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CKJ REVIEW

Sweet dreams: therapeutic insights, targeting imaging and physiologic evidence linking sleep, melatonin and diabetic nephropathy

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ABSTRACT

Melatonin is the main biochronologic molecular mediator of circadian rhythm and sleep. It is also a powerful antioxidant and has roles in other physiologic pathways. Melatonin deficiency is associated with metabolic derangements including glucose and cholesterol dysregulation, hypertension, disordered sleep and even cancer, likely due to altered immunity. Diabetic nephropathy (DN) is a key microvascular complication of both type 1 and 2 diabetes. DN is the end result of a complex combination of metabolic, haemodynamic, oxidative and inflammatory factors. Interestingly, these same factors have been linked to melatonin deficiency. This report will collate in a clinician-oriented fashion the mechanistic link between melatonin deficiency and factors contributing to DN.

Keywords: diabetes, diabetic nephropathy, inflammation, kidney disease, melatonin, oxidative stress

INTRODUCTION

Melatonin is an indolamine that is present in almost every organism from bacteria to humans [1]. In mammals, the site of hormonal melatonin production is the pineal gland, but melatonin is also produced in peripheral tissues for local autocrine and paracrine actions. Pineal melatonin production is restricted to the night and its production duration follows the duration of

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the night. Melatonin mainly regulates biological rhythms and has a role in coordinating behavioural and physiological adaptations to the night/day cycle and seasons [2].

Diabetic nephropathy (DN) is a microvascular complication of diabetes and is the leading cause of renal failure. Blood pressure regulation, glycaemic control and management of hyperlipidaemia are still the mainstays of therapy. These have not resulted in a cure [3, 4]. Melatonin-based therapy may be another pathway for therapeutic synergy.

DN is driven by the metabolic derangement causing haemodynamic changes, oxidative stress and inflammation. In early stages, DN is characterized by glomerular hyperfiltration and podocyte loss [5, 6]. While melatonin deficiency causes metabolic derangements, haemodynamic changes, oxidative stress and inflammation, the potential nephroprotective effects of melatonin are understudied. In this review we summarized the current literature about the effect of melatonin on the development of DN and the underlying pathophysiology.

Melatonin synthesis is tied to light and the day/night cycle

Melatonin (*N*-acetyl-5-methoxy tryptamine) is a tryptophan-derived small molecule showing pleiotropic actions, including antioxidant properties [7]. In mammals, melatonin is centrally produced by the pineal gland, acting as a hormone and, in addition, melatonin is produced in several central and peripheral tissues (e.g. retina, astrocytes, gastrointestinal tract, bone marrow, lymphocytes and skin) where it acts as a paracrine/autocrine factor [8, 9].

Melatonin secretion is tightly regulated (Figure 1). Pineal gland melatonin strictly with nocturnal production depends on two factors: first, circadian timing by the suprachiasmatic hypothalamic nucleus, and second, the nocturnal production is restricted to the night due the so-called photoinhibition of its production by light acting through the retinal melanopsinergic system originating in the intrinsic photosensitive ganglion cells [10]. However, in spite of being produced only during the night and in the dark, melatonin effects might be expressed not only during the night (immediate effects) but also during the day when melatonin is no longer circulating (prospective effects) [2]. Superior cervical ganglia provide sympathetic innervation to the pineal gland, releasing norepinephrine that stimulates the rate-limiting steps that convert tryptophan to melatonin in the pineal gland [2, 11]. Melatonin is not stored but is immediately released into the bloodstream and cerebrospinal fluid, bathing the brain and organs simultaneously. It has a short (40-min) half-life and is metabolized in the liver and kidneys and excreted renally as 6-sulfatoxymelatonin [12].

Melatonin activates two kinds of G-protein-linked membrane receptors, MT1 (high affinity) and MT2 (low affinity), which are encoded by the *MTNR1A* and *MTNR1B* genes, respectively. These receptors are expressed in multiple tissues such as heart and arteries, adrenal gland, kidney, lung, liver, gallbladder, small intestine, adipocytes, ovaries, uterus, breast, prostate, skin and central nervous system. They are also expressed by T and B lymphocytes [13]. However, receptor-expressing cells and tissues are not the only targets of melatonin physiologic actions since melatonin expresses non-receptor-dependent mechanisms of action such as, e.g., the direct nitrogen and oxygen radical species chelating antioxidant effects. As an antioxidant, melatonin protects DNA from oxidative damage [14–17], especially from mitochondrion-derived free radicals [18]. Melatonin also regulates ubiquitin-linked proteasomes to inhibit Ca^{2+}/calmodulin-dependent protein kinase II activity and decreases protein catabolism [19]. It additionally activates extracellular signal-regulated kinase and G-protein q subunit signalling [19].

Central and peripheral effects of melatonin

Melatonin regulates the circadian sleep–wake and body temperature cycles [20, 21]. This chronobiologic effect involves the hypothalamic suprachiasmatic nucleus as imaged by magnetic-resonance imaging [22, 23].

The metabolic role of melatonin has been studied in rats, where pinealectomy leads to increased body weight owing to increased food intake and reduced energy expenditure [24]. Replacing melatonin in these rats reduced body weight and food intake and increased brown fat activation [25, 26]. Interestingly, post-menopausal women taking daily melatonin supplementation in a randomized placebo-controlled trial reduced fat mass and increased lean mass [27]. In addition to these indirect antidiabetic effects, melatonin directly increases pancreatic beta cell survival and function [28–30] by increasing insulin secretion through glucagon-like peptide-1 sensitization [31]. In a population-based study, lower melatonin levels were independently associated with the risk of developing type 2 diabetes, possibly because melatonin regulates glucose tolerance [2, 32–34], and of insulin release in a complex feedback loop [35, 36].

Melatonin also regulates haemodynamic equilibrium. Pinealectomized rats became hypertensive, and this was resolved with melatonin supplementation [37]. Separately, 24-h light exposure (and the resultant melatonin suppression) causes hypertension via sympathetic drive and renin–angiotensin system activation (vasoconstriction and volume retention) [37, 38]. These mechanisms are activated by cardiovascular system melatonin receptors [39]. Also, melatonin acts on mitochondria regulation to maintain a healthy cardiovascular system [40]. In addition, direct brain actions of melatonin also reduce sympathetic tone and downregulate adrenal gland activity via the hypothalamus [41, 42]. Melatonin also modulates the baroreflex set point [43] and regulates heart rate via the medulla [44] and vasoconstriction and vasodilation via direct activation of vessel melatonin receptors [39, 45, 46]. In this regard, melatonin deficiency leads to blood pressure non-dipping or reverse dipping at night [47, 48]. In summary, because melatonin has cardiovascular and metabolic effects, derangements can result in diabetes and obesity (Figure 2).

DN occurs inconsistently and shortens lifespan

Technically, DN is defined as decreased glomerular filtration rate (GFR) and/or elevated urinary albumin excretion (30–300 mg/day microalbuminuria, >300 mg/day macroalbuminuria). Not all diabetics develop DN, but the reasons are unclear. Type 2 diabetics are more likely than type 1 diabetics to develop DN, although there are confounders such as older age and more frequent cardiovascular disease and atherosclerosis [49]. In any case, DN increases the risk of death in both type 1 and type 2 diabetics [50, 51] and ultimately progresses to end-stage kidney disease requiring renal replacement therapy by dialysis or transplantation [52]. However, albuminuria is inconsistently associated with a DN progression and some patients progress without albuminuria [53]. DN biopsies show a variety of pathologic findings involving almost every portion of the nephron, notably basement membrane thickening, podocyte loss and interstitial fibrosis [49]. A key pathogenic pathway is hyperglycaemia increasing

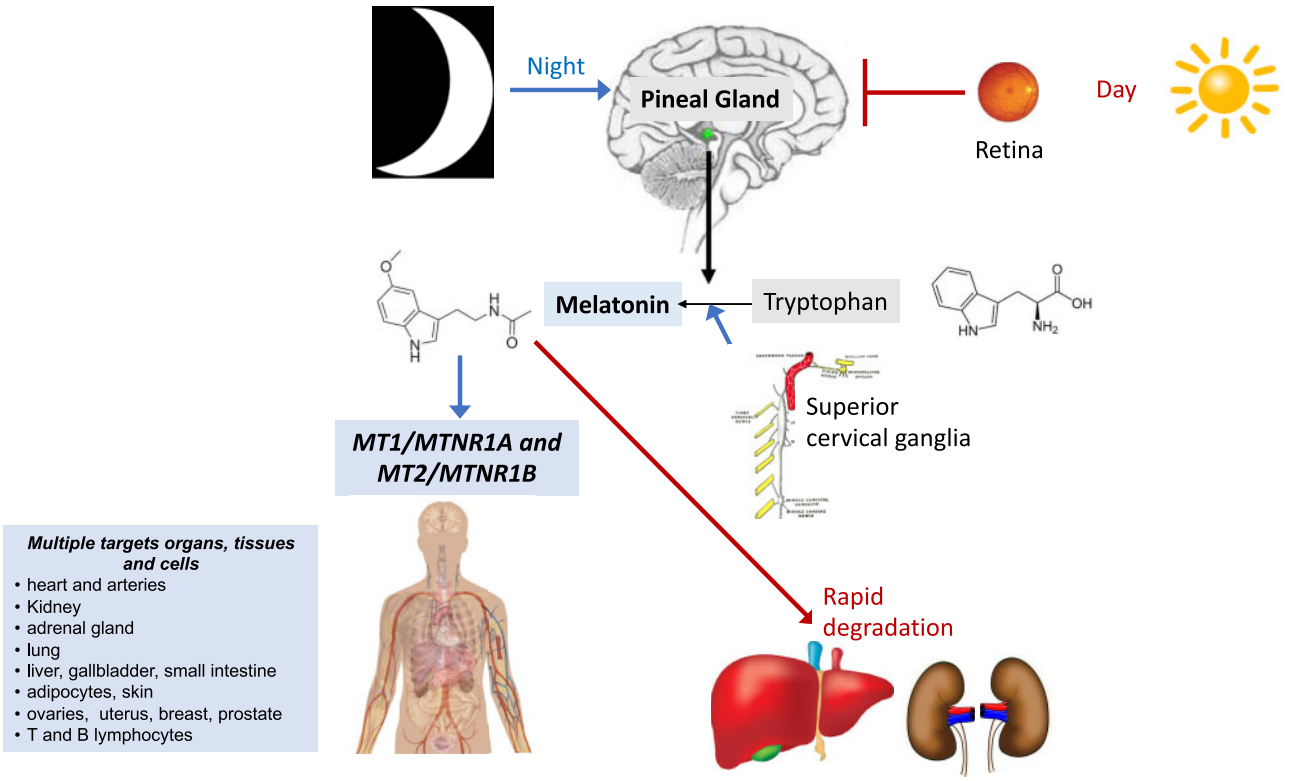

FIGURE 1: Melatonin secretion is regulated by diurnal rhythms of the body. Its secretion is increased during sleep and decreased during the daytime. Its systemic effects are regulated by activating MT1 (high affinity)/MT2 (low affinity) receptors. It is degraded in the liver and kidney.

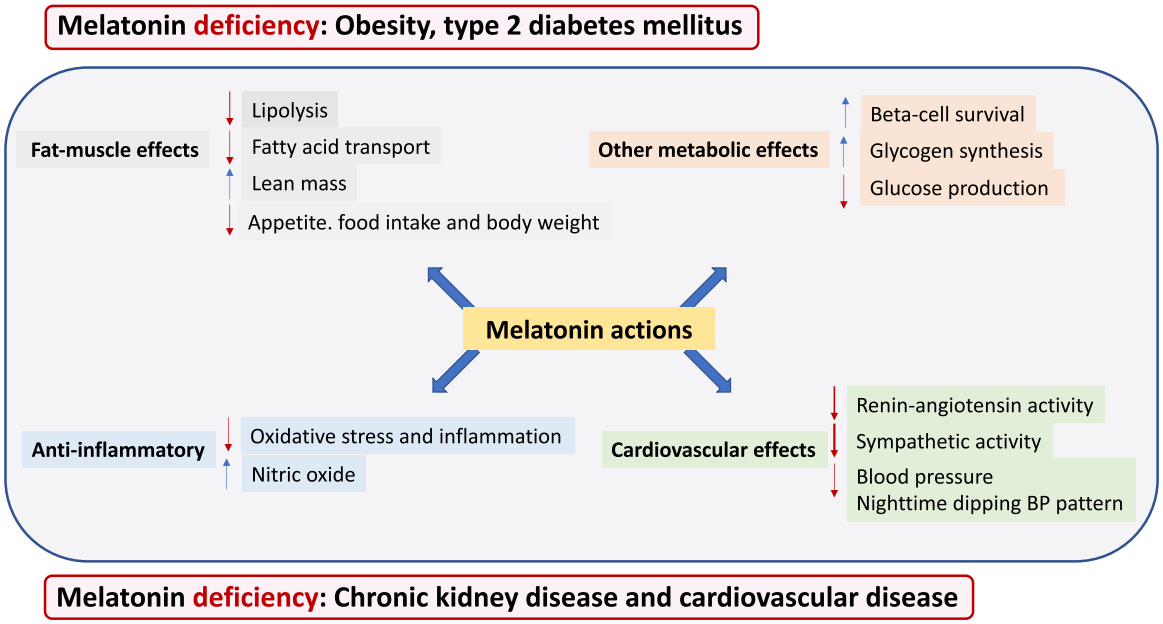

FIGURE 2: Melatonin deficiency may increase the risk for development of diabetes, obesity, cardiovascular and kidney disease. Melatonin has multiple health benefits on multiple organs.

mitochondrial substrate oxidation [49] to activate the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, thus uncoupling nitric oxide synthase [54], resulting in the generation of reactive oxygen species (ROS). Excess ROS causes cell dysfunction, apoptosis and inflammation, and decreasing ROS exposure is beneficial [49, 55].

Other factors contributing to chronic kidney disease (CKD) progression include hypertension and impaired autoregulation, leading to hypoperfusion and inappropriate renin–angiotensin system activation [56] in both type 1 and 2 diabetes [57, 58]. Loss of renal autoregulation allows systemic hypertension to directly hit the glomerulus [59, 60]. Glucose-mediated endothelial

dysfunction promotes microvascular rarefaction and renal hypoxia [49, 61]. Not surprisingly, lowering blood pressure is protective in hypertensive DN [62–64]. Insulin resistance is linked to CKD [65, 66]. Thus insulin receptor deletion in podocytes leads to glomerular damage similar to that observed in DN [67]. Compensatory insulin hypersecretion promotes kidney fibrogenesis through actions in insulin-responsive cells, further contributing to progressive renal disease [68]. Obesity independently promotes inflammation and growth factor activity, thus promoting CKD progression [49]. In this regard, inappropriate recruitment of activated T cells and macrophages favours glomerular and tubulointerstitium inflammation and DN progression [69, 70]. Therapeutic approaches targeting inflammatory mediators decrease albuminuria and GFR loss in animals and humans with DN [71, 72].

Melatonin measurement in diabetes

Melatonin levels are known to vary in a diurnal pattern, with secretion in humans occurring mostly at night. Interestingly, the complications of diabetes impair this secretion. Retinal perception of light may disturb melatonin dynamics in patients with diabetic retinopathy. Autonomic neuropathy may impair innervation of pinealocytes, which leads to altered melatonin haemodynamics in diabetes. These diabetic consequences are less discussed than the common cardiovascular and lower extremity peripheral vascular consequences [73].

Hikichi *et al.* [74] compared both the night- and daytime melatonin secretion in non-diabetic and diabetic subjects. They found that diabetics had lower melatonin at night but daytime levels were not affected by diabetes. In another study, Tutuncu *et al.* [73] designed a study to determine melatonin dynamics in type 2 diabetic patients and its relationship with the autonomic nervous system. They measured melatonin levels between 2 and 4 a.m. and 4 and 6 p.m. and compared these in 36 diabetics versus 13 non-diabetics. Again, like with Hikichi *et al.*, diabetics had lower nighttime melatonin levels and less of a melatonin surge into nighttime, both statistically significant findings. Patients carrying a diagnosis of autonomic neuropathy showed lower night- and daytime melatonin levels compared with non-diabetics (both statistically significant). Retinopathy did not affect the findings but the authors suggested that the participants' degree of retinopathy was not severe enough to generate a signal [73]. Prior to these studies, O'Brien *et al.* [75] had already shown that a physiological increase in nocturnal plasma melatonin concentration is not observed in diabetic patients with neuropathy compared with age-matched non-diabetic controls. The compilation of studies supports the hypothesis that melatonin dysregulation is a novel diabetic complication. Future studies may focus on melatonin dynamics graded by the severity of diabetic neuropathy.

Melatonin and DN

Sleep patterns are linked to diabesity via insulin resistance and metabolic syndrome [76, 77] and the disturbed sleep–diabetes link [78] is likely driven by melatonin deficiency [79]. In fact, type 2 diabetics with decreased sleep had higher 24-h urinary albumin and protein excretion as markers of more severe DN [80]. Moreover, diabetes-derived hyperglycaemia induces a reduction in melatonin production, aggravating sleep and metabolic medical conditions [81].

Peschke *et al.* [82] showed that serial nocturnal plasma melatonin levels were significantly lower in six diabetic patients compared with five non-diabetic controls. Although this study involves only a small number of patients, the performance of serial measurements improves the validity of the study [82]. Melatonin levels also vary with microvascular diabetic complications. Nocturnal plasma melatonin levels were studied in 56 patients by Hikichi *et al.* [74]. Interestingly, they found that the patients with diabetic proliferative retinopathy had lower melatonin levels than healthy patients. However, non-retinopathy diabetics did not demonstrate this finding. Kor *et al.* [83] compared the melatonin levels in 40 type 1 diabetic children and 30 non-diabetic controls. The mean melatonin level in the diabetic group was 6.75 ± 3.52 pg/mL and the mean melatonin level in the control group was 11.51 ± 4.74 pg/mL ($P < 0.01$). In their relatively small cross-sectional study, Robeva *et al.* [84] showed that nocturnal insulin and plasma melatonin levels correlated positively in metabolic syndrome patients but not healthy control patients. Melatonin deficiency may predispose to DN via vasoactive, metabolic, inflammatory, apoptotic and fibrogenic pathways (Figure 3).

Activation of Rho-associated kinases promotes endothelial–mesenchymal transformation [85–87] and DN progression, which is prevented by inhibiting this pathway [88]. In cultured cells, microRNA 497 attenuated Rho-associated kinase signalling [89]. Mesenchymal stem cell therapy improved renal function in rat DN and melatonin improved renal recovery by increasing antioxidant defences and decreasing immune activation [90].

Melatonin also modulates renin–angiotensin system activation, in general, and particularly in DN [91–93]. Thus the renin–angiotensin system was upregulated in CKD patients with impaired melatonin secretion at night [94]. In subtotally nephrectomized rats, treatment with melatonin for 4 weeks improved remnant kidney function and decreased intrarenal renin–angiotensin activation and interstitial fibrosis [95]. In cultured cells, melatonin reduced the expression of apoptotic proteins in response to a diabetic milieu, resulting in increased podocyte numbers. Melatonin prevented angiotensin-2-driven pro-apoptotic protein transcription and protected mitochondrial membranes in a dose-dependent manner [96]. In rats with streptozotocin-induced DN, the combination of melatonin and taurine decreased glomerular inflammation and proteinuria, independent of serum glucose levels [97]. In the same model, melatonin also increased nitric oxide availability and nephroprotective protein levels, including those of antioxidant proteins such as superoxide dismutase [98], and also decreased kidney cell apoptosis [99], improving histological kidney damage [100]. Nephroprotection by melatonin is not limited to DN, but extends to potential clinical complications of diabetic patients. Thus melatonin reduced the inflammation marker interleukin-33 (IL-33) in streptozotocin-induced DN rats with contrast-induced nephropathy [101] and protected against adriamycin-induced podocytopathy [102]. It additionally inhibited and normalized NADPH oxidase activity, a key driver of oxidative stress that is upregulated in obese Zucker diabetic rats [103–105].

Macrophages are the predominant kidney infiltrating cells in DN [106, 107] and macrophage infiltration in biopsy specimens predicts GFR loss in DN [108]. Therapeutic manoeuvres that decrease macrophage infiltration also decrease albuminuria and slow DN regression [109, 110]. The nuclear factor κB (NF-κB) transcription factor is a master regulator of inflammation, contributing to DN progression by promoting macrophage recruitment and activation [111]. Macrophages secrete transforming growth factor β1, a pro-fibrotic factor that plays a key role in DN-

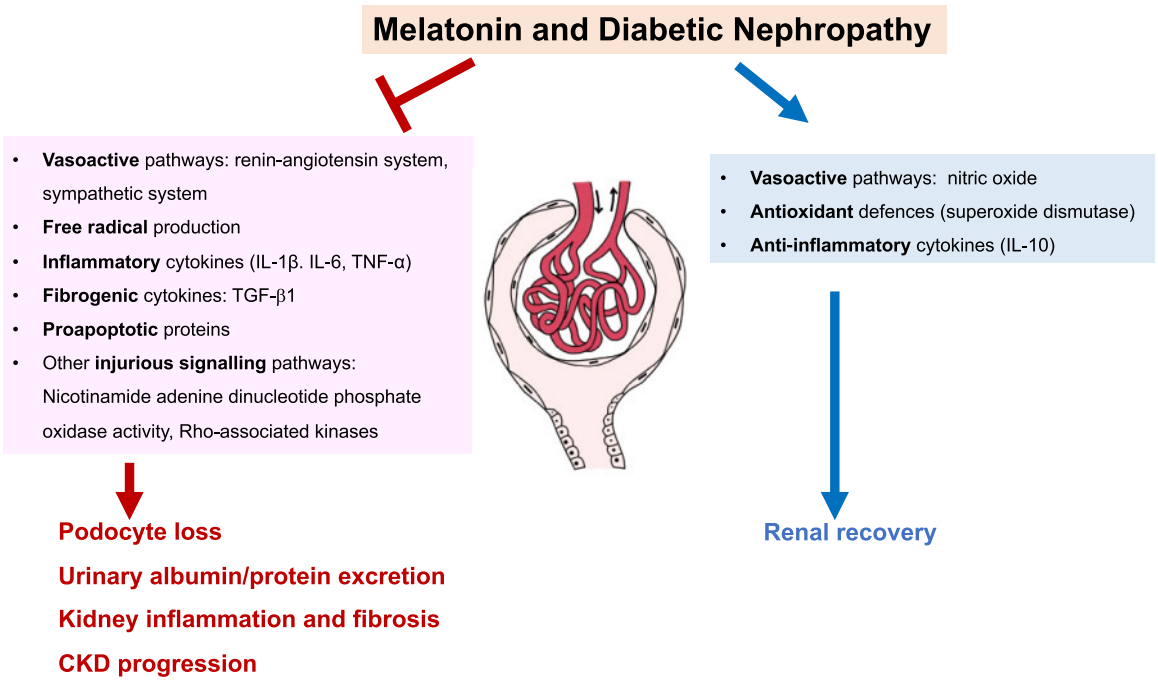

FIGURE 3: Postulated mechanisms of melatonin in the prevention and treatment of diabetic nephropathy. Melatonin may decrease podocyte loss, urinary albumin/protein excretion, kidney inflammation and fibrosis and may increase renal recovery.

associated kidney fibrosis [112]. Melatonin modulates macrophage recruitment and activation via multiple pathways, including NF-κB activation [1]. Thus melatonin decreases M1 pro-inflammatory macrophage and increases M2 anti-inflammatory and reparative macrophages [113], blunting inflammatory cytokine secretion (IL-1β, IL-6 and tumour necrosis factor α) and decreasing free radical production, while increasing the release of anti-inflammatory cytokine such as IL-10 from M2 macrophages [1].

Finally, it is important to correlate melatonin deficiency with obesity and hypertension since these are commonly discussed predisposing factors for DN. Obesity and hypertension frequently coexist [114] and are associated with oxidative stress and inflammation, especially at the vascular level. Specifically, kidney oxidative stress and inflammation contribute to hypertension [115].

As suggested above, melatonin has both anti-inflammatory and antioxidant effects due to cyclooxygenase synthase inhibition and multilevel inflammasome inhibition for cytokines, chemokines and adhesion molecules [116]. Melatonin decreases blood pressure via reduced NF-κB activation and reduced renal inflammation in spontaneously hypertensive rats [117]. Qiao *et al.* [118] demonstrated that melatonin reduced hypertension and inflammatory cellular infiltration of the renal tubules.

Melatonin has many antioxidant effects. Those highlighted in the literature include reduction of oxidative stress, renal inflammation, proteinuria and progression of renal damage in rats with low renal mass [119]. Melatonin exerts renoprotective and antihypertensive effects by increasing nitric oxide bioavailability [120]. Melatonin deficiency is also related with obesity. Melatonin reduces body fat content, especially visceral fat, and improves metabolic condition via reduced free fatty acids, reduced hyperglycaemia and reduced insulin levels alongside improved high-density lipoprotein and adiponectin levels [25, 121–123].

It was shown that the amplitudes of the nocturnal pineal [124] and serum melatonin peaks decreased significantly in obese animals. Daily melatonin supplementation significantly reduced body weight as well as plasma glucose, leptin, triglyceride and total cholesterol levels of the rat models of high-fat diet-induced obesity [125, 126]. The summary of evidence supports the hypothesis that melatonin deficiency plays a role in the development of kidney disease vis-à-vis obesity and hypertension.

CONCLUSION

Melatonin links sleep to metabolic and haemodynamic equilibrium. Melatonin activates the cardiovascular system and kidney receptors to protect from DN in preclinical models. Furthermore, melatonin levels are associated with human DN outcomes. Only human randomized controlled trials will confirm whether melatonin improves renal outcomes in diabetics and increases survival.

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CONFLICT OF INTEREST STATEMENT

None declared.

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