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Original Article

Assessment of diurnal melatonin, cortisol, activity, and sleep–wake cycle in patients with and without diabetic retinopathy

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ABSTRACT

Objective: To assess the diurnal melatonin, cortisol, and activity/rest levels, as well as sleep quality, in patients with and without nonproliferative diabetic retinopathy (DR).
Methods: We included 25 diabetic patients with DR and 29 without DR. A total of 21 healthy subjects constituted the control group. We assessed the circadian rhythm by actigraphy and diurnal salivary melatonin and cortisol measurements. Sleep quality was evaluated by actigraphy and the Pittsburgh Sleep Quality Index (PSQI) and Epworth Sleepiness Scale (ESS) questionnaires. Light exposure was quantified by actigraphy. The primary outcome was peak salivary melatonin level. Secondary outcomes were mean melatonin and cortisol levels during dark hours, activity–rest rhythm, sleep quality, as well as level of white, red, green, and blue light exposure.
Results: Peak melatonin concentration at 04:00 and mean nocturnal melatonin level were significantly reduced in all diabetic patients, regardless of retinopathy stage ($p < 0.001$). Levels of light exposures during dark hours were not significantly different in patients with and without DR and healthy controls. Only patients with DR showed increased intradaily variability in their activity–rest interval, indicating circadian misalignment ($p = 0.04$). Neither the objective actigraphic sleep quality parameters nor the subjective PSQI or ESS scores were significantly different between healthy controls and diabetic patients.
Conclusions: Reduced nocturnal melatonin concentration and increased fragmentation of activity–rest intervals revealed circadian rhythm disturbance in diabetic patients with DR.

1. Introduction

Circadian rhythm is the regulation of biological and behavioral processes in a relatively constant pattern of 24 h to prepare light-dependent organisms for external changes in the environment [1]. Sleep–wake cycles, hormone secretion, blood pressure, metabolism, and seasonal reproduction are among the biological and behavioral events that are regulated by the circadian rhythm. The anatomical basis for the circadian system consists of the brain's suprachiasmatic nucleus (SCN), also called “the master clock,” which acts through neuro-humoral pathways to orchestrate peripheral clocks that in turn control the physiological processes at the tissue level [2]. The master clock and the peripheral clocks are constantly adjusted by internal and external stimuli, of which light exposure, detected by retinal photoreceptors, is the most important entrainment factor [2,3]. Light is detected by specialized subsets of retinal ganglion cells, called the intrinsically photosensitive retinal ganglion cells (ipRGCs) [4–6]. These cells are directly sensitive to light as well as receiving indirect light signals from rods and cones [7]. The direct and indirect light signals are conveyed by ipRGCs to SCN to entrain the master clock [4,8–10].

Diabetes is known to affect different neuronal tissues including the retina, which is one of the most energy-demanding tissues [11]. Na + transport by Na+/K + -ATPase accounts for 50% of retinal energy consumption; phototransduction, neurotransmission, and dark current are other energy-demanding processes in the retina [11]. Recent research indicates that retinal metabolism is regulated

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by circadian rhythm and that, conversely, intact retinal function might be important for the retina itself as well as the circadian photoentrainment [12,13]. Although none of the retinal photoreceptors (ie rods, cones, ipRGCs) are essential to circadian rhythm regulation, all three photoreceptors contribute to circadian photoentrainment [14–16].

Clinically, circadian rhythm can be assessed by actigraphic measurement of activity/rest intervals (actigraphy), melatonin, and cortisol level determination in saliva, urine and plasma, and/or body temperature measurement [17–23]. Previous studies have reported conflicting results regarding the influence of diabetes on the production of melatonin, which is an important endogenous marker of the circadian rhythm [24–29]. In addition, the effect of retinal degeneration as result of diabetes mellitus on circadian rhythm has not yet been investigated. We hypothesized that reduced retinal light perception due to diabetic retinopathy (DR) may lead to circadian misalignment, which in turn results in insulin/glucose dysregulation and eventually deterioration of DR. Thus, in the current study, we investigated the circadian rhythm and the sleep quality in patients with and without moderate nonproliferative DR.

2. Method

This study was approved by the Committee on Health Research Ethics in the Capital Region of Copenhagen (project-id: H-15013160) and was conducted in agreement with the Declaration of Helsinki. Prior to inclusion, subjects received oral and written information, followed by informed consent.

2.1. Subjects

We included 54 patients with diabetes, of whom 25 had moderate nonproliferative DR and 29 did not have DR (Table 1). The diabetic patients were recruited from Steno Diabetes Centre, where a nonmydriatic fundus camera (Topcon TRC-NW8 Non-Mydriatic Retinal Camera; Topcon Corporation, Tokyo, Japan) was used to acquire five overlapping 45-degree fundus photographs of each eye in dilated condition. The five image fields (posterior pole, superior, inferior, nasal, and temporal) were set into a single mosaic image by IMAGEnet software program. Trained readers assessed the images and classified the severity of DR according to Steno Grading.30 Details of Steno Grading are provided elaborated elsewhere [30]. In our study, we included patients with either no DR or moderate nonproliferative DR (microaneurysms, hemorrhages, and soft or hard exudates in the retina). The patients consisted of both type 1 (n = 25) and type 2 (n = 29) diabetic individuals. The inclusion criteria for the patient selection were age between 40 and 80 years, diabetes duration of a minimum of 15 years, and no or moderate DR. The exclusion criteria were ophthalmological diseases other than DR, systemic diseases with a possible effect on the circadian rhythm, that is, hepatic or uremic encephalopathy, psychiatric or neurological diseases, pregnancy, lack of cooperation, and previous history of retinal laser treatment.

2.2. Clinical examinations

Each subject underwent complete ophthalmological examination by the same physician (S.B.). In addition, optical coherence tomography (Heidelberg SD-OCT; Heidelberg Engineering, Heidelberg, Germany or Topcon 3D OCT, Tokyo, Japan) and fundus photography (Topcon Retinal Camera 50DX; Topcon Corporation, Tokyo, Japan) were performed to assess the retina and the degree of retinopathy. Glycated hemoglobin (HbA1c), blood pressure, plasma cholesterol and triglyceride level, plasma creatinine level, and renal function (glomerular filtration rate) were measured.

2.3. Circadian rhythm

2.3.1. Melatonin and cortisol

The participants performed salivary collection using commercially available saliva collection kits (SARSTEDT AG & Co., Nümbrecht, Germany) at home every 4 h in 24 h, starting at 12:00 PM and ending at 12:00 the next day. Each subject collected seven samples in total (Fig. 1).

To ensure better compliance, subjects received written collecting instructions and a diary to record sampling times. Subjects were instructed not to eat, drink, chew gum, or brush teeth for at least 30 min before saliva collection. In addition, they were to refrain from performing heavy activity up to 1 h before the sampling. Saliva samples were stored at 2–8 °C and the samples were delivered to our hospital for analysis after the last saliva collection. The samples were analyzed for diurnal melatonin and cortisol concentrations. Melatonin analysis was performed using commercially available melatonin enzyme-linked immunosorbent assay (ELISA) kits (direct saliva melatonin ELISA RE 54041, IBL-International, Hamburg, Germany). After salivary analysis, we calculated the peak melatonin level and the mean nocturnal melatonin concentration (from 20:00 to 06:00). Due to seasonal variations of light levels throughout the year, we investigated the effect of seasonal half-years (summer and winter) on the melatonin levels.

Cortisol analysis was performed using IDS-iSYS Salivary Cortisol assay, which is based on an in vitro chemiluminescence technology (Imunodiagnostic Systems Limited, Boldon, UK). Similar to

Table 1
Clinical profile of healthy controls and patients with and without diabetic retinopathy (DR).

	Controls ($n = 21$)	Patients with diabetes ($n = 54$)			
		Without DR ($n = 29$)	$p1$[a]	Moderate DR ($n = 25$)	$p2$[a]
Sex (female:male)	15:6	10:19	0.07	11:14	0.17
Age (y, mean ± SD)	60 ± 10	63.1 ± 8.3	0.43	61.6 ± 9.1	0.81
BMI (kg/m^2, mean ± SD)	25 ± 3	27.2 ± 3.6	0.14	28.8 ± 7.0	0.01
Blood pressure (mm Hg)					
Systolic (mean ± SD)	132 ± 14	139.0 ± 13.9	0.19	138.5 ± 14.6	0.25
Diastolic (mean ± SD)	82 ± 7	76.7 ± 74.9	0.09	80.5 ± 8.6	0.83
Smoking (yes:no)	1:20	4:29	1	2:25	1
HbA_{1c} (mmol/mol, mean ± SD)	35 ± 3	57.9 ± 11.5	<0.00001	68.3 ± 12.2[b]	<0.00001
Duration (years, mean ± SD)	–	29.2 ± 10.0	–	29.0 ± 9.3	–
BCVA (ETDRS chart, mean ± SD)	87 ± 4	87.1 ± 4.6	0.998	81.5 ± 7.7	0.004
IOP (mm Hg, mean ± SD)	14 ± 3	14.3 ± 2.4	0.97	15.8 ± 2.7	0.09

BCVA, best corrected visual acuity; BMI, body mass index; ETDRS, Early Treatment Diabetic Retinopathy Study chart; HbA1c, glycated hemoglobin; IOP, intraocular pressure.
[a] The p values refer to comparisons between healthy controls and patients without DR ($p1$) and with DR ($p2$).
[b] Significant difference between patients with and without DR.

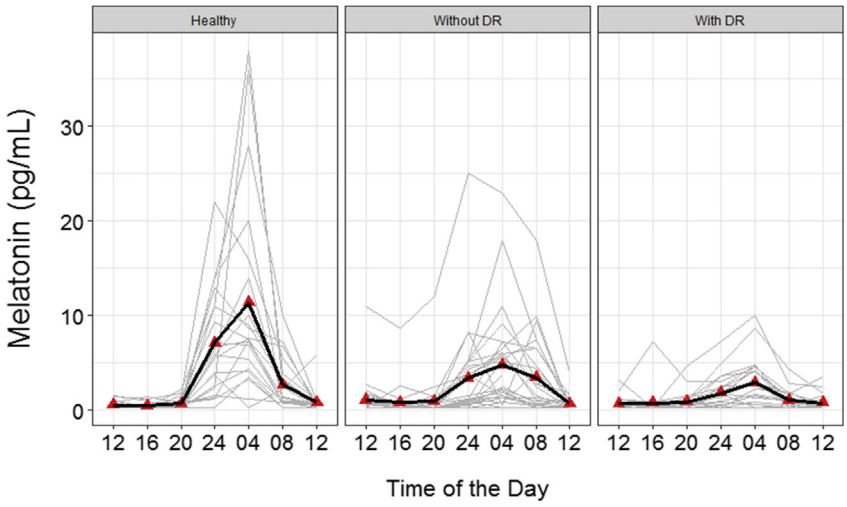

Fig. 1. Average 24-h salivary melatonin profile collected every fourth hour in healthy controls (left) and in patients without (middle) and with (right) diabetic retinopathy (DR). Red triangles indicate mean melatonin level every fourth hour.

melatonin, mean nocturnal cortisol concentration and peak diurnal cortisol level were calculated.

2.3.2. Rest–activity

Rest–activity levels were measured with wrist actigraphy (Actiwatch Spectrum; Respironics, Philips Healthcare, Utrecht, Netherlands), with the actigraph worn by subjects on the nondominant arm for seven consecutive days (Fig. 2).

The Actiwatch measures activity or the absence of activity by registering acceleration in any axis, followed by integration of acceleration measurements above a certain threshold level into one value over an epoch length of 30 s. To ensure a better assessment of rest–activity intervals, subjects were instructed to press an "event-marker button" on the Actiwatches, indicating bedtime, wake-up time, and wrist-off periods. In addition, subjects completed a diary to record bedtime, wake-up time, total sleep time, number of nocturnal awakenings, as well as ingestion of sleep medication as previously described by Brøndsted et al., [18].

Actiwatch data were imported into Philips Actiware 6.0.4 (Philips Respironics) to adjust the start and stop points of the rest intervals based on the event marker, activity level, amount of detected light, and diary information on wake-up and bedtime. Following the quality assessment process, activity-based circadian rhythm outcomes were calculated with the R statistical program (version 3.2.3, The R Foundation for Statistical Computing). Quality of circadian rhythm was assessed by using a

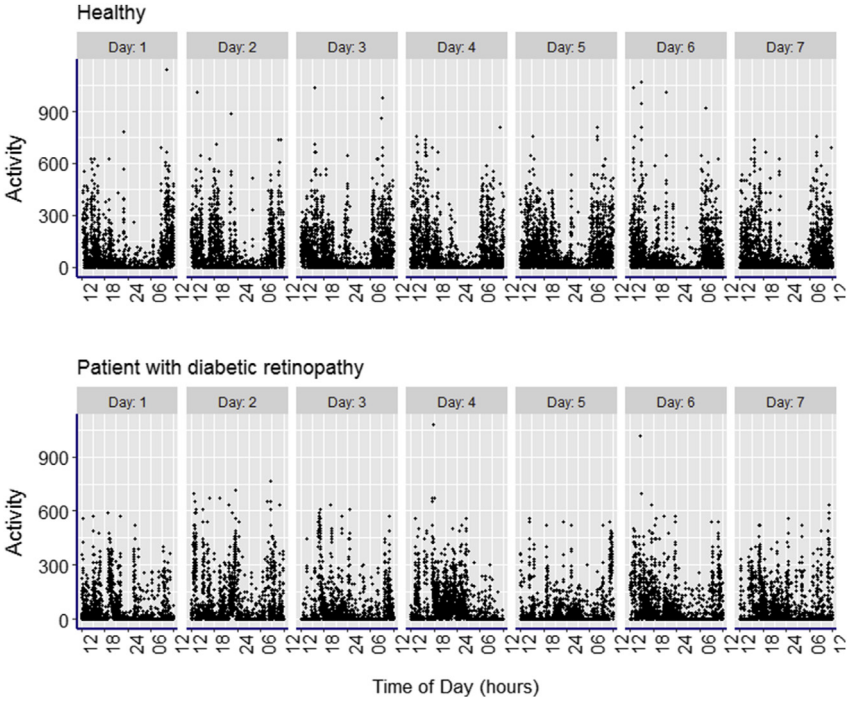

Fig. 2. Measurements of 24-h activity over 7 consecutive days with an actigraph in a healthy subject (upper panel) and a diabetes patient with retinopathy (lower panel). Each dot corresponds to a sum of 30-s activity counts, measured as acceleration in any axis and above a certain threshold level. In healthy subjects, there was a regular intraday variability (IV), whereas in the patients with diabetic retinopathy, the IV was increased, indicating a fragmented activity–rest rhythm ($p = 0.04$).

nonparametric method in which interdaily stability (IS) and the intradaily variability (IV) were circadian rhythm outcomes (see van Sommer et al., for more details [31]). IS corresponds to the ratio of average 24-h variance around the mean and the overall variance and has a theoretical range from 0 to 1, where zero corresponds to Gaussian noise and 1 is a perfect sine wave (ie, a higher value means a more stable rhythm). IV is the ratio of hour-to-hour variability and ranges from 0.5 to 2, where a higher value indicates increased rhythm fragmentation [31]. Additional activity parameters were the average activity, onset of the most active 10-h sequence (M10-onset), and onset of the least active 5-h sequence (L5-onset). M10-onset was reported as decimal hours after 24:00 (midnight), whereas L5-onset was expressed as decimals hours after 12:00 (noon). The M10-onset and L5-onset indicate whether a subject has an advanced or a delayed phase sleep–wake-up onset.

2.3.3. Light exposure

The Actiwatch measured the amount of white, red, green, and blue light exposure in each subject for 24 h over seven days. We used the data from the first six days only, because during the seventh day of the measurement, the subjects performed salivary collection and glucose measurement every 4 h, which could potentially affect the light measurement. Subjects were instructed not to cover their wrists to allow direct light exposure to the Actiwatch sensors.

Following data acquisition, light intensities $\leq$0.0000146 μW/cm^2 for blue and green lights, $\leq$0.00987 μW/cm^2 for red light, and $\leq$0.02 lux for white light were regarded as apparatus noise and thus excluded. The limits were based on light measurement by placing the Actiwatch in a box in a closet located in our dark-adaptation room. The cumulative light levels of each light color from 19:00 to 06:00 were calculated for every subject. After logarithmic transformation, the light levels were compared between healthy subjects and diabetic patients with and without DR.

2.3.4. Sleep quality

Subjective sleep quality was evaluated by Pittsburgh Sleep Quality Index (PSQI) and Epworth Sleepiness Scale (ESS) questionnaires. A global PSQI score of $\geq$5 indicates poor sleep quality, whereas an ESS score of >16 refers to severely excessive daytime sleepiness [32].

Actigraphy was used to objectively assess sleep quality, and sleep parameters were calculated by importing data into the software program Philips Actiware 6.0.04 (Philips Respironics). Major rest intervals were detected automatically, followed by individual assessment of each measurement for errors. When errors were detected, the start and end time of the rest intervals were set manually based on the activity measurement, event marker, light information, and diary information. The software was used to calculate sleep efficiency (percentage of sleep during major rest intervals), sleep onset latency (number of minutes from bedtime to sleep), and wake after sleep onset (WASO; number of minutes awake after sleep onset).

2.4. Outcomes

The primary outcome was peak diurnal melatonin level. Secondary outcomes were mean nocturnal melatonin level, peak diurnal cortisol level, mean nocturnal cortisol concentration, activity-based IS and IV, level of light exposures, and objective sleep quality parameters (sleep efficiency, sleep onset latency, and WASO). PSQI and ESS scores constituted subjective sleep quality parameters.

2.5. Statistical analysis

The R statistical program (version 3.2.3) was used to perform the statistical analysis. Data are presented as mean ± standard deviation unless stated otherwise. Histograms were used to assess whether the data were normally distributed. Logarithmic (base 10) transformation of the data were performed when the data were not normally distributed. Analysis of variance (ANOVA) was used to assess the mean differences in circadian rhythm and sleep quality outcomes between healthy subjects and diabetic patients. A Tukey–honestly significant difference (HSD) post hoc analysis was run to assess the differences among healthy controls, patients with DR, and patients without DR. In addition, we also compared healthy controls with the diabetic patients, grouped by type 1 and type 2 diabetes. A two-way ANOVA with interaction effect was conducted to include a possible synergistic effect between grouping levels. When relevant, p values were adjusted for age, glycated hemoglobin (HbA1c), body mass index (BMI), and sex. Pearson correlation analysis was used to assess the association between melatonin (peak and mean nocturnal levels) and age, HbA1c, BMI, systolic and diastolic blood pressure, diabetes duration, best corrected visual acuity (BCVA), and PSQI and ESS scores.

The generated and/or analyzed data in the current study are available from the corresponding author upon reasonable request.

3. Results

Patients and healthy controls were not significantly different in terms of age, sex proportion, blood pressure, smoking, and intraocular pressure (Table 1). Glycated hemoglobin (HbA_{1c}) was significantly higher in both patient groups compared to healthy subjects ($p < 0.00001$). BMI was higher only in patients with DR ($p = 0.01$) and not in patients without DR ($p = 0.01$). The average diabetes duration was similar in patients with and without DR ($p = 0.99$).

3.1. Circadian rhythm

3.1.1. Melatonin and cortisol

The mean 24-h melatonin profile in healthy subjects and patients with and without DR is illustrated in Fig. 1. Mean nocturnal melatonin concentration was significantly reduced both in patients with ($p < 0.0001$) and without ($p = 0.009$) DR (Table 2).

Mean salivary melatonin level had a peak concentration at 04:00 in all three groups (Fig. 1). The average peak diurnal melatonin concentration was also lower both in patients with ($p = 0.0002$) and without ($p = 0.004$) DR compared to healthy controls. We also tested differences between patients with and without DR, but did not find any significant difference in nocturnal melatonin concentration ($p = 0.23$) or in peak diurnal melatonin level ($p = 0.58$). Furthermore, when conducting two-way ANOVA to check for time and group interaction, melatonin concentration was not significantly different between diabetic patients with and without DR ($p = 0.23$). In healthy controls, the mean nocturnal melatonin level decreased with increasing age (Pearson $r = -0.69$, $p = 0.0007$); the higher the BMI, the lower the nocturnal melatonin level (Pearson $r = -0.58$, $p = 0.008$); and the lower the mean nocturnal melatonin level, the higher the systolic blood pressure (Pearson $r = -0.48$, $p = 0.03$). Peak melatonin concentration was negatively correlated with age (Pearson $r = -0.60$, $p = 0.004$) and BMI (Pearson $r = -0.50$, $p = 0.02$) in healthy controls. However, we did not find statistically significant correlations between melatonin levels (mean nocturnal and peak) and age, HbA1c, BMI, systolic and diastolic blood pressure, diabetes duration, as well as PSQI and ESS scores in patients with diabetes. Moreover, we did not find any

Table 2
Circadian rhythm outcomes presented as mean ± standard deviation.

	Controls ($n = 21$)	Patients ($n = 54$)			
		Without DR ($n = 29$)	$p1$[a]	With DR ($n = 25$)	$p2$[a]
Melatonin (pg/mL)					
Average nocturnal	5.5 ± 4.0	3.2 ± 3.8	0.01	1.7 ± 1.4	<0.001
Peak diurnal (04:00)	11.4 ± 10.7	4.8 ± 5.3	0.01	2.9 ± 2.6	<0.001
Cortisol (μg/dL)					
Average nocturnal	12.0 ± 7.7	11.3 ± 3.9	0.99	11.6 ± 3.7	0.61
Peak diurnal (08:00)	22.8 ± 10.9	24.4 ± 11.6	0.86	18.9 ± 8.9	0.43
Actigraphy – activity					
Average activity	181.3 ± 43.0	172.1 ± 49.2	0.73	160.0 ± 49.2	0.21
Intradaily variability	0.7 ± 0.2	0.8 ± 0.2	0.92	0.9 ± 0.2	0.04
Interdaily stability	0.6 ± 0.1	0.6 ± 0.1	0.92	0.5 ± 0.1	0.32
L5 onset	12.8 ± 1.3	12.3 ± 1.9	0.53	12.7 ± 1.3	0.99
M10 onset	8.9 ± 1.7	8.6 ± 1.9	0.83	9.5 ± 1.6	0.52

DR, diabetic retinopathy; L5 onset, onset of the least active 5-h interval expressed as decimal hours after 12:00 (noon); M10 onset, onset of most active 10-h interval expressed as decimal hours after 24:00 (midnight).
[a] The p values correspond to comparison between healthy controls and patients without retinopathy ($p1$) and with diabetic retinopathy ($p2$). Average nocturnal corresponds to mean melatonin and cortisol levels from 20:00 to 06:00.

effect of seasons (summer and winter half-year) on the peak or nocturnal melatonin levels.

Peak diurnal cortisol level was measured at 08:00, both in healthy subjects and in patients with and without DR. There were no significant differences in the average nocturnal cortisol level or peak diurnal cortisol level between healthy controls and patients with or without DR. We did not find any difference between the two patient groups in regard to peak cortisol concentration ($p = 0.17$) or nocturnal cortisol level ($p = 0.70$). There were no correlations between peak or nocturnal cortisol levels and age, HbA_{1c}, BMI, systolic and diastolic blood pressure, diabetes duration, BCVA, and PSQI and ESS scores.

3.1.2. Rest–activity

Actigraphic measurement of rest activity showed increased IV (ie fragmented activity–rest rhythm) in patients with DR ($p = 0.04$) but not in patients without DR ($p = 0.92$) (Table 2). No significant difference was found in the average activity level or IS between healthy controls and diabetes patients. Furthermore, we did not find any significant difference between patients with and without DR in terms of average activity ($p = 0.63$), IS ($p = 0.47$), or IV ($p = 0.06$). L5 onset was not significantly different between healthy controls and patients with ($p = 0.55$) or without ($p = 0.99$) DR. Similarly, M10 onset was not significantly different between healthy controls and patients with ($p = 0.52$) or without ($p = 0.83$) DR.

3.2. Light exposure

There was no significant difference in the level of white, red, green, or blue light exposures during dark hours (ie, from 19:00 to 06:00) between healthy subjects and patients with or without DR (Table 3).

3.3. Sleep duration and sleep quality

On average, patients with diabetes slept 6.9 h (standard deviation [SD] = 0.7), whereas healthy controls slept 7.2 h (SD = 1.0); hence no significant difference between healthy controls and diabetes patients were found in terms of sleep duration ($p = 0.28$) (Table 4). We also did not find any significant difference between patients with and without DR ($p = 0.33$).

The average sleep efficiency was 84.1% (SD = 6.2) in healthy controls, 84.3% (SD = 7.4) in patients without DR, and 85.6% (SD = 5.7) in patients with DR (Table 3). Hence, no significant difference in the average sleep efficiency was registered between healthy controls and patients with or without DR. There was not any significant difference between healthy controls and patients with or without DR in other objective sleep parameters, ie, sleep onset latency or WASO ($p \geq 0.36$). In addition, PSQI and ESS scores were not significantly different between healthy controls and diabetic patients with or without DR.

4. Discussion

This is the first study to assess circadian rhythm and sleep quality using endogenous, objective, and subjective methods in diabetic patients with and without DR, along with healthy age-matched controls. Our results on daily variations in salivary melatonin showed reduced amplitude and peak levels in patients with and without DR, and fragmented circadian activity–rest rhythm only in patients with DR. However, neither the subjective nor the objective sleep qualities were affected in the diabetic patients.

The reduced nocturnal melatonin levels and fragmented sleep intervals could not be explained by light exposures, because the mean nocturnal light exposures (ie, from 19:00 to 06:00) were not significantly different between healthy controls and patients with or without DR. Moreover, the mean daytime melatonin level was not significantly different between healthy controls and patients with or without DR ($p \geq 0.57$), which further confirms that the significant difference in the melatonin levels during nighttime was independent of the ambient light exposure.

The circadian rhythm drives human behavior and physiological processes including diurnal insulin secretion, glucose metabolism, and eating/fasting patterns [33–35]. Circadian misalignment is associated with glucose intolerance, hyperinsulinemia, obesity, and diabetes [33,36–39]. Meanwhile, the circadian rhythm itself can be affected by many factors, of which light is the most important exogenous cue to entrain the internal biological clock [9]. Environmental light is detected by ipRGCs, which project directly to the master clock, the suprachiasmatic nucleus [SCN] [8,9]. Hence, diabetes-induced neuronal damage in the retinohypothalamic pathway or SCN might result in circadian misalignment, which in turn deteriorates glycemic control and DR.

Melatonin is a neurohormone that is secreted by the pineal gland. It is one of most important endogenous marker of the circadian rhythm, and its plasma concentration is regulated by SCN [40,41]. In our study, we used salivary melatonin concentration as an endogen marker of circadian rhythm, and showed reduced peak melatonin level and reduced mean nocturnal melatonin

Table 3
Light exposure presented as 98% confidence interval in healthy controls and patients with and without diabetic retinopathy (DR).

	Controls ($n = 21$)	Patients ($n = 54$)			
		Without DR ($n = 29$)	$p1$[a]	With DR ($n = 25$)	$p2$[a]
Actigraphy – light					
White light (lux)	591,825 (178,384; 1,005,265)	383,277 (148,918; 617636)	0.99	1,156,905.3 (314,116; 1,999,694)	0.88
Red light ($\mu W/cm^2$)	36,165 (15,942; 56389)	30,345 (15,497; 45194)	0.99	61,064 (22,076; 100051)	0.80
Green light ($\mu W/cm^2$)	56,050 (15,661; 96439)	34,697 (12,603; 56791)	0.99	111,125 (28,943; 19330)	0.90
Blue light ($\mu W/cm^2$)	19,256.38 (4964; 33,549)	11,297.0 (3437; 19,156)	0.99	44,426.1 (10,569; 78283)	0.89

[a] The p values correspond to comparison between healthy controls and patients without retinopathy ($p1$) and with diabetic retinopathy ($p2$).

Table 4
Sleep analysis presented as mean ± standard deviation for controls, overall diabetes patients, and patients categorized into with and diabetic retinopathy (DR).

	Controls ($n = 21$)	Patients			
		Without DR ($n = 29$)	$p1$[a]	With DR ($n = 25$)	$p1$[a]
Objective sleep assessment					
Sleep duration (h)	6.9 ± 0.7	7.0 ± 1.0	0.95	7.4 ± 0.9	0.23
Sleep efficiency (%)	84.1 ± 6.2	84.3 ± 7.4	0.99	85.6 ± 5.7	0.77
Sleep onset latency (min)	18.2 ± 15.1	22.3 ± 35.0	0.85	14.0 ± 9.4	0.86
WASO (min)	48.1 ± 21.9	40.6 ± 12.4	0.34	47.9 ± 17.4	0.36
Subjective sleep assessment					
PSQI global score	4.4 ± 2.4	5.3 ± 3.9	1.00	7.2 ± 4.5	0.054
ESS score	5.0 ± 2.5	5.6 ± 3.1	0.81	7.2 ± 3.6	0.06

ESS, Epworth Sleepiness Scale; PSQI, Pittsburgh Sleep Quality Index; WASO, wake after sleep onset.
[a] $p1$ corresponds to comparison between healthy controls and patients without DR, whereas $p2$ is the result of tests between healthy controls and patients with DR.

concentration in patients with and without nonproliferative DR. Previous studies have shown conflicting results [24–29]. For example, Hikichi et al., found reduced melatonin concentrations in patients with proliferative DR, but not in patients with nonproliferative DR [26]. Aydin et al., did not find any significant difference in plasma melatonin concentrations between healthy controls and patients with proliferative and nonproliferative DR [25]. The reason that the two studies did not find reduced melatonin level in patients with nonproliferative DR might be the low number of patients ($n = 16$ and 13), whereas, in our study, we had a larger study group with nonproliferative DR ($n = 25$) [25,26]. Moreover, Aydin et al., collected plasma melatonin samples during cataract surgery after 18:00, when artificial room light and increased adrenergic conditions during surgery might influence melatonin concentrations [42,43]. Plasma melatonin levels are usually three times higher than salivary melatonin concentrations [44], and although plasma melatonin measurement has greater sensitivity, blood sampling is not appropriate in an outpatient study [20]. In fact, salivary melatonin measurement is the preferred method for the assessment of melatonin level in outpatient settings [20].

Apart from being an important signaling molecule in the regulation of circadian rhythm throughout the body, melatonin plays an important role in the retina, both as an antioxidative molecule as well as a local modulator of retinal circadian rhythm [41,45,46]. Reduced melatonin level leads to disturbed circadian rhythm and a higher level of free radicals, both of which precipitate retinal damage in diabetic patients [41,45,46].

The circadian rhythm, evaluated using actigraphy in the current study, showed a higher fragmentation of activity–rest intervals, that is, increased IV in patients with DR but not in patients without DR. Although circadian misalignment in heart rate, body temperature, and blood glucose have been shown previously, no other study has investigated the relationship between circadian rhythm and DR in humans [47].

Previous studies, using pupillometry, have shown ipRGC damage in patients with DR [48,49]. Retinal damage due to diabetes may lead to the lack of entrainment of SCN to the environmental illumination level. One possible explanation for the disturbed oscillation in melatonin and cortisol levels and in the daily activity of diabetic patients is the decrease in the circadian regulation of gene expression, mainly in clock and clock-controlled genes as well as in dopamine signaling. In addition, it has been shown in a number of papers that there is a tight cross-talk between the molecular clock and the metabolism; and in those cases in which a disruption of the molecular circadian clock occurs, it causes metabolic syndrome, obesity, and type 2 diabetes, as well as higher risk for certain cancer types [47,50–54].

Recently it has been shown that sleep durations ≤ 5 and ≥ 9 h were associated with higher prevalence of diabetic retinopathy in male but not in female subjects [55]. An inverse relationship between sleep duration and fasting glucose, insulin resistance, adiposity, and BMI has been shown both in children and in adults [56,57]. In our study, we did not find any significantly difference in sleep duration between patients with or without DR and healthy controls. Previous studies have also investigated the quality of sleep, using subjective sleep quality assessment. In contrast, the strength of our study is that we used both subjective questionnaire and objective sleep quality assessment (with actigraphy), and did not find any difference between healthy controls and diabetic patients, with or without DR.

This study also has limitations. Although the patients had received detailed instructions on the use of actigraphy, the measurements were performed at the patients' homes in an uncontrolled environment; as such we did not control circadian pattern, light exposure, and physical activity. Another important limitation is that the Actiwatch measures activity rather than sleep, which means that motor activity during sleep due to other causes such as restless legs syndrome may be registered as activity, resulting in an underestimation of sleep [17]. Melatonin level is also prone to undesired changes such as environmental light level, activity, and dietary factors [58]. There was an individual in the group with DR who showed an unusual, seemingly outlying melatonin peak at 16:00, which most likely was due to a disturbed circadian rhythm but could also have been caused by the dimmed light level, reduced activity, or dietary factors.

5. Conclusion

In summary, circadian rhythm is disturbed in patients with diabetes, particularly in those with diabetic retinopathy. Clinical trials administering oral melatonin to volunteers may serve to improve synchronization and rhythmicity in diabetic patients.

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Conflict of interest

H.U. owns stocks in Novo Nordisk A/S and works in advisory boards for Novo Nordisk A/S and Abbott Inc. None of the other authors have any conflict of interest. We recruited our patients from Steno Diabetes Center, which is an outpatient clinic owned by Novo Nordisk. However, our results do not affect the treatment of diabetes patients, and our results were not affected by the company at all. No competing financial interests exist as part of the submission process.

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: https://doi.org/10.1016/j.sleep.2018.10.018.

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