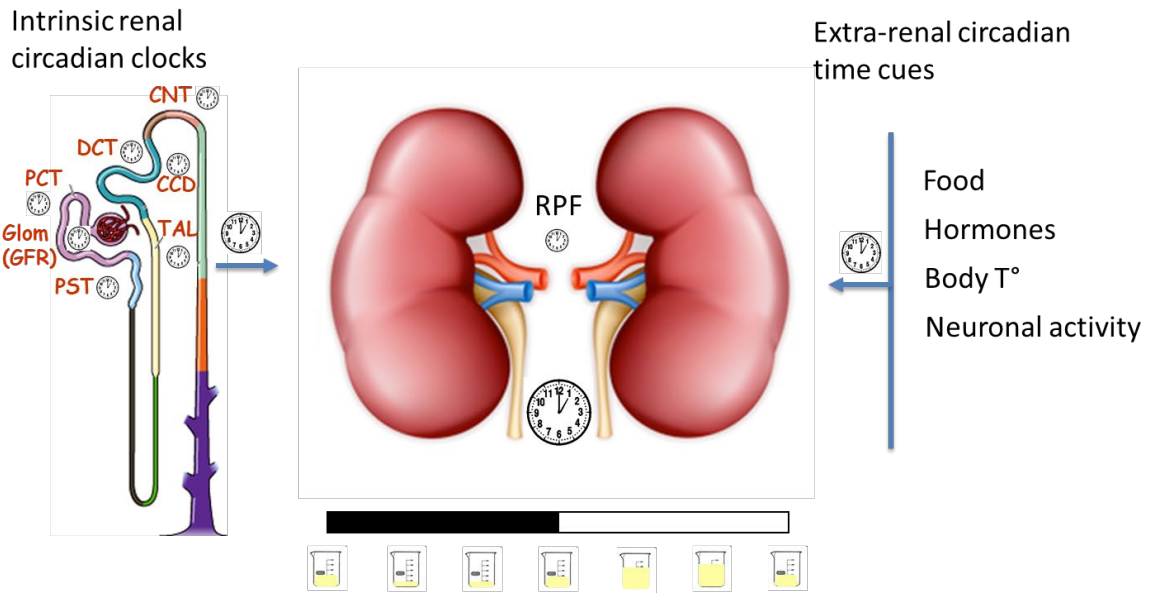
4 External cues (such as light, temperature, humidity and so on) synchronize the central
5 oscillator ('master clock') in the suprachiasmatic nucleus (SCN), which resets all
6 peripheral oscillators present in almost every cell in the body. The oscillator comprises
7 interconnected transcription feedback loops, with BMAL1–CLOCK heterodimers on the
8 activatory loop and PER–CRY heterodimers on the inhibitory loop. The expression of
9 clock-controlled genes (CCGs) is driven by the output of the molecular clock and enables
10 circadian adjustment of renal function. Retinoic acid-related orphan receptors (RORs)
11 and REV–ERB strengthen and stabilize the feedback loops that affect BMAL1 production.
12 ROREs, ROR response elements.



13

14 **Figure 2. Intrinsic circadian clocks in the kidneys.**

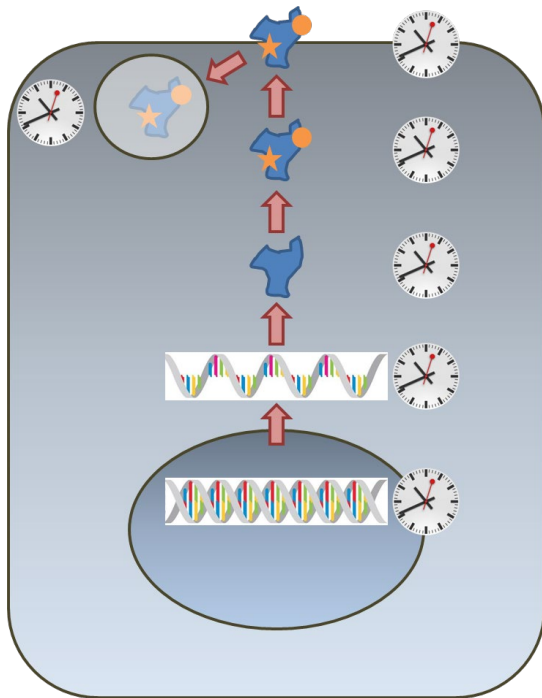
15 Renal plasma flow (RPF), glomerular filtration rate (GFR), podocytes and tubular cells
16 have intrinsic clocks, resulting in a circadian rhythm in urine output. Extra-renal circadian
17 time cues (nutrients, hormones, body temperature, activity of the nervous system and so
18 on) synchronize the rhythms of the intrinsic renal clocks. CCD, cortical collecting duct;
19 CNT, connecting tubule; DCT, distal convoluted tubule; Glom, glomerulus; PCT, proximal
20 convoluted tubule; PST, proximal straight tubule; TAL, thick ascending limb.



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Figure 3. Intracellular circadian rhythms.

Numerous intracellular events display circadian oscillations, including DNA replication, transcription, mRNA translation as well as protein post-translational modifications (acetylation, ubiquitylation, phosphorylation and so on), targeting to the cell surface and recycling and degradation. The circadian rhythms of these different processes may or may not be in phase.



10

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