Oxidative Stress and Light-Evoked Responses of the Posterior Segment in a Mouse Model of Diabetic Retinopathy

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PURPOSE. To test the hypothesis that in a mouse model of diabetic retinopathy, oxidative stress is linked with impaired light-evoked expansion of choroidal thickness and subretinal space (SRS).

METHODS. We examined nondiabetic mice (wild-type, wt) with and without administration of manganese, nondiabetic mice deficient in rod phototransduction (transducin alpha knockout; *GNAT1*^{−/−}), and diabetic mice (untreated or treated with the antioxidant α -lipoic acid [LPA]). Magnetic resonance imaging (MRI) was used to measure light-evoked increases in choroidal thickness and the apparent diffusion coefficient (ADC) at 88% to 100% depth into the retina (i.e., the SRS layer).

RESULTS. Choroidal thickness values were similar ($P > 0.05$) between all untreated nondiabetic dark-adapted groups and increased significantly ($P < 0.05$) with light; this expansion was subnormal ($P < 0.05$) in both diabetic groups. Apparent diffusion coefficient values in the SRS layer robustly increased ($P < 0.05$) in a light duration-dependent manner, and this effect was independent of the presence of Mn²⁺. The light-stimulated increase in ADC at the location of the SRS was absent in *GNAT1*^{−/−} and diabetic mice ($P > 0.05$). In diabetic mice, the light-dependent increase in SRS ADC was significantly ($P < 0.05$) restored with LPA.

CONCLUSIONS. Apparent diffusion coefficient MRI is a sensitive method for evaluating choroid thickness and its light-evoked expansion together with phototransduction-dependent changes in the SRS layer in mice in vivo. Because ADC MRI exploits an endogenous contrast mechanism, its translational potential is promising; it can also be performed in concert with manganese-enhanced MRI (MEMRI). Our data support a link between diabetes-related oxidative stress and rod, but not choroidal, pathophysiology.

Keywords: MRI, retina, choroid, diffusion, choroidal thickness, phototransduction, water

Diabetic retinopathy (DR), a leading cause of vision loss and blindness in patients under the age of 45, is clinically managed based on retinal microcirculation abnormalities. Accumulating evidence has implicated retinal oxidative stress as an important pathogenic factor early in the course of diabetes.^{1,2} Importantly, the major cell type contributing to this oxidative stress is not the endothelial cell, but dysfunctional rod photoreceptors.^{3–6} This newly identified pathogenic role of the rod photoreceptor cell in early DR raises questions about a possible role of the choroid, the essential circulation supporting photoreceptor cells and retinal pigment epithelium located posterior to the retinal pigment epithelium (illustrated in the cartoon in Fig. 1D). In fact, there have been reports of choroidal thinning in diabetes, although it remains unclear whether or not this is due to impaired choroidal vasodilation in diabetes, and/or choroidal injury is linked with diabetes-induced rod-dominated retinal oxidative stress.^{7–18} Few methods are presently available for answering these questions because current imaging methods cannot study both rod function and choroidal anatomy/function at the same time and spatial location in full darkness in a common animal model of DR.

In this study, we developed and applied a noninvasive imaging approach for addressing this gap. Our approach was based on high spatial resolution diffusion-weighted magnetic resonance imaging (MRI) measurements of the apparent diffusion coefficient (ADC) within the retina. Apparent diffusion coefficient MRI has been used to measure choroidal thickness in the rat.¹⁹ Here, we investigate the potential of ADC MRI for monitoring the expansion of choroidal thickness with light, an effect observed in other species but not yet in the mouse.^{20–23} Apparent diffusion coefficient MRI has also been reported to be useful for measuring light-stimulated increases in the subretinal space layer (SRS layer) in rats.¹⁹ The retina is the tissue bounded by the vitreous anteriorly and choroid posteriorly. Within the retina is the SRS, which contains the extracellular fluid around the photoreceptor outer segments and is located at 88% to 100% depth into the retina posterior to the outer limiting membrane (i.e., the end-feet of the Müller cells) and anterior to the retinal pigment epithelium (illustrated in the cartoon in Fig. 1D). Previous microelectrode studies observed that the SRS volume is substantially smaller in dark than in light as a consequence of light-dependent changes in

FIGURE 1. Choroidal thickness analysis outline and ADC profiles in dark-adapted wt mice. (A) Representative image of a mouse eye showing central retinal regions of interest (*white boxes*). (B) Representative flattened central retinal region from anatomic (*left*) and averaged (b100–b990) ADC MRI data (*right*) illustrating greatly reduced signal intensity in the choroidal region. For simplicity, boundaries are illustrated on the anatomic and ADC images (*dotted lines*); objectively determined boundaries were used for actual evaluation of thicknesses as described in Methods. Choroidal thickness was objectively measured as the difference in thickness between the anatomic and averaged ADC data (for details see Methods). (C) Summary of dark-adapted choroidal thickness from five separate groups (Grp) of mice (mean $\pm$ SEM) analyzed as in (B). (D) Summary of central retinal ADC profiles in five groups of dark-adapted wt mice suggests reasonable topographical precision (i.e., reproducibility of the magnitude and spatial features of the profile). Approximate locations of retinal layers (INL, inner nuclear layer; ONL, outer nuclear layer; IS, inner segments; OS/SRS, outer segments and subretinal space; and choroid) are indicated by *dotted vertical blue lines* and the cartoon at the *top* that was modified from a previous study.⁶¹ Profiles are spatially normalized to dark-adapted retinal thickness (0%, vitreous/retina border; 100%, retina/choroid border).

extracellular ion content and thus represents a biomarker of the rod and retinal pigment epithelium unit function.^{24–27} Our working hypotheses are that (1) in nondiabetic mice, ADC MRI will be sensitive to light-evoked expansion of the choroidal thickness and SRS layer; (2) in diabetic mice, light-dependent changes in choroidal thickness and SRS layer will be subnormal^{3,4,28–31}; and (3) antioxidant treatment will correct light-stimulated choroidal and SRS pathophysiology associated with diabetes.

METHODS

All animals were treated in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and Institutional Animal and

Care Use Committee authorization. Animals were housed and maintained in 12 hour:12 hour light:dark cycle laboratory lighting, unless otherwise noted.

Groups

We investigated the following groups (summarized in the Table): nondiabetic 3- to 6-month male C57Bl/6J mice (with and without manganese administration) (wild-type [wt]; Jackson Laboratories, Bar Harbor, ME, USA); nondiabetic male or female α -transducin-1 (T α) knockout mice (*GNAT1*^{−/−}) (kind gift from Janis Lem, Tufts University)^{32–34}; and 2- to 3-month diabetic male C57Bl/6J mice (untreated or treated with the antioxidant α -lipoic acid [LPA]). α -Lipoic acid (50 mg/kg, subcutaneous) was administered 30 minutes prior to anesthesia and imaging. α -Lipoic acid is most commonly administered chronically in experimental diabetic models with the goal of

TABLE. Summary of Anatomic, ADC, and Choroidal Thicknesses in Dark and 20 Minutes of Light (μm , Mean $\pm$ SEM)

Group	Anatomical Thickness		ADC Thickness		Choroidal Thickness	
	Dark	Light	Dark	Light	Dark	Light
wt, $n = 18$	237 $\pm$ 3	264 $\pm$ 4 $\ddagger$	175 $\pm$ 3	181 $\pm$ 4 $\ddagger$	62 $\pm$ 2	86 $\pm$ 3 $\ddagger$
wt+Mn ²⁺ , $n = 6$	216 $\pm$ 4*	235 $\pm$ 8 $\ddagger\ddagger$	157 $\pm$ 3*	163 $\pm$ 2 $\ddagger$	58 $\pm$ 3	72 $\pm$ 8 $\ddagger$
GNAT1 ^{-/-} , $n = 6$	224 $\pm$ 4	257 $\pm$ 5 $\ddagger$	166 $\pm$ 4	177 $\pm$ 5	58 $\pm$ 2	80 $\pm$ 7 $\ddagger$
STZ, $n = 16$	222 $\pm$ 2*	245 $\pm$ 5 $\ddagger\ddagger$	160 $\pm$ 5*	172 $\pm$ 5 $\ddagger$	62 $\pm$ 3	73 $\pm$ 3 $\ddagger\ddagger$
STZ+LPA, $n = 6$	212 $\pm$ 4*	246 $\pm$ 5 $\ddagger\ddagger$	161 $\pm$ 3*	177 $\pm$ 5 $\ddagger$	52 $\pm$ 4	70 $\pm$ 4 $\ddagger\ddagger$

STZ, streptozotocin.

* $P < 0.05$ from dark wt. $\ddagger$ $P < 0.05$ from light wt. $\ddagger\ddagger$ $P < 0.05$ from paired dark/light comparison.

preventing the development of histopathology.^{4,35–39} Because we have previously shown that acute single-dose treatment of 11-*cis*-retinaldehyde (which turns out, unexpectedly, to be an antioxidant [manuscript in preparation]) can specifically impact diabetes-induced rod pathophysiology, in this study we applied LPA acutely (duplicating an experimental design previously used in a sciatic nerve crush injury model) to reduce the oxidative burden.^{5,40}

Diabetes was induced in mice at approximately 2 months of age by streptozotocin (60 mg/kg; 10 mM citrate buffer [pH 4.5]) injection IP once a day for 5 consecutive days; mice were maintained diabetic for 2 months. Body weight and blood glucose levels were monitored twice weekly. Insulin (neutral protamine Hagedorn) was administered to mice as needed, based on body weight and blood glucose levels but not more than twice weekly, to allow slow weight gain while maintaining hyperglycemia (blood glucose levels higher than 400 mg/dL). Mice that lost weight and/or had blood glucose levels greater than 600 mg/dL were given up to 0.2 units of insulin (Humulin N, Eli Lilly and Company, Indianapolis, IN, USA). Normal rodent chow (Purina TestDiet 5001; Richmond, IN, USA), which contains 11.2% fat, 26% protein, and 62.7% carbohydrate, and water were provided ad libitum. Glycated hemoglobin was measured from blood collected after each MRI experiment (Glyco-Tek affinity columns, kit 5351; Helena Laboratories, Beaumont, TX, USA). Blood was drawn from the left ventricle, after puncture, into a capillary tube and stored in an Eppendorf tube with a small amount of heparin to prevent coagulation. The blood was kept in the refrigerator until analysis within one week following the MRI experiment.

Light Exposure Protocol

In our initial description of light-dependent ADC changes, we alternated between immediately collecting light and then dark data at each of the different diffusion gradient strengths.¹⁹ In this study, we simplified the light exposure protocol by first collecting a full diffusion data set in dark-adapted mice and then another set 15 minutes after turning on a light inside the bore of the magnet. In a subset of mice, we immediately collected a full diffusion data set after turning on the light in addition to collecting the data set after 15 minutes in the light.

MRI Procedures

The general mouse preparation for high-resolution MRI is well established in our laboratory.⁵ All animals were maintained in darkness for at least 16 hours before and during the dark phase of the MRI examination. In some mouse groups, MnCl₂ was administered, under dim red light or in darkness, as an intraperitoneal injection (66 mg MnCl₂·4H₂O/kg) on the right side of awake mice.^{3,41} After this injection, mice were

maintained in the dark for another 3.5 to 4 hours. High-resolution anatomic and ADC data were acquired on a 7 T system (Bruker ClinScan; Billerica, MA, USA) using a receive-only surface coil (1.0 cm diameter) centered on the left eye. The end of a fiber optic bundle was attached to a light source (Mark II Light Source; Prescott's, Inc., Monument, CO, USA) placed caudal to the eye, projecting at a white screen $\sim$ 1 cm from the eye, similar to that previously described.¹⁹ We exposed the eye to 0 (i.e., dark) or $\sim$ 500 lux (confirmed outside the magnet using a Traceable Dual-Range Light Meter [Control Company, Friendswood, TX, USA]) placed against a 1-cm-diameter aperture; measured this way, room lighting is $\sim$ 300 lux). Aside from the fiber optic light source, all lights in the MRI room were turned off. In all groups, immediately before the MRI experiment, animals were anesthetized with urethane (36% solution intraperitoneally; 0.083 mL/20 g animal weight, prepared fresh daily; Sigma-Aldrich Corp., St. Louis, MO, USA) and treated topically with 1% atropine to ensure dilation of the iris during light exposure, followed by 2% lidocaine to reduce eye motion. Anatomic and ADC (parallel to the optic nerve, the most sensitive direction for detecting changes at the location of the SRS¹⁹) MRI data sets were collected, first in the dark and then again 15 minutes after turning on the light; since each ADC data set takes 10 minutes to collect, we refer to the midpoint in the ADC collection as 20 minutes of light exposure. The subset of nondiabetic wt mice in which an ADC data set was collected immediately after turning on the light was called the 5 minutes of light exposure time point group. Anatomic images were acquired using a spin echo sequence (slice thickness 600 μm , TR 1000 ms, TE 11 ms, matrix size 192 $\times$ 320, field of view 8 $\times$ 8 mm², NA 2, axial resolution for central retina 25 μm); images sensitized to water diffusion were collected (TR 1000 ms, slice thickness 600 μm , TE 33 ms, matrix size 174 $\times$ 288, field of view 8 $\times$ 8 mm², axial resolution for central retina 27.8 μm ; $b = 0, 100, 250, 500, 600, 750, 990$ s/mm² [collected in pseudorandom order, NA 1 per b value]), registered to the anatomic image, and analyzed (using in-house code) to generate ADC profiles from the central retina. The present resolution in the central retina is sufficient for extracting meaningful layer-specific anatomic and functional data, as previously discussed.⁴² For example, given the present whole-retinal thicknesses of $\sim$ 238 μm (the average thickness across controls in the Table) and the pixel size ($\sim$ 26 μm [mean of 25 and 27.8 μm]), each pixel axially spanned approximately 11% thickness or $\sim$ 9 μm . We also note that the uncertainty in a pixel's thickness can be estimated to be $\sim$ 1/2 pixel thick. The data support this estimate because converting all of the SEM back to SD from the Table and averaging gives a variance of $\sim$ 13.5 μm , which is in reasonable agreement with the 1/2 pixel values of 12.5 to 13.9 μm (from anatomic to ADC images). The data also support our ability to distinguish changes in central retinal thickness on the anatomic images

and significant ADC changes at 88% to 100% depth. In all cases, anesthetized animals were humanely euthanized by cervical dislocation followed by bilateral pneumothorax for assurance of death per our DLAR-approved protocol.

Data Analysis

In each animal, we confirmed ocular dilation based on the iris position on the anatomic MRI data; if eyelid position was closed to a degree likely to impeded the light path, only the dark data from that animal were used.⁴² All images for each animal per lighting condition were registered (rigid body) to the anatomic image. We had previously demonstrated that a simplified estimate of ADC, as the slope describing progressive losses in (log-transformed) signal intensity at progressively higher diffusion weightings (*b* values), was sufficient for observing dark/light changes in the SRS ADC.⁴² Thus, in this study we calculated ADC as previously described.⁴² In all cases, the same central retinal regions of interest (± 0.4 –1 mm from the optic nerve head) were analyzed; thickness and ADC values from the superior and inferior retina were respectively averaged.

In each mouse, thicknesses (μm) from the anatomic and ADC images were objectively determined using the “half-height method” wherein a border is determined via a computer algorithm based on the crossing point at the midpoint between the local minimum and maximum, as detailed elsewhere.^{42,43} The distance between two neighboring crossing points thus represents an objectively defined thickness.

From the anatomic image, thickness were normalized with 0% depth at the presumptive vitreoretinal border and 100% depth at the presumptive retina/choroid border. Note that because of partial volume averaging there are slight contributions from nonretinal tissue anteriorly at the vitreoretinal border and posteriorly at the retina/choroid border. The latter contribution was previously demonstrated with gadolinium-based measurements.⁴⁴ We typically exclude the anterior (0%–8% depth) and posterior (88%–100% depth) regions on the basis of this partial volume averaging argument.^{42,45} However, the SRS exists at the 88% to 100% depth. We reason that light-evoked changes in the SRS ADC will be largely isolated from (i.e., not contaminated by) ADC and thickness changes in the choroid because the fast-flowing blood in the choroid will be suppressed to a greater degree by the diffusion gradients than in the SRS. In support of this assumption we note that *GNAT1*^{−/−} mice clearly show a lack of light-dependent changes in SRS ADC but normal choroidal expansion between dark and light (see Results); a similar disconnect between SRS and choroid was noted in an initial study using a diffusion gradient in the direction perpendicular to the optic nerve (data not shown), similar to that previously reported.¹⁹

Choroidal thickness in each mouse was estimated as follows. We take advantage of the fact that ADC images show a suppressed signal from the vertical vessels between the horizontal segments of the choroid and the choroidal capillaries (i.e., the direction parallel to the optic nerve) and the vitreous (which has higher ADC than retina due to the lack of cellular barriers⁴⁶); such diffusion-induced suppression of the choroid is evident in the images in Figure 1. We then used the same computer algorithm as above to objectively determine the thickness from the portion of retina that was not suppressed in the averaged (to improve signal-to-noise) b100 to b990 diffusion images (Fig. 1); herein this is referred to as the ADC thickness. The thickness difference between that generated from the anatomic image (with, e.g., a choroid contribution) and that generated from the ADC image (with suppressed choroidal contribution) was considered an esti-

mate of the choroid thickness for each mouse (Fig. 1). In this manner, choroidal thicknesses were estimated for dark- and light-exposed conditions for each mouse; the accuracy of this choroidal thickness estimate appears to be reasonable (see Discussion).

In our earlier studies in mice, longer (on the order of hours) dark and light adaptation time did not produce significant differences in the retinal thickness from anatomic images.^{41,47,48} However, in this study, the dark-to-light transition clearly produced a significant increase in anatomic image-derived retinal thickness (Fig. 2; Table). It was clear that this light-evoked thickening was largely due to increases in choroidal thickness (Table).^{20,21,23} Thus, to allow for comparisons between groups and conditions, the ADC profiles in dark and light in each mouse were spatially normalized to the anatomic thickness value in the dark (since our data indicated that the “extra” thickness in the light was primarily via expansion of the choroid in the light).

Evaluations of choroid and SRS were done objectively, based strictly on dark-adapted anatomic thickness values (as described above), and thus data were not masked.

Statistical Analysis

All thicknesses in each group were evaluated for a normal distribution and were compared using a one-way ANOVA test. Due to insufficiently opened eyelids, we could not always collect both dark and light data from all animals. Also, the dark baseline ADC data were averaged from all wt groups regardless of subsequent light exposure. Choroid and SRS layer ADC data from untreated and vehicle-treated diabetic groups were not different, so data sets were respectively combined. Comparison of ADC profile data between groups was first performed using a one-tailed unpaired *t*-test at different locations of the intraretinal profiles to objectively identify regions of interest. Then, a generalized estimating equation (GEE) approach was used to compare selected location ranges, identified from the *t*-tests as significant.^{47,49} The GEE method is a more powerful two-tailed method that performs a general linear regression analysis using contiguous locations in each subject and accounts for the within-subject correlation between contiguous locations. When the initial *t*-test identified a location range as likely significant ($P \leq 0.05$, i.e., *P* less than or equal to 0.05) at the one-tailed level, GEE was performed on the data in that range; regions marked as statistically significant had $P \leq 0.05$ on GEE. Data are presented as mean $\pm$ standard error of the mean (SEM).

RESULTS

Group Summary

Control mouse body weights were 28.8 ± 0.4 g ($n = 18$, ages 3–6 months, mean $\pm$ SEM); in the age-matched controls for the diabetic group, the glycated hemoglobin levels were $5.6 \pm 0.2\%$ ($n = 9$). In manganese-treated control mice, body weights were 26.7 ± 0.4 g ($n = 6$); glycated hemoglobin levels were not evaluated. *GNAT1*^{−/−} mice had body weights of 21.2 ± 0.7 g ($n = 6$); glycated hemoglobin levels were not evaluated. Two-month diabetic mice had body weights of 24.0 ± 0.7 g ($n = 16$) and glycated hemoglobin levels of $11.3 \pm 0.3\%$. Three month diabetic mice acutely treated with LPA had body weights of 23.5 ± 1 g ($n = 6$) and glycated hemoglobin levels of $11.8 \pm 0.3\%$. Body weight in nondiabetic mice given manganese was not different ($P > 0.05$) than in wt mice, but was lower in *GNAT1*^{−/−} and both diabetic mouse groups ($P < 0.05$). As expected, control mice had lower ($P < 0.05$) glycated

FIGURE 2. Summary of objectively determined choroidal thickness (see Fig. 1 and Methods for more details) measured from (A) dark and (B) light conditions for wt control mice ("C"), *GNAT1*^{-/-}, untreated diabetic ("D"), and LPA-treated diabetic (D+LPA) mice. Red *, significant difference ($P < 0.05$) from light wt controls. Error bars: SEM.

hemoglobin levels than both groups of diabetic mice; LPA treatment did not affect glycated hemoglobin levels between diabetic groups ($P > 0.05$).

Thickness

Reproducibility. Thickness values within different dark-adapted nondiabetic wt mouse groups not given manganese were compared, as this afforded the largest number of comparisons; dark adaptation was a common condition to all wt groups regardless of subsequent light exposure protocol (Table; Fig. 1).

Choroid. Choroidal thickness values in the dark were not different between any of the groups studied (Table). All groups demonstrated significantly increased whole-retina thickness over dark values after 20 minutes of light (Table) that was largely due to expansion of the choroid (Fig. 2; Table): wt (38%), *GNAT1*^{-/-} (+38%), diabetic (+17%), diabetic+LPA (+35%). Smaller but still significant increases in choroidal thickness were noted after 5 minutes of light (data not shown). Notably, in the light but not in the dark, both untreated and LPA-treated diabetic groups had smaller ($P < 0.05$) choroidal thickness than wt mice (Fig. 2; Table).

Retina. As summarized in the Table, in the dark, ADC thicknesses in diabetic mice were smaller ($P < 0.05$, one-way ANOVA) than in nondiabetic wt and *GNAT1*^{-/-} mice. Apparent diffusion coefficient thickness appeared to increase with 20 minutes of light exposure in the wt (+6%), *GNAT1*^{-/-} (+7%), diabetic (+8%), and diabetic+LPA (+10%) groups. However, these retinal changes are roughly an order of magnitude smaller than for the choroid (see above); in the absence of a physiologic explanation for retinal expansion, these changes likely represent some residual choroidal contribution using the present MRI resolution.

Effect of Systemic Manganese. Wild-type mice given manganese had a significant light-evoked expansion of

anatomic thickness but not in ADC or choroidal thicknesses (Table); the reason for this is not yet clear.

Apparent Diffusion Coefficient

Reproducibility. All untreated dark-adapted wt mouse groups exhibited similar topography across the retina (i.e., similar magnitudes of spatial features of the profile, Fig. 1) and notable reproducibility ($P > 0.05$) at the SRS layer (88%–100% depth), the region tested for our working hypothesis. All dark-adapted profiles were combined for further comparisons of ADC changes at the SRS layer.

Time Course. Next, ADC profiles across the retina were compared at 5 and 20 minutes of light exposure (Fig. 3). At both time points, light evoked a significant ($P < 0.05$) increase in ADC at the SRS layer from the all-groups mean dark profile. However, the light-dependent change in ADC values at 88% to 100% depth was lower ($P < 0.05$) at 5 minutes of light relative to that at 20 minutes.

Effect of Intracellular Manganese. Apparent diffusion coefficient MRI has the potential to evaluate aspects of rod function complementary to that provided by manganese-enhanced MRI (MEMRI), the imaging modality of choice to study retinal calcium channels.^{50,51} Combining these two methods is expected to provide useful complementary information if the presence of manganese does not somehow alter the ADC MRI measurement at the SRS layer. We thus tested, and confirmed, this possibility. Mn^{2+} -treated wt mice (Fig. 4) were found to have a large light-stimulated change in the ADC at 88% to 100% depth, which was not different ($P > 0.05$) from that in the untreated mice (Fig. 2).

Mice Lacking Rod Phototransduction. Light-dependent changes in rod extracellular ion content increase the SRS volume compared to that in dark.^{24–27} Based on this, we hypothesized that in mice null for phototransduction, the ADC at the SRS layer would not change between light and dark. We confirmed this prediction in *GNAT1*^{-/-} mice (Fig. 4). Consis-

FIGURE 3. Summary of central retinal ADC as a function of retinal depth during dark (closed symbols) and either (A) 5 minutes or (B) 20 minutes of ~500 lux light (open symbols) in untreated nondiabetic wt mice. Data are presented using the conventions in Figure 1D. Red line: region with significant differences ($P < 0.05$) between profiles.

tent with a lack of phototransduction, the ADC values at 88% to 100% depth in *GNAT1*^{-/-} mice were not different ($P > 0.05$) from those in dark-adapted wt mice. We did find a significant ($P < 0.05$) increase in water mobility anterior to the SRS (i.e., 44%–48% depth) in dark- versus light-exposed mice; the interpretation of this change is currently unclear. Nonetheless, these data support the predicted contribution of rod phototransduction to light-stimulated ADC increases at the SRS layer.

Diabetes. Unlike wt controls, untreated or saline-treated diabetic mice had no significant ($P > 0.05$) change in ADC at 88% to 100% depth between dark and light conditions (Fig. 5). A significant ($P < 0.05$) increase in ADC with light was observed at 60% to 68% depth in untreated diabetic mice; the mechanism underlying this change requires additional investigation. The lack of a light-stimulated change in the ADC at 88% to 100% depth likely arises from diabetes-induced changes in rod ion content.^{3,4,28–31} Antioxidant treatment can correct altered ion regulation in the photoreceptor layer of diabetic rodents.^{2–4} Consistent with these previous findings,^{4,41,44–48} systemic treatment with LPA partly corrected light-stimulated increases in ADC at the SRS layer (Fig. 5).^{4,35–38,52,53} We also note that regions anterior to the SRS layer also demonstrated differences between nondiabetic and diabetic mice, although more work is needed to identify the responsible mechanisms.

DISCUSSION

Retinal and Choroid Thickness

Previously, diffusion-weighted MRI was found to be useful for measuring choroid thickness in rats.⁴⁶ The present data in wt

mice further support the precision and accuracy of MRI choroidal thickness measurement.^{19,44,46} For example, our estimated central retinal choroidal thickness from wt C57BL/6 mice (range, 62 [dark]–86 [light] μm , Table) compares reasonably with published values obtained using other methods from the same mouse strain (52–78 μm ^{46,54,55}). In this study, as previously reported, retinal thickness from *GNAT1*^{-/-} mice was not significantly different from that in control mice.^{34,41} Here, we note that choroidal thickness is also not different between *GNAT1*^{-/-} and control mice (Table; Fig. 3).

In diabetic mice, the present data support our previous report of retinal thinning in diabetic mice (Table).³ On the other hand, unlike previous reports showing that the choroid thins in diabetes,^{7,12–14} we find no evidence that diabetes causes choroidal thinning per se since dark-adapted values are normal (Table). Somewhat surprisingly, our data suggested instead that in early diabetes the extent of light-evoked choroidal expansion was subnormal. These results do not rule out the possibility that choroidal thinning might occur at longer durations of diabetes.^{7–10,12–14,16}

In nondiabetic subjects, the mechanism underlying light-stimulated choroidal thickness expansion remains somewhat unclear, although there is a clear brain-based component.^{20,21,23} In support of this interpretation, nondiabetic mice with nonfunctioning rods had a normal extent of choroidal thickness increase with light (Table; Fig. 2). One possible mechanism by which light may centrally regulate choroidal thickness is via melanopsin-containing ganglion cells that project back to the same brain structures (e.g., the paraventricular hypothalamus) from which the choroidal innervation arises; more work is needed to test this hypothesis.^{56,57}

FIGURE 4. Summary of central retinal ADC as a function of retinal depth during dark (closed symbols) and 20 minutes of ~500 lux light (open symbols) in (A) Mn²⁺-treated wt mice or (B) GNAT1^{-/-} mice. Data are presented using the conventions in Figure 1D. Red line: region with significant differences ($P \leq 0.05$) between profiles.

In 2-month diabetic mice, an impaired light-stimulated choroidal thickness expansion was observed. The underlying mechanism is not clear, in part because the endothelial cell, nerve, and neuron contributions to the choroidal response to light in nondiabetic mice are not yet known. Nonetheless, we note that oxidative stress plays a pathogenic role in DR, so it seemed reasonable to examine its role in choroid dysfunction. The impaired light-stimulated expansion of the choroid was not corrected by LPA, suggesting that the underlying mechanism does not involve diabetes-induced oxidative stress, although these results do not rule out the possibility that more long-term application of antioxidants might be necessary for the choroid.

Retinal ADC Topography

Apparent diffusion coefficient MRI was found to be reasonably precise as a likely result of the “vertical” retina micro-architecture being reliably layered. For example, in dark-adapted wt mice, there was a notable diffusion barrier (i.e., greater ADC values) at the inner limiting membrane (between largely acellular vitreous and structured retina) and at ~80% depth into the retina (presumed location of the outer limiting membrane) (Fig. 1).²⁶ Based on this consideration, it appears that the SRS layer maintains a constant length in dark and light because the distance between the vitreal/retinal border and peak of the presumptive outer limiting membrane remains at ~80% depth (Figs. 3, 4).²⁶ It would seem that the SRS increases its volume in light by increasing its width, probably in concert with adaptations to RPE processes and photoreceptor cells to accommodate this change.²⁶

SRS Layer

Based on experiments in rats, we previously suggested that light-dependent expansion of the SRS layer can be monitored via the light-evoked increase in ADC.¹⁹ Here, we found an increase in ADC at the SRS layer with light in wt mice and demonstrate that this effect is regulated at least in part by phototransduction. These data strongly support the use of ADC MRI to monitor light-stimulated expansion of the SRS layer.

In 2-month diabetic mice, we did not find evidence for light-evoked expansion of the SRS layer during the dark-to-light transition based on our ADC measurement. The mechanism underlying normal SRS volume expansion depends on light-dependent changes in extracellular ion content, including hydrogen ions, since acidification dramatically decreases the SRS volume via increased fluid absorption by the retinal pigment epithelium.^{24–27,58} Intriguingly, diabetes-induced acidification has been observed in acutely hyperglycemic nondiabetic rats and in diabetic rats (Dmitriev AV, et al. *IOVS* 2014;55:ARVO E-Abstract 1049; Henderson DI, et al. *IOVS* 2012;53:ARVO E-Abstract 5379). More work is needed to understand the role of different ions in nondiabetic mice. Nonetheless, the impaired light-stimulated ADC increase at the SRS layer was significantly restored by LPA, suggesting involvement of an oxidative stress mechanism.

In conclusion, ADC MRI, even after systemic injection of manganese, appears sensitive to light-evoked expansion of choroidal thickness and SRS layer. In diabetes, no evidence was found for choroidal thinning, and diabetes-induced oxidative stress appeared to impair only light-stimulated expansion in the SRS layer and not the light-dependent choroidal expansion.

FIGURE 5. Summary of central retinal ADC as a function of retinal depth during dark (closed symbols) and 20 minutes of ~500 lux light (open symbols) in (A) wt control mice (from Fig. 3B), (B) untreated diabetic mice, (C) saline-treated diabetic mice, and (D) LPA-treated diabetic mice. Data are presented using the conventions in Figure 1D. Red line: region with significant differences ($P < 0.05$) between profiles.

Future studies will investigate the possibility that additional information about photoreceptor function is achievable from diffusion MRI by including much higher b values, fitting the higher b value data to a biexponential function, and examining the full diffusion tensor. Potential applications of ADC MRI include (1) combined ADC MRI and MEMRI study of experimental retinopathy models; (2) longitudinal studies of age-related retinal degeneration models where impaired rod function and/or abnormal water handling of the retina is considered to be a critical sight-threatening problem but cannot be investigated using existing electrode assays with an extracellular marker^{59,60}; and (3) translational clinical studies.

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