Light Modulates Ocular Complications in an Albino Rat Model of Type 1 Diabetes Mellitus

Elias Andrawus1, Gizi Veildbaum1,2, Esther Zemel1,2, Rina Leibu3, Ido Perlman1,2, and Naim Shehadeh1,2,4

1 The Ruth and Bruce Rappaport Faculty of Medicine, Technion, Haifa, Israel
2 Rappaport Family Institute for Research in the Medical Sciences, Haifa, Israel
3 Department of Ophthalmology, Rambam Health Care Campus, Haifa, Israel
4 Department of Pediatrics A and the Pediatric Diabetes Unit, Rambam Health Care Campus, Haifa, Israel

Correspondence: Ido Perlman, The Ruth and Bruce Rappaport Faculty of Medicine, Technion, POB 9649, Efron Street, Haifa 31096, Israel. e-mail: iperlman@technion.ac.il

Received: 18 May 2016
Accepted: 3 April 2017
Published: 3 July 2017

Keywords: diabetes type 1; diabetic retinopathy; cataract; electroretinogram; light damage; glial fibrillary acidic protein (GFAP)


Copyright 2017 The Authors

Purpose: The purpose of the study was to assess potential interactions of light exposure and hyperglycemia upon ocular complications in diabetic rats.

Methods: Streptozotocin-induced (STZ-induced) diabetic rats (N = 39) and non-diabetic rats (N = 9) were distributed into eight groups according to the irradiance and color of the light phase during the 12/12-hour light/dark regime. Follow-up lasted 90 days and included assessment of cataract development and electroretinogram (ERG) recordings. Stress to the retina was also assessed by glial fibrillary acidic protein immunocytochemistry.

Results: Cataract development was fast in diabetic rats that were exposed to unattenuated white light or to bright colored lights during the light phase. Diabetic rats that were kept under attenuated brown or yellow light during the light phase exhibited slower rate of cataract development. Electroretinogram responses indicated very severe retinal damage in diabetic rats kept under bright colored lights in the blue-yellow range or bright white light during the light phase. Electroretinogram damage was milder in rats kept under bright red light or attenuated yellow or brown light during the light phase. Glial fibrillary acidic protein expression in retinal Müller cells was consistent with ERG assessment of retinal damage.

Conclusions: Attenuating white light and filtering out short wavelengths have a protective effect on the eyes of diabetic rats as evident by slower rate of cataract formation and a smaller degree of retinal damage.

Translational Relevance: Our findings suggest that special glasses attenuating light exposure and filtering out short wavelengths (400–530 nm) may be beneficial for diabetic patients.

Introduction

Ocular complications in patients suffering from diabetes mellitus are the leading causes of acquired visual loss in the working-age adults (20–70 years old) worldwide.1,2 Diabetes mellitus is one of the most common risk factors that has been linked with cataract formation.3,4 The diabetic-induced lens changes, which are indistinguishable from age-related cataracts in non-diabetic patients, tend to occur at a younger age. The pathogenesis of diabetes-induced cataract is not yet fully understood, but the role of the polyol pathway in the initiation of the disease has been extensively studied, and the use of aldose-reductase inhibitors and antioxidants has been proven beneficial in prevention and treatment of this condition.4

Although diabetic retinopathy (DR) is considered primarily a vascular phenomenon with alteration of the blood retinal barrier,5 recent works suggest that diabetic-related retinal pathology starts earlier and is expressed as reduced function of retinal neurons, probably due to hyperglycemia, as has been shown in a rat model of type 1 diabetes6,7 and in diabetic patients.8–10 Diabetic retinopathy progresses in an orderly fashion from mild (nonproliferative) stages to...
severe (proliferative) stages that can lead to blindness. Hyperglycemia is the major risk factor for diabetic-related ocular complications; however, controlling the glucose level can reduce the prevalence and progression of retinal vascular pathology but does not prevent it.\textsuperscript{11,12} Therefore, other factors probably also play a role in the development of diabetic cataract and DR.

Additional established stress that affects visual function and is encountered by every individual is exposure to light.\textsuperscript{13–15} The ocular optical system focuses the visual world onto the retina, exposing it to a wide range of radiation during daytime. UV-B and UV-C are absorbed by aromatic amino acids and nucleotides in the cornea and lens, whereas infrared radiation-B (IR-B) and IR-C are absorbed by water molecules and also do not pass the cornea. Therefore, radiation that reaches the retina includes mainly UV-A, visible, and IR-A. Radiation becomes harmful to ocular structures when it is absorbed by molecules, including heme proteins, flavoproteins, cytochrome c, melanin and lipofuscin in retinal pigment epithelial cells, and visual pigments in photoreceptors, leading to heat dissipation to surrounding tissue. The light energy and its rate of deposition (exposure time) determine the type of damage: mechanical, heat, or photochemical.\textsuperscript{14} Ultraviolet light is a risk factor for cataract development\textsuperscript{15,16} and retinal damage, while visible light, especially in the blue region of the spectrum, is regarded as the primary risk factor for retinal function.\textsuperscript{17}

In experimental animals, exposure to bright light for a short period of time (up to 24 hours)\textsuperscript{18} or daily exposure to high levels of light in a 12/12-hour light/dark cyclic regime\textsuperscript{19} can cause major reduction in visual function due to photoreceptor degeneration. Light-induced damage is a multifactorial pathology, depending upon the parameters of exposure to light (irradiance, wavelength, and duration), time of the day at which light exposure occurs, age, history of lighting conditions prior to exposure, rhodopsin content, and photoreceptor status with regard to the balance between generation of reactive oxygen species and antioxidants.\textsuperscript{18–21}

Since light has been discovered as a potential harmful factor to the retina, several studies have tested potential links between light exposure and retinal diseases. Retinitis pigmentosa is an inherited disease causing photoreceptor degeneration, leading eventually to blindness. It has been hypothesized that exposure to light might speed up the rate of photoreceptor degeneration.\textsuperscript{22} However, a 5-year experimental trial in two patients did not support this hypothesis.\textsuperscript{23} In glaucoma, researchers suggested that blue light might be a risk factor through the generation of reactive oxygen intermediates following light absorption by cytochrome c oxidase in mitochondria occupying the axons of retinal ganglion cells.\textsuperscript{24} Age-related macular degeneration is a macular disease leading to central scotoma and reduced visual acuity. It is a multifactorial disease, with aging being the major factor, but exposure to light also has been suggested as a contributing factor.\textsuperscript{25,26}

As discussed above, DR is believed to develop due to two major risk factors. In the initial stages, hyperglycemia is the prominent risk factor leading to reduced function of retinal neurons, while in more advanced stages, hypoxia due to compromised retinal vascular system becomes the major risk factor. It was therefore hypothesized that high energy usage by the rod photoreceptors under dark-adapted conditions exacerbated retinal hypoxia, thus speeding up the development of DR.\textsuperscript{27} Accordingly, the use of nocturnal dim light during night sleep to prevent dark adaptation was suggested to reduce the energy needs of the rod photoreceptors and thus to slow down the development of DR.\textsuperscript{28} In fact, a phase III clinical trial is now underway to test the effects of using specialized goggles equipped with dim light during night sleep on development of early diabetic edema.\textsuperscript{29}

Since oxidative stress has been linked to DR\textsuperscript{30,31} and to light-induced photoreceptor damage,\textsuperscript{19,21} we hypothesized that stress to retinal cells by hyperglycemia, as is the case in diabetes, and stress to retinal cells by light exposure can interact to cause more damage than expected from a simple summation of the effects of each of these two stress factors alone. To test this hypothesis, we kept groups of STZ-induced diabetic albino rats and control albino rats under 12/12-hour light/dark cyclic regime, in which the composition of light during the light phase varied between the groups, and assessed cataract formation and retinal function.

### Methods

#### Animals

Forty-eight male, approximately 6-weeks-old, albino Sprague-Dawley (SD) rats (Harlan Laboratories Ltd, Jerusalem, Israel), weighting 120 to 150 g, were used in this study. After delivery, the rats were allowed a period of 1 week to adjust to the new environment. Each group of rats was randomly divided into four subgroups, with 12 rats in each subgroup. After 1 week of acclimatization, diabetic rats were induced with streptozotocin (STZ) intravenously (25 mg/kg body weight) dissolved in citric acid (pH 4.5) and control rats were injected with citric acid alone. The rats were then allowed 1 week to adjust to the new environment and were then housed in a 12/12-hour light/dark cyclic regime. The rats were subjected to light exposure for 4 weeks starting on the 1st day after delivery, and the light exposure was continued for 5 days a week for 4 weeks. The rats were divided into four groups: control + light, diabetic + light, diabetic + dark, and control + dark. The control + light group was exposed to light for 4 weeks, the diabetic + light group was exposed to light for 4 weeks, the diabetic + dark group was exposed to light for 4 weeks, and the control + dark group was exposed to light for 4 weeks. The rats were housed in individual cages and were provided with water and food ad libitum. The animals were weighed weekly and their weight was recorded. The rats were anesthetized with ketamine (100 mg/kg body weight) and xylazine (10 mg/kg body weight) and their eyes were examined using slit lamp biomicroscopy (Zeiss, Jena, Germany) to assess cataract formation and retinal function.

The retinal function was assessed using multifocal electroretinography (mERG). The mERG was recorded using a charge-coupled device (CCD) camera (Diagnosys, Lowell, MA) and a 12-bit digitizer (National Instruments, Austin, TX). The mERG was recorded using a 3-mm saline drop applied to the cornea. The stimulus was a 15-Hz flickering pattern with a frequency of 15 Hz and an intensity of 300 cd/m². The mERG was recorded from the light-exposed and non-light-exposed eyes of the rats.

The rats were then sacrificed by cervical dislocation and the eyes were removed. The retina was isolated and processed for histological examination. The retina was fixed in 4% paraformaldehyde and embedded in paraffin. The retina was sliced and stained with hematoxylin and eosin (H&E) for histological examination. The retinal thickness was measured using an image analysis system (Zeiss, Jena, Germany). The results were expressed as the mean ± standard deviation (SD). The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey’s post-hoc test. A p-value < 0.05 was considered statistically significant.
environment. During this period, the rats were maintained on a standard diet and water ad libitum, under environmental temperature of 24°C and humidity of 60%. Lighting conditions consisted of 12/12-hour light/dark cyclic regime where white light of 350-lux illuminance was used during the light phase. For the induction of diabetes and for ERG recording, rats were anesthetized with intramuscular injection (0.5 mL/kg body weight) of a mixture composed of ketamine hydrochloride (100 mg/mL), acepromazine maleate (10%), and xylazine (2%) in a ratio of 0.3:0.2:1. At termination of the follow-up period, rats were euthanized by an overdose (80 mg/kg body weight) of sodium pentobarbital injected intraperitoneally, and the eyes were enucleated in order to prepare the retinas for immunocytochemistry.

Rats were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and according to Israeli and institutional guidelines.

**Induction of Diabetes**

One week after receiving the rats and getting them acclimated to the new environment, 39 rats were rendered diabetic by intraperitoneal injection of STZ at a dose of 60 mg/kg body weight after overnight fasting. This drug is toxic to insulin-secreting pancreas cells and is a widely accepted animal model for type 1 diabetes. Blood glucose was measured in nonfasting rats with a glucometer (ACCU-CHEK Performa Nano; Roche, Germany). Diabetes was confirmed from blood glucose measurement 72 hours after STZ injection and again 1 week later. A rat was labeled as diabetic if blood glucose level exceeded 300 mg/dL. Control rats had blood glucose level around 80 mg/dL. Blood glucose was monitored once a week in all the rats throughout the entire period of follow-up.

**Cataract Assessment**

A trained ophthalmologist, unaware of our working hypothesis and the lighting conditions in which rats were maintained, was responsible for assessment of cataract formation. For cataract assessment, the eye was illuminated with white light, and the magnitude of opaqueness, its density, and location were evaluated. Quantitative cataract assessment ranged from 0 (no cataract) to 5 (advanced mature cataract) according to a previous report.

**Immunocytochemistry**

Glial fibrillary acidic protein (GFAP) is normally expressed in astrocytes that are located in the retina–vitreous border around blood vessels, but not in retinal Müller cells. However, following retinal stress of any kind, Müller cells are activated as evident by induction of GFAP expression. Therefore, GFAP expression in retinal Müller cells serves as a sensitive molecular marker for retinal pathology.

The enucleated eyes of the rats were fixed with paraformaldehyde, washed in 0.1 M PBS and then cryoprotected with escalating sucrose overnight. Retinal cryosections of 16-μm thickness were labeled with primary GFAP antibody (monoclonal mouse anti-rat GFAP antibody) and were washed three times with PBS. The sections were then incubated with the secondary antibody (sheep anti-mouse antibody attached to fluorescein isothiocyanate) as a fluorescent indicator to identify GFAP. The same retinal sections were also incubated with 4',6-diamidino-2-phenylindole (DAPI) (1 mg/mL) at 1:1000 PBS to stain cell nuclei in order to identify the different retinal layers and to localize the sites of GFAP expression.

**Electroretinogram (ERG)**

The ERG measures the electrical response of the retina to a light stimulus. It is composed of two major waves, the negative a-wave reflecting light-evoked electrical activity in the photoreceptors and the positive b-wave that is generated by electrical activity in second-order neurons, mainly on-center bipolar cells. Therefore, the ERG is widely used to assess function of the distal retina, including photoreceptors, bipolar cells, and synaptic transmission between them.

Before ERG recordings, the rats were kept overnight in total darkness for dark adaptation. The pupils were fully dilated (cyclopentolate hydrochloride 1%) and topical anesthesia (benoxinate HCl 0.4%) was administered in order to prevent any potential discomfort. A heating pad was used to maintain normal body temperature.

The ERG responses were recorded simultaneously from both eyes using contact lens-type corneal electrodes (Medical Workshop, Groningen, The Netherlands). A drop of methylcellulose (cellospan, Dr. Fischer, Israel) was applied to maintain corneal hydration and to ensure electrical contact. Surgical needles, inserted into the ears, served as reference and ground electrodes. The ERG responses were recorded.
in response to Ganzfeld white-light stimulations of different strength, which were controlled by the data acquisition system.

The ERG responses were amplified by a factor that depended upon the amplitude of the ERG (40K, 10K, and 4K for small-, intermediate-, and large-amplitude ERGs, respectively) and were filtered (1–500 Hz). The amplified and filtered ERGs were digitized (2 kHz), and averaged by the data acquisition system (UTAS 3000; LKC Technologies, Gaithersburg, MD). Several responses (4–10), elicited by identical light stimuli, were delivered at different interstimulus intervals (2–30 seconds) depending upon stimulus strength, were recorded, and were averaged to improve signal-to-noise ratio. The parameters for ERG averaging (number of repeated stimuli and interstimulus intervals) were similar to those recommended by the International Society for Clinical Electrophysiology of Vision ERG guidelines.37

For ERG analysis, we measured the amplitude of the a-wave from the baseline to the trough of the wave, and the amplitude of the b-wave was determined from the trough of the a-wave to the peak of the b-wave. In order to assess the combined damage of hyperglycemia and light exposure, we compared the maximal amplitude of the dark-adapted ERG b-wave of the diabetic rats kept under different light/dark conditions to corresponding values recorded from control, non-diabetic rats kept under the same lighting conditions.

Research Plan

Diabetic and control rats were kept in specially designed chambers with efficient circulation of air under 12/12-hour light/dark cycle using different light composition for the light phase of the cycle, as listed in Table 1. Rats’ cages were placed on one shelf having the same distance from the light, which was composed of several identical sources spread evenly above the cages.

There were eight groups of diabetic (experimental) rats and three groups of healthy, non-diabetic (control) rats. In compartments 1, 2, and 3, illumination during the light phase of the cycle was unattenuated white light, attenuated brown light, or attenuated yellow light, respectively. Three diabetic rats and three non-diabetic healthy rats were kept in each of these compartments for comparison. Thus, for each specific level and color of illumination in compartments 1, 2, and 3, the healthy, non-diabetic rats served as control for the diabetic rats. In order to assess the effects of changing the illumination regime upon retinal function, we compared the diabetic rats between the three compartments and the control rats between the three compartments. The spectral properties of the filters used in compartments 2 and 3 are shown in Figure 1. The yellow filter transmits only 10% of light at wavelengths below 490 nm and then starts to transfer more for longer wavelengths: 50% transmission for 580 nm and 80% transmission for wavelengths longer than 625 nm. The brown filter (Fig. 1) transmits minimally (less than 10%) at wavelengths below 530 nm. At 580 nm the filter transmits only 20% of the light and at 670 nm, only 40% of the light. Maximal transmission of this filter in the visible range is less than 70% for 690 nm. The goal of this experiment was to test the effects of broadband filters and reduced irradiance on retinal function of non-diabetic and diabetic albino rats in comparison to unattenuated white light.

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Dominant Color, Wavelength</th>
<th>Irradiance, mW/m²</th>
<th>Illuminance, Lux</th>
<th>Number of Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>White</td>
<td>1500–2000</td>
<td>2600–3500</td>
<td>3 diabetics + 3 normal</td>
</tr>
<tr>
<td>2</td>
<td>Brown filter (Fig. 1)</td>
<td>1000–1500</td>
<td>1500–2300</td>
<td>3 diabetics + 3 normal</td>
</tr>
<tr>
<td>3</td>
<td>Yellow filter (Fig. 1)</td>
<td>1000–1500</td>
<td>2000–3000</td>
<td>3 diabetics + 3 normal</td>
</tr>
<tr>
<td>4</td>
<td>Violet (440–460 nm)</td>
<td>1500–2000</td>
<td>1160–1550</td>
<td>6 diabetics</td>
</tr>
<tr>
<td>5</td>
<td>Blue (460–490 nm)</td>
<td>1500–2000</td>
<td>1875–2500</td>
<td>6 diabetics</td>
</tr>
<tr>
<td>6</td>
<td>Green (490–520 nm)</td>
<td>1500–2000</td>
<td>2526–3369</td>
<td>6 diabetics</td>
</tr>
<tr>
<td>7</td>
<td>Cyan (520–550 nm)</td>
<td>1500–2000</td>
<td>1863–2484</td>
<td>6 diabetics</td>
</tr>
<tr>
<td>8</td>
<td>Red (630–655 nm)</td>
<td>1500–2000</td>
<td>3.8–5.1</td>
<td>6 diabetics</td>
</tr>
</tbody>
</table>

a The range of irradiance (illuminance) in the compartment reflects measurements at different locations within the compartment.
In each of the other five compartments (4–8), we kept six diabetic rats and no healthy rats. Colored light-emitting diodes (LEDs) of specific wavelength determined the irradiance and color of the illumination in these compartments during the light phase (Table 1).

The irradiances in the white-, brown- and yellow-light compartments (1, 2, 3) were adjusted by adding neutral density filters, and the irradiance in the colored light compartments (4–8) was adjusted by the current to the LEDs. The irradiance in all the compartments, in watts per square meter, was measured with a handheld laser power meter (Edmund Optics, Barrington, NJ), and the illuminance was measured in lux only in the unattenuated white light and attenuated brown and yellow lights using a multifunctional light meter (Starlite; Gossen, Nürnberg, Germany). The illuminance in the colored light chambers was estimated from the known irradiance, the dominant wavelength, and the watt-to-lumen conversion factor for that wavelength. We used the human scotopic conversion factors table because the rat is a night animal with rods having an action spectrum similar to that of human scotopic vision. Since illuminations in the compartments were not exactly uniform, a range of irradiance and illuminance values are given in Table 1.

It should be noted that the illuminance in all the compartments during the light phase of the light/dark cyclic regime was considerably lower than that measured in the shade of an average Israeli summer day (6000 lux) and about three to four times higher than the illuminance in a typical lighted office room (500–900 lux).

Starting at 3 weeks after induction of diabetes, development of cataract was assessed weekly by an experienced ophthalmologist who was not aware of our working hypothesis. Retinal function was assessed from the ERG responses that were recorded at day 42 and day 90 after induction of diabetes in order to assess diabetes-induced reduction in retinal function and its susceptibility to the conditions of illumination. At termination of the follow-up period, the rats were euthanized using an overdose (80 mg/kg body weight) of sodium pentobarbital injected intraperitoneally, the eyes were enucleated, and the retinas were prepared for immunocytochemistry.

Results

Cataract Development

Figure 2 summarizes the development of cataract in diabetic rats that were kept under 12/12-hour...
light/dark cyclic regime in which the light phase was of similar irradiance (1500–2000 mW/m²), but the peak wavelength differed in the range between 400 nm and 700 nm. Cataract was rated from the opacity of the lens on a scale from 0 to 5, based upon previously published parameters. Minimal signs of cataract could be detected about 3 weeks after induction of diabetes, and cataract continued to develop with time, almost linearly, until leveling off toward the end of the follow-up period. All diabetic rats, subjected to bright colored light (compartments 4–8) during the light phase developed fully opaque cataract (rated 5) within 90 days from induction of diabetes (Fig. 2).

We also raised diabetic rats under illumination conditions in which the light phase was either unattenuated white light (1500–2000 mW/m²) or attenuated light by about 30% (1000–1500 mW/m²) with a broadband filter that also limited the wavelength composition of the light; one compartment was illuminated with brown light (compartment 2) and the other with yellow light (compartment 3). Figure 3 shows fast cataract development in the diabetic rats kept under unattenuated white light during the light phase (compartments 4–8) during the light phase; the degree of cataract in all eyes (N = 6 for three rats) reached a score of 5, indicating dense cataract of the entire lens within 90 days of induction of diabetes.

Figure 3. Development of cataract in diabetic (filled symbols) and non-diabetic healthy control (empty symbols) albino rats that were kept under identical conditions of illumination; unattenuated white light (blue squares), attenuated brown light (brown circles), attenuated yellow light (yellow triangles). The grading scale of cataract is modified after a previous study by Taylor et al.32

Figure 4 shows bright (I = 2.5 cd-s/m²) flash ERG responses that were recorded 42 days after the induction of diabetes from one eye of control albino rats and one eye of diabetic albino rats (upper and lower rows, respectively) that were kept under similar light conditions. Large differences can be observed between the diabetic rats and the control rats for each condition of illumination, being largest for unattenuated white light and smallest for attenuated brown light (Fig. 4, first and second columns, respectively). With attenuated yellow light during the light phase, the control versus diabetic rat differences were intermediate (Fig. 4, third column).

We recorded the ERG responses from each rat (diabetic or control) using light stimuli of different strengths in order to construct the response–log stimulus strength relationship and to derive the maximal response amplitude of the dark-adapted ERG a-wave and b-wave. However, in many rats, the ERG responses were severely depressed, and we could only use the response to the brightest light stimuli as a measure of the maximal response amplitudes.

The dark-adapted ERG responses of the diabetic rats that were kept for 42 days in conditions of bright colored lights during the light phase of the light/dark cycle (compartments 4–7, Table 1) were nonmeasurable and therefore are not discussed here. Figure 5 shows the ERG data of the six diabetic rats that were kept under bright (1500–2000 mW/m²) red (630–655 nm) light during the light phase of the light/dark cycle.
in compartment 8 (Table 1). These diabetic rats suffered a moderate degree of retinal damage as evident by maximal ERG responses that were reduced by about 50\% compared to mean maximal ERG b-wave of about 500 \(\mu V\) that had been measured in diabetic rats kept in normal laboratory conditions (350-lux white light) during the light phase.\(^6\)

In the compartments where the light cycle was either unattenuated (1500–2000 mW/m\(^2\)) white light or filtered and attenuated (1000–1500 mW/m\(^2\)) brown or yellow light, we kept control rats (\(N = 3\)) and diabetic rats (\(N = 3\)) in each compartment for comparison. The ERG responses of the diabetic and non-diabetic healthy control rats in these compartments were measured twice; once on day 42 and again at termination of the experiment, on day 90. The data for the ERG records on day 42 are summarized in Figure 6. Control rats, kept under unattenuated white light, exhibited moderate to severe light-induced retinal damage of about 70\% ERG deficit compared to our experience with control rats kept under normal laboratory conditions,\(^6\) while the diabetic rats in the same compartment exhibited very severe retinal damage, as indicated by nonrecordable ERG responses. Light-induced retinal damage progressed with time, and when the ERG responses were recorded again after additional 48 days (total of 90 days), they were too small to be measured in the control rats also, indicating very severe light-induced retinal damage (data not shown here). The attenuated brown light or yellow light caused less damage to the retinas of the control rats and diabetic rats, as can be assessed from the ERG data in Figure 6. This is expected from previous studies on factors affecting light damage to the albino rat retina\(^18,20,21\) because the irradiance of the light was attenuated and the wavelength composition was dominated by long-wavelength light. The ERG responses of the diabetic rats that were kept in the brown-light compartment were less affected compared to the diabetic rats kept in the yellow-light compartment, while the ERG responses of the control rats were better preserved in the yellow-light compartment compared to the brown-light compartment (Fig. 6). However, the small number of rats in each group (three diabetic and three control) and the variability in the ERG responses did not allow reliable statistical tests between the different groups. When the ERG responses were measured 48 days later (90 days after induction of diabetes), the ERG responses of the diabetic rats showed further progression of light damage (not shown here).
GFAP Immunocytochemistry

At termination of the follow-up period (90 days) following the ERG recording session, the rats from compartments 1, 2, and 3 were euthanized; the eyes were enucleated, and the retinas were processed for GFAP immunocytochemistry. Figure 7 shows representative retinal sections of six rats (three controls and three diabetic) that were kept in compartments 1, 2, and 3. In all retinas, GFAP immunoreactivity is seen in astrocytes, located at the retinal surface, just above the ganglion cell layer that normally expresses GFAP. Expression of GFAP in Müller cells is seen in the three retinas from the diabetic rats (Fig. 7, right column), appearing as red radial structures, indicating retinal gliosis, probably secondary to retinal damage. The degree of GFAP expression in Müller cells varies between the different retinas, probably signaling different degrees of retinal damage. The most apparent GFAP expression in Müller cells is seen in the retina of the diabetic rat that was kept in attenuated brown light (Fig. 7D) compared to the one raised in the compartment where the light phase consisted of unattenuated yellow light (Fig. 7F). The retinal micrograph from the diabetic rat kept under unattenuated brown light (Fig. 7D) showed lesser degree of GFAP expression in Müller cells. In all retinal micrographs, which were obtained from healthy non-diabetic control rats that were raised under the same lighting condition as the diabetic rats, GFAP expression in Müller cells was low (Figs. 7A, C, E).

Observations similar to those shown in Figure 7 were seen in all the retinas tested for GFAP immunocytochemistry. In order to get a quantitative measure of GFAP expression in Müller cells, we graded the magnitude of GFAP expression in the retinas of all the rats (control and diabetic) that survived the 90 days of follow-up as follows: 0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining. The average score for the rats in the different compartments is summarized in Table 2.

Discussion

We tested in this study the working hypothesis that two, seemingly unrelated stressful factors, hyperglycemia and bright light, can interact in the eye to produce more damage than expected from summation of the effects of each stress alone. In order to test this hypothesis, we kept diabetic rats and non-diabetic healthy control rats under 12/12-hour light/dark cyclic regime where the 12-hour light phase differed in irradiance and wavelength composition (Table 1).

Our findings support the working hypothesis. The ERG responses of diabetic rats that were kept for 42 days under 12/12-hour light/dark cycle where the light phase consisted of bright (1500–2000 mW/m²) white light indicate severe deterioration of retinal function to a degree that the ERG responses were not recordable. Also, the control rats, which were studied in parallel to the diabetic rats, suffered from light-
Figure 7. Immunocytochemistry (red, GFAP; blue, DAPI) of representative retinal micrographs from non-diabetic healthy control rats (A, C, E) and diabetic rats (B, D, F). Each pair of micrographs, in each row, was obtained from a control rat and a diabetic rat that were kept under similar light conditions as indicated to the left of each row. Retinal layers are indicated in each micrograph: ONL, outer nuclear layer; INL, inner nuclear layer. Calibration bar in each micrograph has a length 100 μm except for micrograph (B) where the calibration bar has a length of 50 μm.
attributed to the use of bright (1500–2000 mW/m²) light during the 12-hour light phase of the illumination suffered a moderate degree of retinal damage (Fig. 5), about 50% compared to diabetic rats that were kept in normal laboratory conditions.6 These findings are consistent with previous reports on the spectrum of light-induced retinal damage.17 Some studies showed that light-induced retinal damage had an action spectrum similar to that of rhodopsin and argued that light absorption by rhodopsin in rod photoreceptors was the step leading to light-induced retinal damage. Others supported the short-wavelength action spectrum theory showing that light-induced retinal damage reduced monotonically from short- to long-wavelength light. Regardless of the exact theory, red light at 630–655 nm is expected to be considerably less effective in inducing retinal damage, as was found here for diabetic rats (Fig. 5).

The effects of filtering and attenuating the white light on retinal function of diabetic rats is also consistent with either of the above theories regarding the action spectrum of the light-induced retinal damage.17 We found that attenuating the bright white light with either a brown filter or a yellow filter partially protected the diabetic rat retina from light-induced damage, with the brown filter being more effective compared to the yellow filter (Fig. 6). The brown filter and the yellow filter used here were similar in irradiance, but differed in the wavelength composition of the transmitted light (Fig. 1). The yellow filter transmitted more light in the short- and medium-wavelength range of the spectrum compared to the brown filter (Fig. 1) and therefore caused a more severe retinal damage (Fig. 6). Furthermore, the light illuminance (in lux) of the yellow and brown lights were higher than that in the violet-light compartment and similar to that in the blue-light compartment (Table 1), yet retinal damage, as assessed from the ERG responses, was considerably smaller. Thus, our findings indicate that illuminance level and wavelength composition are the important factors in light damage for diabetic rats.

Another sight-threatening diabetic complication is the early formation of cataract that is caused mainly by hyperglycemia that activates the sorbitol metabolic pathway in the cells of the lens, producing products that cannot diffuse out of the cells.38 As a result, intracellular osmolarity increases, leading to water influx and cataract formation. However, the level of oxidative stress and the irradiance and spectral composition of light exposure are also known

### Table 2. Average Value for Assessment of GFAP Expression in the Retinas of Rats Kept under the Same Light/Dark Conditions as Assessed from the Degree of GFAP Staining: 0, No Staining; 1, Weak Staining; 2, Moderate Staining; 3, Strong Staining

<table>
<thead>
<tr>
<th>Group</th>
<th>Average Grade of GFAP Immunoreactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic control rats in unattenuated white light</td>
<td>1.5</td>
</tr>
<tr>
<td>Diabetic rats in unattenuated white light</td>
<td>2.67</td>
</tr>
<tr>
<td>Non-diabetic control rats in attenuated brown light</td>
<td>0.5</td>
</tr>
<tr>
<td>Diabetic rats in attenuated brown light</td>
<td>1.5</td>
</tr>
<tr>
<td>Non-diabetic control rats in attenuated yellow light</td>
<td>1.0</td>
</tr>
<tr>
<td>Diabetic rats in attenuated yellow light</td>
<td>2.33</td>
</tr>
</tbody>
</table>

induced retinal damage, but to a considerably lesser extent, and exhibited about 70% ERG damage (Fig. 6). The cataracts in diabetic rats cannot account for these ERG differences between the diabetic and control rats. At day 42 post diabetes induction, the day of ERG recording, the cataract score was ~1.5 in the diabetic rats and ~0.5 in the control rats (Fig. 3). This difference is too small to produce significant differences in the light intensity reaching the retina to account for the ERG amplitude differences.

We found previously that diabetic rats kept under regular laboratory conditions, 350-lux white light in the light phase of the cyclic light/dark regime, exhibited 20% to 30% reduction in the ERG responses, and therefore we suggest that 12-hour exposure to bright white light during the light/dark cycle plus hyperglycemia induces a more severe retinal damage than the summation of the effects of each stressful factor alone.

Our attempt to determine the spectral sensitivity of light damage in diabetic rats failed, since all diabetic rats kept under purple (440–460 nm), blue (460–490 nm), green (490–520 nm), or cyan (520–550 nm) light during the 12-hour light phase exhibited nonrecordable ERG responses when measured 42 days after induction of diabetes. This result is attributed to the use of bright (1500–2000 mW/m²) colored lights containing mainly medium and short wavelengths. In contrast, diabetic rats kept under red (630–655 nm) light of the same irradiance (1500–2000 mW/m²), but considerably lower illuminance (3.8–5.1 lux), during the 12-hour light phase of the illumination suffered a moderate degree of retinal damage (Fig. 5), about 50% compared to diabetic rats that were kept in normal laboratory conditions.6 These findings are consistent with previous reports on the spectrum of light-induced retinal damage.17

<table>
<thead>
<tr>
<th>Group</th>
<th>Average Grade of GFAP Immunoreactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic control rats in unattenuated white light</td>
<td>1.5</td>
</tr>
<tr>
<td>Diabetic rats in unattenuated white light</td>
<td>2.67</td>
</tr>
<tr>
<td>Non-diabetic control rats in attenuated brown light</td>
<td>0.5</td>
</tr>
<tr>
<td>Diabetic rats in attenuated brown light</td>
<td>1.5</td>
</tr>
<tr>
<td>Non-diabetic control rats in attenuated yellow light</td>
<td>1.0</td>
</tr>
<tr>
<td>Diabetic rats in attenuated yellow light</td>
<td>2.33</td>
</tr>
</tbody>
</table>

...
In our experiments, cataract development in diabetic rats depended mainly upon the state of hyperglycemia, but also reflected the irradiance of the light phase during the light cycle. Cataract formation was fast in diabetic rats raised under bright (1500–2000 mW/m²) white, purple, blue, green, cyan, or red light during the light cycle and was slowed in diabetic rats kept under attenuated brown light or attenuated yellow light by about 2 weeks (Figs. 2, 3). These observations suggest that diabetic cataract formation is exacerbated by high level of irradiance and not on illuminance or spectral composition.

In summary, our findings are consistent with the hypothesis that diabetic cataract is caused mainly by the hyperglycemic state but is exacerbated by light exposure, with irradiance being the important factor. In contrast, retinal damage is caused mainly by exposure to light, with illuminance and spectral composition being the important factors, and the hyperglycemic state in diabetes exacerbated light-induced damage. Therefore, the best way to protect the eyes from light-induced damage is to use glasses with appropriate lenses that attenuate light irradiance and filter out short- and medium-wavelength light can be beneficial to diabetic patients at risk of developing cataract and DR.

Acknowledgments

Supported in part by a grant from Diabetes Good View Company, Nazareth, Israel.

Disclosure: E. Andrawus, None; G. Veildbaum, None; E. Zemel, None; R. Leibu, None; I. Perlman, (I, C, P); N. Shehadeh, (I, C, P)

References

13. Sacca SC, Roszkowska AM, Izzotti A. Environmental light and endogenous antioxidants as the


