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The role of melatonin and circadian rhythms in the pathogenesis of diabetic retinopathy: A systematic review

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ABSTRACT

Aims: This review investigates literature on systemic melatonin levels and circadian timing in diabetic retinopathy (DR), examining their associations with DR.

Methods: Our search was conducted in March 14, 2024, and included the databases Medline, Web of Science, Scopus, ProQuest Health, Latin American and Caribbean Health Sciences Literature (LILACS), Cochrane, International Standard Randomised Controlled Trial Number (ISRCTN) registry, and International Clinical Trials Registry Platform (ICTRP).

Results: Our review analysed twelve articles measuring melatonin concentration in saliva, blood serum, urine, or aqueous humour. Studies measuring melatonin levels in saliva found no significant differences in the average nocturnal or daytime melatonin levels between type 2 diabetes (T2D) patients with and without DR. The studies comparing serum melatonin levels in patients with different stages of DR and controls showed inconsistent results. Only two studies measured the endogenous onset of melatonin secretion, known as dim light melatonin onset (DLMO), a highly accurate biomarker for circadian regulation. These studies showed that only 33% and 57% of patients with DR had detectable DLMO in saliva and serum, respectively. All studies evaluating overnight melatonin production using urinary aMT6s (urinary 6-sulfaoxymelatonin) levels found that DR was associated with lower nocturnal melatonin production.

Conclusions: Our review results showed evidence of reduced nocturnal melatoin production in DR with no significant changes in melatonin circadian timing.

1. Introduction

Diabetes is a major cause of death and disability globally, impacting individuals across all countries, age groups, and gender [1]. There has been a rapid increase in the prevalence of type 2 diabetes (T2D) in the past few decades especially among children, adolescents and younger adults [2]. Diabetes places considerable pressure on healthcare systems [3]. According to the International Diabetes Federation (IDF), 537 million people worldwide had diabetes in 2021, leading to health expenditures of US $966 billion, with projections exceeding $1054 billion by 2045 [4]. Diabetes is the leading cause of blindness, heart attacks, stroke, kidney failure and peripheral arterial disease. Diabetic retinopathy (DR), a common complication of diabetes, affects up to 35 % of adults with T2D [5]. Globally, it is the fifth most common cause of moderate to severe visual impairment in those aged 50 years and above [6], and one of the leading cause of preventable blindess in the working age population [7,8]. Risk factors associated with an increased risk of DR development include poor glycaemic control, hypertension, dyslipidaemia and diabetes duration [9,10].

Circadian rhythms are ubiquitous intrinsic rhythms that follow a near 24-h cycle and underlie several biological and behavioural functions, including regulation of the sleep-wake cycle, metabolism, vascular and cardiometabolic function, and immune modulation [11–17]. Circadian rhythm timing is regulated by the central endogenous clock in the hypothalamus, termed the Suprachiasmatic nuclei (SCN). These central circadian rhythms synchronise peripheral clocks and circadian rhythms in various tissues and organs, regulating physiological processes throughout the body. A specialised set of light sensitive retinal cells, known as intrinsically photosensitive retinal ganglion cells (ipRGCs), relay environmental light information to the SCN, which in

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turn regulates the endogenous release of the neurohormone melatonin [18,19]. Therefore, the timing of systemic melatonin circadian rhythms is commonly used as a marker for the timing of the endogenous circadian clock [20]. Circadian rhythms are synchronised to an external environmental stimulus ("zeitgeber," German word for "time giver") that influences circadian rhythm timing and maintains the optimal, stable relationship between biological rhythms and environmental/behavioural interactions. For most organisms, the strongest zeitgeber is ambient light (or daily light: dark cycle) [21].

Large-scale cross-sectional studies and meta-analyses have demonstrated a U-shaped relationship between sleep duration and the risk of T2D, suggesting that 7–8 h of sleep is optimal for minimising this risk [22,23]. Importantly, T2D is strongly associated with disruptions in circadian rhythms, which impair insulin sensitivity and glucose regulation [24–27]. These circadian effects on glucose regulation are primarily driven by peripheral clocks [26]. The SCN preserves peripheral clock synchrony through neuroendocrine mechanisms that directly influence metabolic processes. It communicates with the autonomic nervous system and regulates the secretion of cortisol, growth hormone, and melatonin, all of which play distinct roles in modulating insulin sensitivity [28]. Irregular sleep patterns, shift work, and exposure to artificial light at night can desynchronise peripheral and central circadian rhythms, leading to insulin resistance and hyperglycaemia [25]. Consistent with this, large-scale genome-wide association study (GWAS) studies have shown polymorphisms in many clock genes such as *CLOCK*, *BMAL1* and *CRY* increase the risk of T2D [29].

Circadian rhythm disturbances and related vascular dysfunction among individuals with T2D may contribute to DR by disrupting the retinal microvasculature [15,25,26,30,31]. DR has been associated with a reduction in the density of ipRCGs, which could affect both peripheral retinal rhythms and processes, as well as central circadian rhythms regulated by the SCN [32]. It has been hypothesised that circadian rhythm dysfunction at structural, physiological, metabolic, and cellular levels, plays a critical role in the development and progression of DR [33]. However, recent studies investigating melatonin concentration and circadian timing in individuals with DR, T2D without DR, and healthy controls have found equivocal results [34–45]. Notably, only few studies have measured the endogenous starting point of melatonin secretion, also known as the dim light melatonin onset (DLMO), which is regarded as the most important biomarker for circadian timing and circadian phase in human sleep [46]. Furthermore, the potential mechanisms underlying circadian dysregulation in DR remain unclear. Understanding these mechanisms is crucial to uncover how poor sleep and circadian dysfunction may contribute to the pathogenesis of DR. This systematic review is the first to critically evaluate the existing literature on systemic melatonin levels and circadian timing in humans with DR, aiming to explore the associations between melatonin levels, circadian rhythms, and DR and its severity.

2. Methods

This systematic review adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and was registered with the International Prospective Register of Systematic Reviews (PROSPERO; CRD42023473338). The review included studies that measured melatonin concentrations in bodily fluids such as blood, saliva, urine, or aqueous humour among participants with T2D, both with and without DR. Studies focusing exclusively on type 1 diabetes (T1D), using animal models, case reports, case series, review articles, or conference abstracts were excluded. The search was conducted in March 2024, without any restrictions on prior dates. The following databases were used for the systematic review: Medline, Web of Science, Scopus, ProQuest Health, Latin American and Caribbean Health Sciences Literature (LILACS), Cochrane, International Standard Randomised Controlled Trial Number (ISRCTN) registry, and International Clinical Trials Registry Platform (ICTRP). We used the following search terms for our study: *'diabetic retinopathy' OR 'diabetic eye disease' OR 'diabetic macular edema' OR 'diabetic macular edema' OR'diabetic papillopathy' OR 'diabetic maculopathy' OR 'diabetic retinal angiopathy' OR 'diabetic microvascular complications' AND 'sleep'OR'melatonin' OR 'circadian rhythm' OR 'period circadian proteins'OR 'sleep disorders, Circadian rhythm' OR 'DLMO' OR '"Dim Light"' OR 'Urinary 6-Sulfatoxymelatonin'* (Supplementary material 1). The titles and abstracts of the articles were reviewed by authors ED and RS. Any disagreements regarding study inclusion were resolved by two additional reviewers, RC and MPS. After reviewing the full texts, the reviewers extracted information on the study title, design, authors, publication year, study populations (including age and severity of DR), reported melatonin concentrations and timing, and any relevant secondary outcomes. In addition, data on the statistical analysis and study limitations were also extracted. All studies reported melatonin concentrations in pg/mL, except those examining urinary melatonin, which were expressed in ng/mg due to the significantly higher concentration of melatonin [47]. The quality of the studies included in the review was evaluated using the National Institute of Health's Study Quality Assessment Tools for case-control studies and observational cohort and cross-sectional studies.

3. Results

An initial search identified 1411 records from electronic databases, of which 869 were duplicates. The abstracts of 542 records were screened by the two reviewers independently, excluding 508 records that did not meet the selection criteria (Fig. 1). The remaining 36 records underwent a full review. Twelve studies met the final inclusion criteria for this review (Table 1). All studies measured melatonin concentration and/or melatonin circadian timing in

Human participants with and without DR. Sample size varied from 23 to 300 participants between 40 and 80 years of age. Based on the source of the biological samples analysed, the studies were categorised into melatonin measured in blood serum, saliva and urine. Two studies examined melatonin concentration in saliva samples [41,42], four analysed urine samples to assess 6-sulfatoxymelatonin (aMT6s, a major metabolite of melatonin) [36,37,39,43], and four analysed blood serum for measuring melatonin concentration [38,44,45] and DLMO timing [35]. One study examined melatonin in both saliva (DLMO) and urine (aMT6s) samples [34], and another examined melatonin levels in the eye's aqueous humour as well as blood serum [40]. In terms of analytical platforms, nine studies employed enzyme linked immunosorbent assay (ELISA), one employed high-performance liquid chromatography (HPLC), and two used radioimmunoassay (RIA). The risk of bias assessments of the articles included in the review is summarised in the Supplementary material 2.

3.1. Studies that assessed salivary melatonin levels and diabetic retinopathy

Two studies examined and compared the salivary melatonin levels among T2D patients (with and without DR) and healthy controls [41, 42]. In one study, saliva samples were taken once in the morning between 06:00 and 06:30 a.m. [42], while in the other study, samples were collected every 4 h throughout a 24-h period [41]. Melatonin levels in both studies were measured using ELISA. In their study, Ba-Ali et al. reported that the peak melatonin concentration occurred at the same time (04:00 a.m.) for participants with T2D regardless of their DR status as well as for healthy controls. However, the peak melatonin concentration was significantly reduced in the T2D patients, both without (4.8 $\pm$ 5.3 pg/mL) and with (2.9 $\pm$ 2.6 pg/mL) DR, when compared to healthy controls (11.4 $\pm$ 10.7 pg/mL). Overall, both studies found no significant differences in the average nocturnal (Ba-Ali et al. without DR: 3.2 $\pm$ 3.8 pg/mL, with DR: 1.7 $\pm$ 1.4 pg/mL) or morning melatonin (Kalere et al., without DR: 3.3 pg/mL, with DR: 7.2 pg/mL) levels between T2D patients with and without DR.

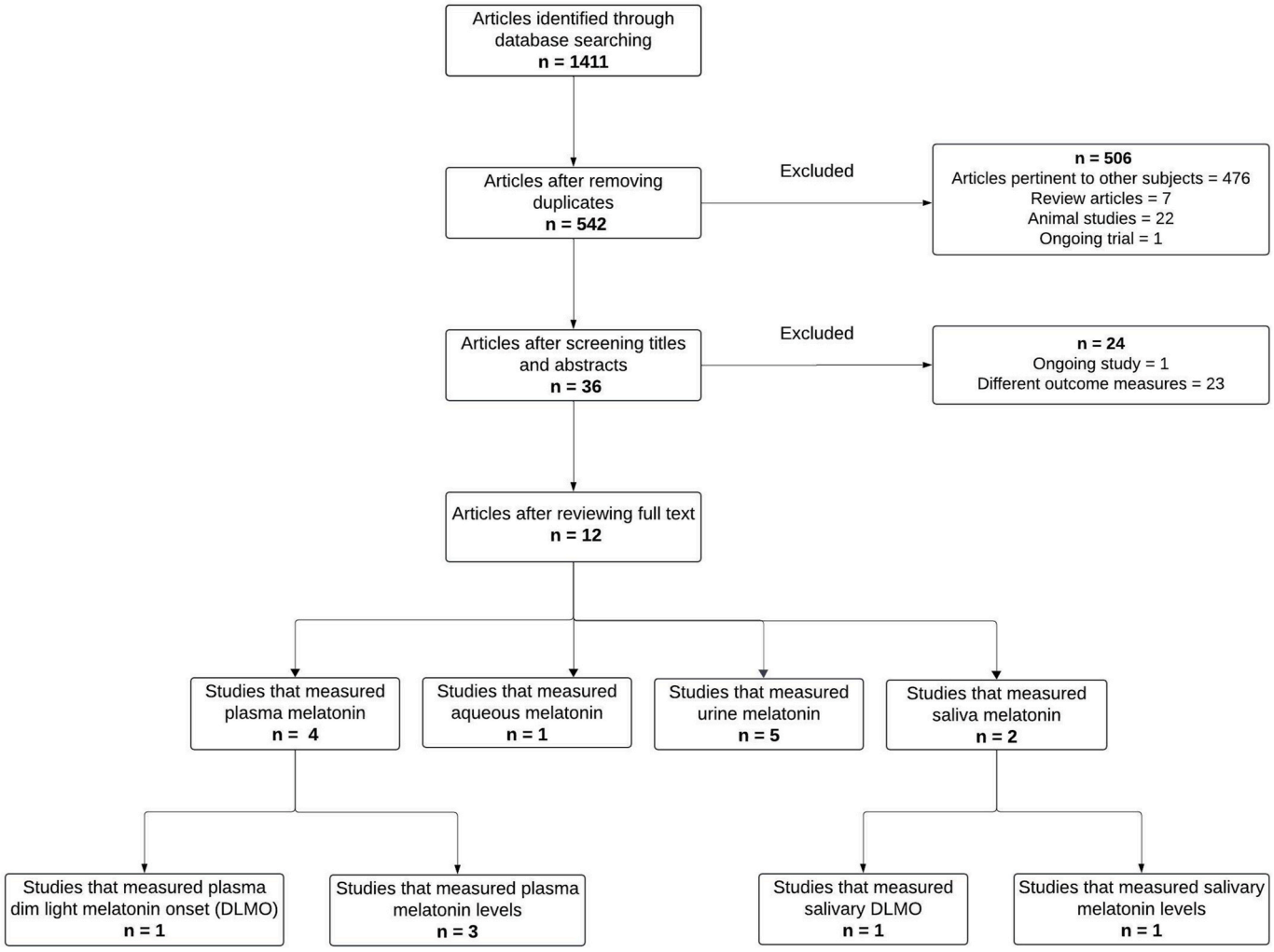

Fig. 1. Flow chart illustrating the process involved in the systematic review.

3.2. Studies that assessed salivary dim light melatonin onset and diabetic retinopathy

Only one study compared the salivary DLMO among T2D patients with and without DR and healthy controls [34]. The authors collected saliva samples every 30 min, starting 7 h before participants' self-reported bedtimes until 2 h after, and analysed them using RIA to determine DLMO timing [34]. They found that only 33 % of patients with DR had detectable DLMOs compared to 83 % in healthy controls and 86 % in T2D patients without DR. Because of the small number of DR patients with detectable DLMO, timing comparisons between groups were not feasible. No significant difference was observed in the detectability of DLMO between T2D patients without DR and healthy controls.

3.3. Studies that assessed serum melatonin levels and diabetic retinopathy

Four studies investigated melatonin concentration in blood serum in patients with diabetes and healthy controls [38,40,44,45]. In these studies, blood samples were collected at different time points throughout the day. Wan et al. [44] and Saeed et al. [45] measured melatonin concentrations from blood samples collected at a single time point in the morning between 8:00 a.m. and 12:00 p.m. Hikichi et al. [38] collected blood samples at two time points; midnight and 03:00 p.m., while Aydin et al. [40] did not specify the collection times. Three studies used ELISA [40,44,45] while one study used HPLC [38]. Both studies that collected morning blood samples found significantly lower serum melatonin levels in patients with DR compared to T2D patients without DR and age-matched healthy controls [44,45]. Notably, Wan et al. [44] reported that serum melatonin levels were lower in patients with both non-proliferative diabetic retinopathy (NPDR, 44.48 ± 10.30 pg/mL) and proliferative diabetic retinopathy (PDR, 44.69 ± 8.95 pg/mL) compared to those without DR (60.38 ± 13.43 pg/mL) and healthy controls (72.83 ± 16.25 pg/mL). Conversely, Aydin et al. [40] found no significant differences in serum melatonin concentrations between patients with PDR (5.37 ± 1.74 pg/mL), NPDR (6.11 ± 1.90 pg/mL), and healthy controls (6.15 ± 1.91 pg/mL). Hikichi et al. [38] in their study reported no significant difference in daytime (03:00 p.m.) melatonin levels among patients with NPDR and PDR. However, midnight melatonin levels were significantly lower in the PDR (10.9 ± 11.4 pg/mL) compared to the NPDR (31.1 ± 26.5 pg/mL) group and healthy controls (37.5 ± 30.8 pg/mL).

3.4. Studies that assessed serum dim light melatonin onset and diabetic retinopathy

In a recent study, Reutrakul et al. conducted blood sampling every 30 min for a 24 h period to measure the DLMO from blood serum in diabetes patients with and without DR and analysed using RIA. The DLMO was present in all T2D patients without DR, but only in 57.1 % of those with DR [35]. The melatonin output across 24 h was significantly lower in patients with DR (346.7 pmol/l per 24 h) than in those without DR (1033.8 pmol/l per 24 h). However, the timing of DLMO (average

Table 1
Summary of the studies used in the systematic review.

Authors	Year	Country	Study-design	Population	Bio-fluid measure	Technique	Results
Hikichi et al. [38]	2011	Japan	Case-control	T2D = 30, Controls = 26	Plasma	HPLC	Nighttime melatonin levels were significantly lower in the T2D group than in the control group ($p < 0.03$) and lower in the PDR group than in the control and NPDR groups ($p < 0.01$ and $p < 0.03$ respectively), but no significant difference was found between the control and NPDR groups.
Chen et al. [39]	2014	China	Case-control	Controls = 16, T2D with no DR = 10, NPDR = 19, PDR = 38	Urine	ELISA	The urinary aMT6s was significantly lower in PDR group compared to controls, NPDR and T2D groups. Urinary aMT6s could be associated in the pathogenesis of PDR
Reutrakul et al. [36]	2017	Thailand	Cross-sectional	T2D = 56	Urine	ELISA	DR was significantly associated with reduced nocturnal urinary aMT6s-to-creatinine ratio, and an increase in glycated haemaglobin percent (HbA1c) by 1.013 % of its original value (B = −0.013, 95 % CI: −0.038, −0.005).
Aydin et al. [40]	2016	Turkey	Case-control	DR = 26 Controls = 14	Aqueous & Serum	ELISA	Melatonin levels significantly lower in the aqueous in patients with PDR but not in NPDR compared to controls. Plasma levels of melatonin was reduced in patients with PDR but no significant differences between the groups.
Ba-Ali et al. [41]	2019	Denmark	Case-control	T2D with DR = 25, T2D without DR = 29, Controls = 21	Saliva	ELISA	Peak melatonin concentration at 04:00 a.m. and the mean nocturnal melatonin levels were significantly reduced in all T2D patients, regardless of retinopathy stage. Levels of light exposure during dark hours were not significantly different in patients with and without DR and controls.
Kalere et al. [42]	2019	Latvia	Case-control	T2D = 26 Controls = 12	Saliva	ELISA	T2D patients showed significantly lower levels of melatonin than the control group: 6.1 (0.78; 12.2) pg/ml vs 17.8 (8.2; 25.5) pg/ml, $p = 0.003$. No significant differences in the average morning melatonin levels between T2D- patients with (7.2 pg/mL) and without DR (3.3 pg/mL).
Reutrakul et al. [34]	2020	USA	Case-control	T2D without DR = 15, T2D with DR = 15, Controls = 15	Urine, DLMO	ELISA RIA	Patients with T2D and DR had lower urinary aMT6s than other groups ($p < 0.001$). Patients with DR were more likely to have no detectable DLMO ($p = 0.049$).
Sirisreetreerux et al. [37]	2021	Switzerland	Case-control	T2D without DR = 10, T2D with DR = 15 Controls = 10	Urine	ELISA	Patient with DR had significantly lower urinary aMT6S compared to controls. However, no significant difference between DR and no DR groups.
Tanaka et al. [43]	2021	Japan	Case-control	T2D = 167, Controls = 27	Urine	ELISA	The natural logarithmically scaled 6-SMT level (Ln 6-SMT) was significantly lower T2D patints (1.9–1.1) compared to patients without T2D (2.8–1.0, $P < 0.001$). Multivariate linear regression analysis identified duration of diabetes, smoking status, urinary albumin-to-creatinine ratio, retinopathy and coronary heart disease as factors that could influence Ln 6-SMT levels in patients with T2D.
Wan et al. [44]	2021	China	Case-control	T2D without DR = 57, NPDR = 64, PDR = 61, Controls = 118	Plasma	ELISA	Low melatonin levels were found in the no DR group compared with controls. Lower melatonin was found in the NPDR and PDR compared to controls.
Saeed et al. [45]	2022	Saudi Arabia	Case-control	T2D = 100, Controls = 100	Plasma	ELISA	Lower serum melatonin levels in patients with DR compared to T2D patients without DR and age-matched healthy controls
Reutrakul et al. [35]	2024	USA	Case-control	T2D with DR = 14, T2D without DR = 9	Plasma & DLMO	RIA	Participants with DR compared to participants without DR had lower 24 h serum melatonin output ($p = 0.042$) and greater day-to-day sleep variability ($p = 0.012$). Six individuals with diabetic retinopathy had no detectable dim-light melatonin onset. PIPR correlated with 24 h mean melatonin levels ($r = 0.555$, $p = 0.007$). ipRCG dysfunction in diabetic retinopathy is associated with disruptions of the 24 h melatonin rhythm, suggesting circadian dysregulation in diabetic retinopathy.

T2D, type 2 diabetes mellitus; DR, diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; HPLC, high-performance liquid chromatography; DLMO, dim-light melatonin onset; ELISA, enzyme linked immunosorbent assay; RIA, radioimmunoassay; aMT6s, 6-sulfatoxymelatonin; OSA, obstructive sleep apnoea; PSQI, Pittsburgh Sleep Quality Index; ESS, Epworth Sleepiness Scale; ipRCG, intrinsically photosensitive retinal ganglion cells; PIPR, post illumination pupil response.

time, 09:49 p.m.), peak time of melatonin rhythm, and peak melatonin level were all similar between the two groups.

3.5. Studies that assessed urinary melatonin levels and diabetic retinopathy

Five studies assessed the association between urinary melatonin levels and DR [34,36,37,39,43]. Collectively, the studies found that the presence of DR was significantly associated with lower nocturnal urinary aMT6 concentrations. Four studies collected overnight urine samples including the first next morning void and measured urinary aMT6 levels using ELISA [34,36,37,39]. Sirisreetreerux et al. [37] and Reutrakul et al. [34] reported that the median urinary aMT6s-to-creatinine ratio was significantly lower in patients with DR (7.1 ± 6.5 and 1.2 ± 2.6 ng/mg, respectively) compared to T2D patients without DR (16.1 ± 25.1 and 15.5 ± 26.1 ng/mg) and healthy controls (37.1 ± 27.7 and

11.3 ± 15.7 ng/mg). These findings were consistent with mediation analyses performed by Reutrakul et al. [36] that found that DR was significantly associated with reduced nocturnal urinary aMT6s-to-creatinine ratio, and an increase in glycated haemaglobin percent (HbA1c) by 1.013 % of its original value (B = −0.013, 95 % CI: −0.038, −0.005). The study by Tanaka et al. [43], which analysed the first morning urine sample from patients with and without T2D using ELISA, found that higher urinary melatonin levels were associated with a decreased odds of DR after adjusting for all relevant confounding factors (odds ratio: 0.56, 95 % confidence interval: 0.37–0.85, $p < 0.01$).

3.6. Studies that assessed aqueous humour melatonin levels and diabetic retinopathy

A single study by Aydin et al. examined melatonin concentration in aqueous humour, which was collected at the time of cataract surgery and analysed using ELISA [40]. The study team found that melatonin levels were significantly elevated in the aqueous humour of patients with PDR (18.57 ± 2.67 pg/mL), but not with NPDR (13.79 ± 2.56 pg/mL), in comparison with age-matched healthy controls (13.63 ± 2.71 pg/mL).

4. Discussion

This review summarised the existing literature on melatonin and circadian rhythm regulation in DR. A small number of studies found a significant association between DR and reduced nocturnal melatonin secretion. However, current data do not provide strong evidence of significant changes in melatonin circadian timing in DR, or changes in melatonin concentration in relation to the severity of DR.

Studies that examined melatonin concentration based on a single saliva sample [42] or blood serum samples [44,45] collected in the morning showed inconsistent results. Whilst there were no significant differences in the salivary melatonin levels between T2D patients with and without DR, serum melatonin levels were significantly lower in patients with DR compared to T2D patients without DR and healthy controls. It is noteworthy that daytime salivary and serum melatonin concentrations are generally comparable (<10 pg/mL) and show a strong correlation [48,49]. Therefore, these differences may be attributed, at least in part, to variations in age [50,51]. The participants in serum melatonin studies, including those in the T2D, DR, and control groups, were all aged between 50 and 66 years [44,45], whereas the participants in the saliva melatonin study ranged from 26 to 86 years with no information on age demographics for the disease and control groups [42]. The variations in age could influence the measured concentration of melatonin in both DR and control groups. Furthermore, the two studies that measured melatonin in blood serum did not adequately control for critical factors, such as dietary consumption and light levels during blood collection [52,53], which could affect the measured melatonin concentrations. Most importantly, the melatonin levels in the morning only represents the relapsing or descending phase of the melatonin curve [54], which can be extremely variable and unreliable [55]. Moreover, the measurement of morning melatonin levels using ELISA, as conducted in all three studies, demonstrates poor reliability and has limited clinical validity [56]. Therefore, future studies assessing melatonin levels in patients with DR should avoid reporting only morning levels and instead provide comprehensive information on total melatonin production and timing [55].

DLMO is the endogenous starting point of melatonin secretion that typically occurs ~2 h before a person's bedtime, provided the lighting is dim (i.e., approx. <10 lux) [46]. It is regarded as the gold-standard assessment of circadian rhythm timing in humans [46,57]. Only two studies, employing robust methodologies, have examined DLMO among T2D patients with and without DR, and healthy controls, using saliva [34] and blood serum [35], respectively. In both studies, DLMO was detectable in a small proportion of patients with DR: 33 % in saliva and 57.1 % in blood serum, compared to over 86 % in T2D subjects without DR. The low detection rate of DLMO in patients with DR may be attributed to ipRGC dysfunction, which impairs the efficacy of photic entrainment of circadian rhythms, eventually leading to abnormal melatonin circadian timing and secretion [34]. Additionally, lower melatonin level may be associated with poor sleep quality, sub-optimal sleep durations (≤6 h or ≥ 8 h), and insomnia in patients with DR [35, 58–60].

Serum melatonin is rapidly metabolised and excreted as urinary metabolite, aMT6s. Nocturnal excretion of aMT6s is a reliable measure of serum melatonin, and provides an overview of the amount of melatonin secreted during the night [61,62]. In this review, several studies across various populations and age groups reported that the presence of DR was strongly associated with reduced overnight melatonin secretion [34,36,37,39,43]. In two studies, reduced aMT6s levels were significantly associated with increased odds of DR after adjusting for several relevant confounding factors such as age, gender, smoking history and coronary heart disease (odds ratio, 0.56 [43] and 0.25) [39]. These findings were also in agreement with another recent study [35] that conducted blood sampling every 30 min for a 24-h period and found significantly reduced serum melatonin levels in patients with DR compared to those without DR. The exact mechanism behind the reduced production of melatonin in DR is not well understood but may involve several potential pathways (Fig. 2). These factors could be associated with ipRGC dysfunction in individuals with T2D, leading to circadian dysregulation and reduced overnight melatonin production [34]. In addition to the pineal gland, melatonin is also synthesised in the retina [63,64] where its production is regulated by a circadian clock [65]. The retina and brain exhibit similar circadian rhythms, with some degree of interaction between the two (see review) [64]. Central melatonin dysregulation may contribute to local vascular dysfunction, leading to oxidative stress and microvascular damage in DR. Alternatively, it may cause peripheral clock desynchronisation and altered retinal melatonin physiology, further exacerbating retinal microvascular damage in DR [66–68].

Lastly, sub-optimal glycemic control and melatonin dysregulation may exacerbate retinopathy in patients with diabetes [36].

Some studies in this review explored whether melatonin levels differ based on the severity of DR. In a study by Ba-Ali et al. [41], saliva samples were collected every 4 h over a 24-h period, allowing for a comprehensive assessment of melatonin production and circadian timing in patients with different stages of DR. The study found that peak melatonin concentration at 04:00 a.m. and mean nocturnal melatonin levels were significantly reduced in all T2D patients, regardless of DR stage. However, because all patients in this study had mild-to-moderate NPDR, the study may not have been sensitive enough to detect melatonin differences across various stages of DR. Supporting this hypothesis, Hikichi et al. [38], and Chen et al. [39] found that nocturnal plasma melatonin concentration and overnight melatonin production (measured by aMT6s levels), respectively, were significantly lower in PDR patients compared to those with NPDR and healthy controls. Both studies found no significant differences in melatonin levels between patients with NPDR and healthy controls. The former study by Ba-Ali and colleagues also used ELISA kits for melatonin analysis, several of which may be poorly validated and can lead to significant inaccuracies in melatonin measurement compared to other methods, such as HPLC (employed by both Chen and Hikichi) and RIA [56]. Collectively, these findings suggest reduced nocturnal melatonin secretion in patients with T2D, which could be further decreased in those with PDR, but may require sensitive assays or analyses (such as HPLC) for accurate detection.

In one study, Aydin et al. [40] reported higher melatonin levels in the aqueous humour of patients with PDR compared to those with NPDR and healthy controls. However, this study did not control for the time of sample collection, which could impact the results due to the diurnal fluctuations in ocular melatonin levels [69]. Additionally, inflammation

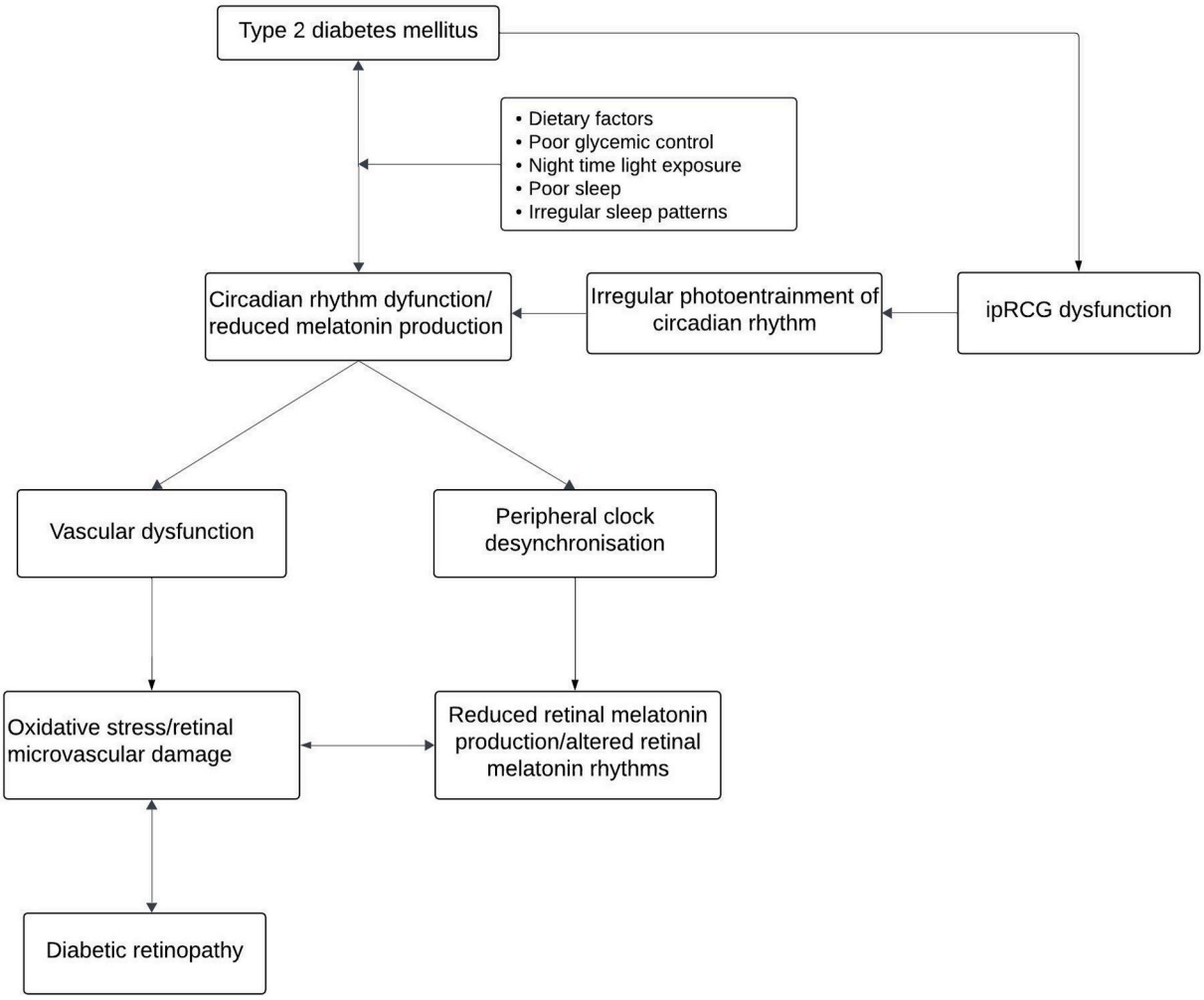

Fig. 2. A hypothetical framework that incorporates circadian rhythm dysfunction, altered melatonin production, and intrinsically photosensitive retinal ganglion cell (ipRGC) dysfunction in the pathogenesis of diabetic retinopathy in patients with type 2 diabetes mellitus (T2D).

or injury to the eye during cataract surgery could influence ocular melatonin levels [70]. Since melatonin is also synthesised in ocular tissues, such as the retina [63,64], the findings from this study may reflect local melatonin changes rather than changes in pineal melatonin secretion.

Our review provides novel insights into the role of melatonin and circadian dysregulation in DR. It highlights evidence of reduced nocturnal melatonin synthesis and some degree of circadian rhythm dysfunction in individuals with DR, which may contribute to the abnormal duration and irregular sleep patterns commonly observed in this population [59,71]. Importantly, addressing irregular or abnormal sleep duration requires understanding these biological mechanisms. Targeted interventions, such as melatonin supplementation or bright light therapy [72,73], may help realign circadian rhythms and improve sleep patterns. These findings underscore the need to evaluate sleep habits and night-time light exposure as strategies to mitigate DR progression in at-risk individuals.

Some of the strengths of our study include being the first systematic review to examine the existing literature on systemic melatonin levels and circadian timing in individuals with DR and its severity. Additionally, we employed study quality assessment tools to rigorously evaluate the methodological quality of the included studies. The current study has some limitations. First, all included studies were case-control (n = 10) or cross-sectional studies (n = 1). Future longitudinal studies are warranted to investigate the association between melatonin concentration, circadian rhythm, and the onset and progression of DR. Second, the small number of studies included in this review employed very different methods for collecting biofluids (single morning blood or saliva sample, 24 h diurnal variation in blood and saliva, DLMO and urinary aMT6s) and measuring melatonin (ELISA, RIA and HPLC), which likely contributed to some inconsistent findings. Third, only two studies measured the DLMO, the most important biomarker for circadian timing and circadian phase in humans. As a result, this review provides limited insight into the potential link between DR and changes in circadian rhythm timing. Fourth, majority of the studies had low to moderate sample size (Table 1), limiting the generalisability of the findings to larger populations. Finally, these studies were all conducted in different geographical locations and populations, which may have also influenced the observed melatonin profiles and circadian rhythms [74].

In conclusion, studies have investigated the potential role of melatonin circadian dysregulation in the pathogenesis of DR. DR appears to be associated with reduced nocturnal melatonin production, which might explain poorer sleep quality and increased sleep variability in patients with DR [60]. Although some studies found reduced melatonin production with increased severity of DR, there was inconsistency in the findings across the studies reviewed, with variations in study design, sample size, and methodologies potentially contributing to the discrepancies observed. Based on the current data, there is no strong evidence of change in melatonin circadian timing in DR. Future large cohort and longitudinal studies with robust methodological approaches (e.g., analysis of DLMO with sensitive assays) are required to ascertain the causal relationship between melatonin dysfunction and DR in

humans. Whether melatonin supplementation could be beneficial in preventing the onset or progression of DR remains an open question that requires empirical investigation [75,76].

Author contributions

All authors contributed to the study design and conceptualisation. Ranjay Chakraborty and Mallika Prem Senthil conducted the literature search. Eilish Devlin, Joshua Barclay, Royston Yi Sheng An, Eugene Lee, Steven Oh, Muhammad Husnain and Abbas Hassani did the data curation and analysis. The risk of bias assessment was executed by Eilish Devlin and Mallika Prem Senthil. The initial manuscript draft was written by Eilish Devlin and Mallika Prem Senthil with input from all authors on previous versions. All authors reviewed and endorsed the final manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2025.103202.

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