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Outer Retinal Structure and Function Deficits Contribute to Circadian Disruption in Patients With Type 2 Diabetes

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PURPOSE. Light transmitted by retinal photoreceptors provides the input for circadian photoentrainment. In diabetes, there is a high prevalence of circadian and sleep disruption but the underlying causes are not well understood. Patients with diabetes can exhibit dysfunctional photoreceptors but their role in circadian health is not known. Here we quantify photoreceptor function and contributions to circadian health and sleep in patients with diabetes without diabetic retinopathy and healthy controls.

METHODS. Rod, cone, and melanopsin function was derived using chromatic pupillometry in 47 participants including 23 patients with type 2 diabetes and 24 age-matched healthy controls after an ophthalmic examination including retinal thickness assessment using optical coherence tomography. Circadian health was determined using dim light melatonin onset (DLMO) and sleep questionnaires; light exposure was measured using actigraphy.

RESULTS. Compared with the control group, the patients with diabetes had a significantly earlier DLMO (1 hour) ($P = 0.008$), higher subjective sleep scores ($P < 0.05$), a reduction in pupil constriction amplitude for red stimuli ($P = 0.039$) and for the early postillumination pupil response (PIPR) for blue ($P = 0.024$) stimuli. There were no between-group differences in the light exposure pattern, activity levels, and intrinsic melanopsin-mediated PIPR amplitude ($P > 0.05$). A significant correlation was evident between outer retinal thickness and DLMO ($r = 0.65$, $P = 0.03$) and the pupil constriction amplitude ($r = 0.63$, $P = 0.03$); patients with thinner retina had earlier DLMO and lower pupil amplitudes.

CONCLUSIONS. We infer that the observed changes in circadian function in patients with no diabetic retinopathy are due to structural and functional outer retinal rod photoreceptor deficits at early stage of diabetic eye disease.

Keywords: pupil light reflex, melanopsin, melatonin, photoreceptors, diabetes

Diabetes mellitus is a chronic systemic disease characterized by hyperglycemia that frequently involves the eye and leads to the development of neuroretinal and microvascular abnormalities.1,2 Patients with diabetes often exhibit abnormal circadian rhythms with a higher prevalence of sleep disturbances, poor sleep quality, and excessive daytime sleepiness.3,4 Impaired sleep causes poor glycemic control and insulin resistance that can exacerbate complications such as diabetic retinopathy (DR) and neuropathy.5,6 The underlying causes of circadian and sleep disruption in diabetes are not well understood and have been associated with blood glucose levels, autonomic dysfunction, neuropathic pain, emotional stress,7–9 and lower melatonin concentrations.10,11 There is evidence that photoreceptor dysfunction, in particular, the impairment of melanopsin containing intrinsically photosensitive retinal ganglion cells (ipRGCs) can contribute to sleep disturbances in eye diseases such as glaucoma and AMD.12–15 However, such relationship between ipRGC function and sleep has not been investigated in patients with diabetes, although there is evidence for reduced melanopsin function16 and anatomical loss of ipRGCs in advanced DR.17

In early stages of diabetes, retinal neurodegeneration occurs before the clinical manifestation of retinopathy.18 At pre-retinopathy stage, anatomic alterations in the outer and inner retina can be detected,19,20 and it has been proposed that rod photoreceptors are more vulnerable due to their higher oxygen demand than cones.21 Functionally, the electrophysiological responses in patients with no DR are reduced, indicative of outer retinal deficits.22–25 One study reported that plasma dim light melatonin onset (DLMO) is normal in a small cohort of male individuals ($n = 5$) with type 2 diabetes;26 however, none of these studies evaluated melanopsin or rod function, or the link between photoreceptor function and circadian health and sleep.

Although melanopsin primarily contributes to circadian photoentrainment including the regulation of pineal melatonin release and sleep-wake cycles,27 animal studies show that rods are also required for normal circadian photoentrainment.28 The pupil light reflex is mainly driven by the outer retinal rod and cone inputs to ipRGCs during light stimulation,29,30 at light offset the postillumination pupil response (PIPR) initially receives rod input during the early-redilation phase and is then controlled by the intrinsic melanopsin response.31,32 Pupillo-
metric studies in patients without DR have been limited to case studies and smaller sample sizes and are therefore inconclusive as to whether there is a deficit in the intrinsic melanopsin response.35-37 No study has investigated how the extrinsic inputs to melanopsin and intrinsic melanopsin function in preretinopathy diabetic individuals may affect their sleep behavior. This is important because it allows the determination of photoreceptor pathomechanisms involved in the early stages of disease and could provide the foundation for potentially treating diabetes-related sleep disorders with light.34

In this study, we aimed to determine inner (melanopsin) and outer retinal (rod and cone) photoreceptor contributions to circadian health in patients with diabetes and no clinical DR. We evaluated the relationship between photoreceptor structure and function and circadian health, sleep, and light exposure through objective measures of salivary DLMO, actigraphy, and subjective sleep questionnaires (Pittsburgh Sleep Quality Index and Epworth Sleep Scale).

**METHODOLOGY**

**Participants**

Twenty-five patients with type 2 diabetes without DR (17 men, 8 women) (as diagnosed by their treating practitioners) with mean (±SD) age of 63.4 ± 11.8 years and 25 age-matched healthy controls (7 men, 18 women) with a mean age of 60.6 ± 10.8 years were recruited from the Queensland University of Technology (QUT) health clinics. Participants with diabetes had a disease duration between 0.5 and 25 years (9.5 ± 8.2 years; mean ± SD). The study was conducted in accordance with tenets of the Declaration of Helsinki and received approval from QUT. University Human Research Ethics Committee (Approval No: 1700000699). Study protocol and procedures were explained in detail and an informed written consent was obtained from all the study participants.

Individuals diagnosed by an eye specialist as having diabetes without DR, or healthy individuals with no systemic or ocular pathologies, no ocular surgeries (except cataracts) or sleep or mood disorders, were eligible for inclusion in the study. Recruited participants completed the study in two separate laboratory visits. All patients with diabetes self-reported their blood glucose levels and Hb1AC that were recorded 3 to 4 days before the first visit. On the first visit, an ophthalmic examination was performed to determine the participant’s eligibility; this included visual acuity (Bailey-Lovie LogMAR chart), slit lamp, tonometry (iCareTA01; Icare Optics, Singapore) and indirect ophthalmoscopy, retinal thickness with optical coherence tomography (OCT) (RS 3000-Advance, HD OCT; Scan type: Macula map 512 × 128; Nidek, Fremont, CA, USA) and fundus photography (Canon Non-Mydriatic Retinal Camera, CR-DGi; Canon Inc., Tokyo, Japan) to exclude DR based on the Early Treatment Diabetic Retinopathy Study Classification (ETDRS). Patients with poor glycemic control, restless leg syndrome, sleep apnea, crystalline lens opacification of nuclear sclerosis (NS) > Grade 2 (based on Lens Opacities Classification System III), blue blocking, and multifocal IOL implants were excluded from the study. Eligible participants then received an actigraphy device and a sleep diary to record light exposure, activity, and daily sleep and wake time for the next 14 days. The determination of the salivary melatonin for DLMO was conducted at the participant’s home the afternoon/night before the visit. Pupillometry with natural pupils (no dilation) was performed at both visits to assess rod, cone, and melanopsin function.

**Pupillometry**

A custom-built pupillometer based on an extended Maxwellian view arrangement32 was used to measure the rod, cone, and melanopsin contributions to the pupil light reflex (for review see Feigl and Zele15). The pupillometry setup consists of three optically aligned light-emitting diodes (LEDs); a short (blue, $\lambda_{max} = 460$ nm; full width at half maximum [FWHM] = 23), medium (green $\lambda_{max} = 519$ nm; FWHM = 34), and long wavelength (red $\lambda_{max} = 630$ nm; FWHM = 16) light that was imaged on the pupil plane of the left eye via two Fresnel lenses (100-mm diameter, 127-mm and 70-mm focal lengths; Edmund Optics, Singapore) and a 5° light-shaping diffuser (Physical Optics Corp., Torrance, CA, USA). A stimulus irradiance of 15.5 log.quanta.cm$^{-2}$s$^{-1}$ was presented in a 50° field and the consensual response (right eye) were recorded through a telecentric lens (Computar 2/3, 55-mm and 2X extender G-Mount; Computar; Singapore) using a PixelINK infrared camera sampled at a 60-Hz frame rate (IEEE-1394, PLB741 Fire Wire, 640 × 480 pixels, 60 frames per second; PixelINK, Ottawa, ON, Canada).

Pupillometry procedures were performed in a darkened room (< 1 lux) and the participants adapted for 10 minutes before testing. The current study followed a standard protocol developed in our laboratories, using a 1-second prestimulus as baseline pupil diameter followed by 1-second light pulse (pupil light response [PLR]) and 40 seconds after light offset (postillumination pupil response [PIPR]).32-35 In the current study, medium (green $\lambda_{max} = 519$ nm) wavelength was included because it limited the effect (if present) of crystalline lens opacification of the stimulus on retinal irradiance that could occur with the short wavelength (blue) stimulus. There is negligible lenticular attenuation of the long-wavelength (red) stimulus. The pupil measurements were repeated twice for all wavelengths (red, blue, and green) with an interval of 2 minutes between each stimulus to ensure that the PIPR had returned to baseline before starting the next recordings.32 Pupil measurements were performed at both visits and at the same time of the day between 9 AM and 4 PM to minimize circadian variation.35

The camera was calibrated using a 10-mm artificial pupil placed in the plane of observer’s pupil. Calibrated pixel values fed into custom-designed pupil tracking software that extract-ed the pupil diameter from the infrared video recording; blink and lid artifacts from participant data were extracted and linearly interpolated.32 The analysis metrics from the pupil model included the transient phase of the PLR between 180 and 500 ms,30,31,36,37 and the peak pupil constriction amplitude during light stimulation, the PIPR amplitudes (after light offset) of the average between 0 and 1.7 seconds,32 at 6 seconds, and plateau (derived from the exponential model).30,31,36,37 All pupil measurements and analysis metrics were completed in accordance with the standards in pupillography.38

**Dim Light Melatonin Onset**

DLMO is a standard biomarker for estimating circadian phase.39 In this study, DLMO was determined from the salivary samples of participants collected using a sparse sampling technique,40 a robust methodology for natural settings, including at-home sampling.41 At the first laboratory visit, participants were provided with a saliva kit including seven labeled Salivettes (Salivettes, Sarstedt AG & Co, Adelaide, Australia) and an instruction manual on how to collect and handle the samples. On the evening before the second visit, the participants received either hourly phone calls or text reminders to ensure compliance with the saliva collection procedures. As light suppresses melatonin production, salivary samples were...
collected in dim illumination (e.g., a bed lamp produces a room illumination of approximately 10 lux) using the sparse sampling that started 6 hours before the individual’s habitual sleep time and continued 1 hour after habitual bedtime. The lights were turned off after the last saliva collection. Saliva samples were collected every hour by chewing the cotton swab for 1 minute and stored in a freezer overnight. Participant compliance with the procedures was verified by using the actigraphy measurements (see below) to determine the mean light exposure during salivary collection period; if an individual’s average light exposure was ≥2 SD above 10 lux, the data were excluded. This was performed to ensure data quality and reliability.

Melatonin concentrations were determined by radioimmunoassay according to the standard procedures. The salivary melatonin data were plotted as a function of time, and using a skewed baseline cosine function (SBCF) model. Melatonin onset was defined as the time when melatonin level raised by 0.01 pM above the baseline salivary melatonin. In this SBCF model,

\[
Y(t) = b + \left( H / \left( 2 \ast \left( 1 - c \right) \right) \right) \ast \left( \cos \left( t - \phi + v \ast \cos \left( t - \phi \right) \right) - c \right) + \left[ \cos \left( t - \phi + v \ast \cos \left( t - \phi \right) \right) \right] / C_0 \]

where \( t \) = time (radians), \( b \) = baseline salivary melatonin, \( H \) = height of the amplitude above baseline, \( c \) = width, \( \phi \) = phase (radians), and \( v \) = skewness (radians). In every participant, the melatonin data were fitted with a SBCF model after floating all parameters and minimizing the sum-of-square differences between the data and the model parameters with an Excel solver module.

**Actigraphy**

Participants were provided with a wrist-worn actigraphy device (GENE Active; Activinsights, Kimbolton, Cambridge-shire, UK) to record light exposure and physical activity during continuous 60-second epochs for a 14-day period between the first and second laboratory visit. Participants were instructed not to cover the sensor with their clothing and only remove the device during a shower or while swimming. Artifacts such as light exposure data generated from the nonwear times and data indicating darkness (0 lux) during daytime with activity were eliminated. The measurements were determined in a 60-second sampling frequency to reliably quantify and record a stable estimate of light exposure and activity. Global solar exposure (GSE) and the time of first and last light was obtained from the Willy Weather Web site for Brisbane, Australia, to calculate the daily day and night light exposure in every participant.

**Sleep Questionnaires**

Subjective sleep behavior was assessed using two questionnaires: the Pittsburgh Sleep Quality Index (PSQI) for sleep quality and sleep disturbances and the Epworth Sleep Scale (ESS) for excessive daytime sleepiness. A global PSQI score was calculated, and a cutoff of >5 was applied to differentiate between “good” and “poor” sleepers with a higher number indicating poorer sleep; a two-factor model was used to derive perceived sleep quality (PSQI-SQ) and sleep efficiency (PSQI-SE). The ESS applies a cutoff of >10 to report excessive daytime sleepiness. In addition to this, a sleep diary was maintained to record daily sleep and wake timings that included time to bed, time taken to fall asleep, number of minutes awake at night, first wake time, final awakening, afternoon naps, and nonwear times. Sleep efficiency was calculated from the sleep diary (Actual sleep time/Total time in bed’100). The sleep and wake timings from the sleep diary were used to determine the individual differences in chronotypes (based on diurnal preference either morning type or evening type).

**Statistical Analysis**

The data were screened for normality using Shapiro-Wilk test. The statistical difference between disease and control groups was analyzed using the means of the parametric data (transient pupil light reflex, peak pupil constriction amplitude, PIPR, light exposure, activity, the PSQI global score, the ESS, sleep length, sleep latency, sleep efficiency, and number of minutes awake at midnight) with an independent t-test and nonparametric data (number of minutes exposed to different photopic illumination [lux], sedentary activity, and afternoon naps) with Mann-Whitney U test. The correlation among sleep parameters, pupil metrics, retinal thickness, and melatonin data were tested using Pearson’s correlation and Spearman’s correlation.

The effect of age on the sleep, pupil, and melatonin data were evaluated using linear regression.

**Results**

We excluded two patients with diabetes due to poor glycemic control and one control participant due to the frequent use of sleep medications; this resulted in a sample of 23 diabetic individuals without DR (15 men, 8 women) and 24 healthy control (7 men, 17 women) participants. The mean age (±SD) of the diabetic (62.9 ± 12.2 years) and control groups (60.2 ± 10.8 years) were not significantly different (\( t_{45} = -0.86, P = 0.42 \)). All patients with diabetes had well-controlled blood glucose levels (Hb1AC level = 6.4% ± 1.0%; mean ± SD) with a regular diet (\( n = 1 \)), oral medications (\( n = 19 \)), or insulin-dependent treatment (\( n = 3 \)) and none had obstructive sleep apnea. Neither group had a previous history of ocular pathology, all participants had best corrected visual acuity of 6/6, normal IOP (≤ 21 mm Hg), and no retinal abnormalities on ophthalmoscopy or fundus photography. The OCT imaging, however, detected a significant retinal thinning at 3-mm retinal eccentricity in the patient group with diabetes with no DR (mean ± SD; diabetes 325 ± 19 μm versus controls 358 ± 16 μm, \( P = 0.018 \)); segmentation analysis identified a significant reduction in the outer retinal layer thickness at 3 mm in an annulus (diabetes 171.4 ± 16 μm versus controls 207.2 ± 11.0 μm, \( P = 0.001 \)) in the patient group, whereas the inner retinal nerve fibre layer thickness (diabetes 97.7 ± 8 μm versus controls 98.5 ± 9 μm) was not significantly different between the two groups.

**Pupil Light Reflex**

The PLR metrics (transient pupil response, peak constriction amplitude) and the PIPR metrics (early PIPR amplitudes average between 0 and 1.7 seconds, at 6 seconds, and plateau) were quantified and derived from the best-fitting linear and exponential model of the pupil data as described previously in our laboratories. The average data (mean and 95% CI) for each metric are given in the Table and box plots with the individual data are shown in Figure 1. The average baseline pupil diameter was not significantly different between groups (mean ± SD; diabetes 5.26 ± 0.83 mm and controls 5.53 ± 0.54 mm). The transient PLR to the red, blue, and green wavelengths were similar between the groups (Fig. 1A–C). The peak pupil constriction amplitude to the red stimulus was significantly reduced and led to an earlier redilation in the diabetic groups (\( t_{41} = 0.006, P = 0.039 \)) (Figs. 1D, 2A).
indicative of outer retinal impairment; there was no group difference in the constriction amplitude with the green and blue stimuli (Figs. 1E, 1F) that includes melanopsin contributions. The early PIPR (average between 0 and 1.7 seconds) to blue stimuli (Figs. 1E, 1F) that includes melanopsin contributions was significantly lower in amplitude to any of the three wavelength stimuli, which was there a difference in the 6-second or plateau PIPR significantly different between groups (Figs. 1G, 1I), neither PIPR amplitudes to the red and green stimuli were not different illumination ranges based on light therapy studies. We evaluated the daylight exposure time (measured from the time to last light and first light). Given that both groups experienced similar daylight illumination, we evaluated the daylight exposure time (>1000 lux) at three different illumination ranges based on light therapy studies. The mean number of minutes exposed to between 1000 and 4999 lux (median diabetes: 73 minutes versus controls: 69 minutes; \( P = 0.82 \)) and 5000 and 9999 lux (median diabetes: 21 minutes versus controls: 28 minutes; \( P = 0.47 \)) and illuminations >10,000 lux (median diabetes: 22 minutes versus controls: 27 minutes; \( P = 0.07 \)), and was similar between groups.

There was no difference in the physical activity recorded in counts per minute; in addition, similar levels of sedentary activity (median: diabetes: 596 minutes, controls: 537 minutes; \( P = 0.10 \)) (Fig. 4). The peak daylight levels reached 18,500 lux with an average daylight exposure of 1200 lux across 14 days with a daytime illumination equivalent to overcast day and an average night light exposure of 28 lux (measured from the time to last light and first light). Given that both groups experienced similar daylight illumination, we evaluated the daylight exposure time (>1000 lux) at three different illumination ranges based on light therapy studies. The mean number of minutes exposed to between 1000 and 4999 lux (median diabetes: 73 minutes versus controls: 69 minutes; \( P = 0.82 \)), 5000 and 9999 lux (median diabetes: 21 minutes versus controls: 28 minutes; \( P = 0.47 \)) and illuminations >10,000 lux (median diabetes: 22 minutes versus controls: 27 minutes; \( P = 0.07 \)), and was similar between groups.

**Dim Light Melatonin Onset**

A total of 329 saliva samples from 47 participants (23 diabetes and 24 controls) were assayed. The mean dim light exposure (lux) during sample collection was similar between groups (diabetic individuals: \( 4.0 \pm 3.0 \) lux versus controls: \( 3.5 \pm 4.0 \) lux; mean \( \pm \) SD) and no data were excluded based on light exposure measurements. The lowest limit of quantification of the melatonin level is 4.3 pM. Participants assigned with nonresponder status (controls = 5 and diabetes = 5) due to melatonin concentrations <4.3 pM at all time points, and participants with melatonin concentrations below the melatonin onset criterion <3 pg/mL at all time points (controls = 4 and diabetes = 5) were excluded from the melatonin analysis. Melatonin concentrations from the remaining 28 participants (diabetic individuals: \( n = 13 = 91 \) saliva samples and controls: \( n = 15 = 105 \) saliva samples) were plotted as a function of time and modeled using a SBCF. Using the SBCF model, the DLMO time was defined when the individual’s melatonin level first raised by 0.01 pM above baseline salivary melatonin (Fig. 3A). The mean melatonin onset time relative to the habitual sleep time was 2 hours 22 minutes (>1 hour) earlier for the diabetic individuals and 1 hour 15 minutes (±40 minutes) earlier for healthy controls; DLMO in the diabetic group was 1 hour earlier than controls (\( t_{25} = -3.55, P = 0.008 \)) (Fig. 3B). The mean habitual sleep time, however, was not significantly different between groups (clock time: diabetes 22 hours 28 minutes ± 55 minutes versus controls 22 hours 46 minutes ± 45 minutes) indicating that, on average, both groups went to bed at a similar time.

**Actigraphy**

The mean light exposures in patients with diabetes and control participants were not significantly different (daylight exposure: \( t_{13} = 0.27, P = 0.78 \) and night light exposure: \( t_{13} = 1.64, P = 0.10 \) (Fig. 4). The peak daylight levels reached 18,500 lux with an average daylight exposure of 1200 lux across 14 days with a daylight illumination equivalent to overcast day and an average night light exposure of 28 lux (measured from the time to last light and first light). Given that both groups experienced similar daylight illumination, we evaluated the daylight exposure time (>1000 lux) at three different illumination ranges based on light therapy studies. The mean number of minutes exposed to between 1000 and 4999 lux (median diabetes: 73 minutes versus controls: 69 minutes; \( P = 0.82 \)), 5000 and 9999 lux (median diabetes: 21 minutes versus controls: 28 minutes; \( P = 0.47 \)) and illuminations >10,000 lux (median diabetes: 22 minutes versus controls: 27 minutes; \( P = 0.07 \)), and was similar between groups.

**Subjective Sleep Assessment**

The PSQI global score (mean \( \pm \) SD) was on average higher in patients with diabetes (7 ± 4) than in the control group (3 ± 2), indicating a greater number of poorer sleepers in the diabetic group (2) indicating a greater number of poorer sleepers in the diabetic group (1 vs. 0.5) compared with controls (3 ± 2) and was similar between groups. The sleep diary revealed that all the participants had similar sleep and wake times with similar chronotypes (morning preferences) between and within groups. Sleep efficiency was reduced (mean \( \pm \) SD: 78% ± 9% and 90% ± 3%; \( t_{40} = 5.34, P = 0.01 \) and showed a significant correlation with the mean number of minutes awake at night (Spearman’s \( r = -0.9, P = 0.01, R^2 = 0.78 \)) in patients with diabetes. The other parameters calculated from the sleep diary, including sleep...
Figure 1. Box and whisker plots show the median, interquartile, and range of pupil constriction during light stimulation and after light offset in patients with diabetes ($n=23$) (filled circles) and controls ($n=24$) (unfilled circles). The PLR and PIPR metrics plotted as a function of percentage (%) pupil diameter for red (left panels), blue (center panels), and green (right panels) stimuli for patients with diabetes and controls. The peak pupil constriction for red stimuli was significantly reduced in addition to the combined rod-melanopsin PIPR response for the blue stimuli. Statistical significance is denoted by an asterisk (* $P < 0.05$).
Figure 2. The average and 95% CI of pupil light reflex in response to long \((\text{red})\) wavelength \((\lambda = 630 \text{ nm})\) and short \((\text{blue})\) wavelength \((\lambda = 460 \text{ nm})\) stimuli for diabetes \((n = 25)\) and control individuals \((n = 24)\). The mean for diabetes \((\text{solid red line})\) and 95% CI \((\text{shaded})\) for controls in response to long-wavelength \((A)\) and short-wavelength stimuli \((B)\). The red peak pupil constriction amplitude and the blue PIPR amplitude average between 0.0 and 1.7 seconds \((\text{early PIPR})\) were significantly different between diabetes and control individuals \((\text{independent } t\text{-test, } *P < 0.05)\).

Figure 3. \((A)\) Box and whisker plot shows the median, interquartile, and range of duration of melatonin onset \((h)\) relative to bedtime. Each data point indicates the individual onset times in patients with diabetes \((\text{red squares})\) and controls \((\text{blue circles})\). \((B)\) A representative example of the SBCF model for a 68-year-old female patient with diabetes \((\text{red squares})\) and a 64-year healthy control \((\text{blue circles})\) who had similar sleep onset \((\text{blue and red arrows})\). The red and blue vertical line indicate the melatonin onset times, which is at 1 hour 53 minutes and 1 hour before their habitual sleep time for the diabetic and healthy control, respectively \((\text{independent } t\text{-test, } *P < 0.05)\).

Figure 4. Light exposure \((\text{lux})\) is presented in 60-second sampling for a 50-Hz frequency \((14 \text{ days})\) for diabetic \((\text{red traces})\) and control \((\text{blue traces})\) individuals as a fraction of time \((\text{hours})\). The average rise \((\text{wake})\) and sleep times were similar, and both groups received similar light levels across different illuminations.
Correlations Between Structural Measures (OCT), DLMO, and Pupil Metrics

The outer retinal thickness as measured with the OCT significantly correlated with the DLMO (Spearman’s r = -0.65; P = 0.03) and with the red peak pupil constriction amplitude (Pearson’s r = 0.63; P = 0.024) indicating that outer retinal photoreceptor damage and dysfunction may contribute to earlier melatonin onset. DLMO also correlated with disease duration (Spearman’s r = 0.65; P = 0.026) showing that there was an earlier DLMO with longer disease duration. There were no significant correlations in the control group for structural measures (OCT), DLMO, and pupil metrics. Linear regression analysis showed no effect of age on sleep, pupil, and melatonin onset data.

Discussion

Patients with type 2 diabetes without DR have a significantly earlier melatonin onset time (Fig. 3A) and poorer sleep behavior, even though their daily light exposure levels are similar (Fig. 4). We detected outer retina structural (OCT) and functional (pupil) defects (Figs. 1D, 2A) and dysfunctional extrinsic rod inputs to melanopsin ganglion cells (Figs. 1H, 2B), suggestive of contributing to the observed circadian and sleep disruption. The intrinsic melanopsin response, however, was normal in patients without DR (Fig. 2B).

The role of retinal photoreceptors in regulating circadian rhythms and sleep is largely derived from mice studies; ipRGCs signal to the suprachiasmatic nucleus for circadian photoentrainment.52,53 and rods are substantial contributors to circadian photoentrainment.28 As cones and ipRGCs become insensitive under scotopic conditions, rods signal to ipRGCs through rod-bipolar pathway and use rod-cone pathways under mesopic-photopic levels to mediate circadian photoentrainment.28 The contribution of rod and melanopsin photoreceptors to the human pupil light reflex has been previously established using spectral sensitivity measures50,51 with extrinsic rod inputs to melanopsin dominating the early-redilation phase of the PIPR after offset of a 1-second short wavelength (blue) stimulus,50 as used in this study. Hence, our observation that the early-phase PIPR is significantly reduced in diabetic individuals indicates that the rod inputs to ipRGCs are impaired (Fig. 2B), consistent with the reduced peak constriction amplitude to the long-wavelength stimulus (Fig. 2A, left), which is dominated by outer retinal inputs.55 These findings of rod dysfunction are supported by electrophysiological studies demonstrating altered rod signaling in patients with diabetes without DR.56 In addition to this, early DR is also known to be first associated with rod dysfunction.57,58

Changes in melatonin levels have been linked with the pathogenesis of diabetes,59 showing either no difference in plasma melatonin63 or significantly reduced peak melatonin concentrations,64 which might be due to differences in the measurement of melatonin at different time points during the night in these studies. The determination of DLMO allows a better and more reliable estimate of circadian phase.59 There is only one study that measured DLMO in a small cohort of five patients with diabetes.26 This study found no differences in the plasma melatonin onset time compared with controls but did not stratify the sample according to the presence or grade of DR. Participants also experienced high light exposures (440-825 lux) before bedtime when the plasma was collected; hence, melatonin onset could have been suppressed.65 Our study ensured salivary samples were collected in dim light (<10 lux) using a sparse sampling methodology that allowed participants to complete the procedure at home. This is a valid approach,41 and the current DLMO sample was sufficient to determine the statistical significance between groups with a power of 80%, assuming a type 1 error of 5% (two-tailed). In addition to this, we verified the reliability of the data with the light exposure recordings from the actigraphy device during sample collections for every individual. Given that chronotype can influence the melatonin onset, we determined the sleep and wake timings from the individual’s sleep diary, and there was no significant difference between and within groups in their sleep-wake schedule that could have affected the melatonin results. We also excluded patients with poor glycemic control, restless leg syndrome, and obstructive sleep apnea that can cause sleep disruption.50,66 Pupil size and sleep quality reduces with increasing age, particularly above 65 years,67 and both groups were age-matched to correct for the effect of age. Moreover, the analysis of the average baseline pupil diameter showed that it was not significantly different between groups. As light exposure can shift a person’s circadian phase, we determined the light exposure levels and found no differences in the day and night light exposure between and within groups consistent with the previous study findings in diabetes.10 We ensured both diabetes and control participant recruitment was during the same seasonal period, and there were no differences in the mean GSE in Brisbane, Australia, between October and March (spring and summer seasons) with average temperatures ranging between 15°C and 28°C.

In conclusion, this study demonstrates that reduced photoreceptor function and altered retinal structure may contribute to an earlier melatonin onset and poorer sleep behavior in people with early-stage diabetic eye disease. This finding provides a foundation for developing light therapies to selectively target rod photoreceptors to maintain regular photic signaling to the suprachiasmatic nucleus to improve sleep-wake cycles in patients. Potential light therapy strategies with adequate amount of light intensity, duration, and wavelength exposure to treat the diabetic cohort are yet to be explored.

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References


