Manganese-Enhanced MRI of the DBA/2J Mouse Model of Hereditary Glaucoma

David J. Calkins, Philip J. Horner, Robin Roberts, Marius Gradianu, and Bruce A. Berkowitz

PURPOSE. To test the hypothesis that manganese-enhanced magnetic resonance imaging (MEMRI) is a sensitive approach for measuring of age-related ocular changes in experimental pigmentary glaucoma.

METHODS. Four groups of light-adapted mice were studied using MEMRI: young (2–3 months), C57BL/6 (negative controls), and DBA/2J mice and aged (10–11 months) C57BL/6 and DBA/2J mice. In all mice, eye perimeter, optic nerve head width, iridocorneal angle, ciliary body area, and total and inner retinal thickness, and a surrogate of retinal ion regulation (intraretinal uptake of manganese) were assessed from MEMRI data and compared. Axon counts were obtained from optic nerves harvested from MEMRI-assessed eyes.

RESULTS. As the C57BL/6 and DBA/2J mice aged, differential and significant changes in ocular perimeter, retinal thickness, iridocorneal angle, ciliary body area, and optic nerve head width were readily measured from MEMRI data ($P < 0.05$). In C57BL/6 mice, only inner retinal thickness and perimeter were correlated. In DBA/2J mice, ocular perimeter was correlated with total and inner retinal thickness, ciliary body area, optic nerve head width, and iridocorneal angle. Comparison of young and aged mice revealed a subnormal intraretinal manganese uptake ($P < 0.05$) in aged DBA/2J mice, but not in aged C57BL/6 mice. Manganese uptake did not correlate with the ocular perimeter. Axon density in the optic nerve correlated with MEMRI-measured optic nerve head width ($P < 0.05$).

CONCLUSIONS. These studies provide a baseline of noninvasive MEMRI-detectable changes associated with age in a common animal model of hereditary glaucoma that may be useful in the longitudinal evaluation of therapeutic success. (Invest Ophthamol Vis Sci. 2008;49:5083–5088) DOI:10.1167/iovs.08-2205

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presents prominently in many DBA/2J mice by 4 to 6 months and increases in probability of occurrence with increasing age. However, at each age, even in older animals, there is considerable variability in the degree of ocular and nerve disease.

**METHODS**

The animals were treated in accordance with the Principles of Laboratory Animal Care (publication no. 85–23, revised 1985; http://grants1.nih.gov/grants/olaw/references/plsblpol.htm), as well as the ARVO Statement for the use of Animals in Ophthalmic and Vision Research.

**Groups**

The C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME) and maintained at the Wayne State School of Medicine. The DBA/2J mice were obtained from Jackson Laboratories, maintained in a specific pathogen-free facility at Harborview Research and Training, University of Washington, and regularly backcrossed with fresh founders from Jackson Laboratories to minimize uncontrolled genetic drift. At select times, these mice were shipped to the Wayne State School of Medicine. In all cases, the mice were maintained in a 12-hour light-dark cycle with standard rodent chow available ad libitum. Four groups of light-adapted mice were studied by using manganese-enhanced magnetic resonance imaging (MEMRI): young (2–3 months) C57BL/6 (negative controls, \( n = 4 \)) and DBA/2J (\( n = 9 \)) mice and aged (10–11 months) C57BL/6 (\( n = 7 \)) and DBA/2J (\( n = 6 \)) mice. The aged group was chosen as 10 to 11 months to maximize the probability of observing differences from the young group in this first MEMRI study. The aged DBA/2J mice were chosen for similarity in IOP, as measured monthly by a handheld tonometer (TonoPen; Medtronic, Jacksonville, FL). The range of mean IOPs for the left eye of these animals (which we imaged) was 16.1 to 17.9 mm Hg (mean ± SD: 16.75 ± 0.63), and a Kruskal-Wallis one-way analysis of variance using ranks indicated no significant difference between monthly IOP measurements in these eyes (\( F = 0.56, P = 0.82 \)). By comparison, mean IOP in 3-month DBA/2J mice in our colony at the time of harvesting was 15.5 mm Hg.

**High-Resolution MRI**

All mice were housed in normal 12-hour cycled laboratory lighting until the end of the experiment. Then they were maintained in darkness for 16 to 20 hours and then lab lighting for 20 minutes before manganese injection. MnCl\(_2\) was administered as an intraperitoneal injection (66 mg/kg) on the right side of awake mice. After this injection, the mice were maintained in light conditions for another 3.5 to 4 hours and were anesthetized for MEMRI examination. Immediately before the MRI experiment, the mice were anesthetized using urethane (36% solution, 0.083 mL/20 g animal weight IP; prepared fresh daily; Aldrich, Milwaukee, WI) and xylazine (1–8 mg/kg IP). We found that urethane alone tended to increase the respiratory frequency of the mice, while the amount of the muscle relaxant xylazine minimized these artifacts. Because of the use of urethane, separate groups of mice were used at each time point. Core temperatures were maintained with a recirculating heated water blanket. MRI data were acquired on a 4.7-T system (Avance; Bruker BioSpin Corp., Billerica, MA) using a two-turn transmit-receive surface coil (1.0 cm diameter) placed over the eyes. A single transverse slice through the center of the eye (based on sagittal localizer images collected using the same adiabatic pulse sequence as above) was obtained for each mouse. Transverse images were then acquired using an adiabatic spin-echo imaging sequence (repetition time, TR 350 seconds; echo time, TE 16.7 ms; number of acquisitions, NA 16; sweep width, 61,728 Hz; matrix size, 512 × 512; slice thickness, 620 \( \mu \)m; field of view, 12 mm\(^2\)).

**Data Analysis**

**Ocular Anatomy.** From each image, the following structures were identified and measured: ocular perimeter, ciliary body area, iridocorneal angle, optic nerve head width, and retinal thickness.

**Ocular Perimeter.** Previously, we, and others, found that changes in intraocular pressure could be estimated on very high resolution MRI by, for example, changes in ocular perimeter. We reported that reasonable agreement between the MRI-derived perimeter of rat eyes and applanation tonometry readings (Tono-Pen XL; Medtronic) were found.

**Ciliary Body Area.** As shown in Figure 1, in each image, both superior and inferior ciliary bodies were readily identified. The area of each body was determined in NIH image (available by ftp at zippy.nimh.nih.gov/ or at http://rsb.info.nih.gov/nih-image; developed by Wayne Rasband, National Institutes of Health, Bethesda, MD). No differences between superior and inferior values were noted (data not shown), and so these were averaged together for further analysis.

**Iridocorneal Angle.** Both superior and inferior angles were readily identified on each image. Angles were measured using the angle function in NIH image Superior and inferior values were not different (data not shown) and were averaged together for comparison.

**Optic Nerve Head Width.** In each image, the cross-section of retina at the optic nerve head was identified by the relative lack of manganese uptake. The distance from superior to inferior retina at the inflection point defines the optic nerve head width used in this study.

**Retinal Thickness.** Total and inner (nerve fiber layer+ganglion cell layer+inner plexiform layer+inner nuclear layer) retinal thicknesses were manually measured from superior and inferior retina at six different locations per side as the radial distance between the anterior and posterior edges of the retina or anterior and border of postreceptor and receptor retina, respectively, at 0.4 to 1 mm from the optic nerve. No differences between superior and inferior hemiretinal thickness values were noted (data not shown). Thus, averaged data were used for comparisons.
The ocular anatomic data were consistent with a normal distribution.

### Statistical Analysis

The ocular anatomic data were consistent with a normal distribution, and comparisons between groups were mostly performed with an unpaired two-tailed t-test. Comparisons of MEMRI retinal signal intensities were performed with a generalized estimating equation (GEE) approach. GEE performs a general linear regression analysis on all the pixels in each subject and accounts for the within-subject correlation between adjacent pixels. In all cases, $P < 0.05$ was considered statistically significant. The data are presented as the mean ± SEM unless otherwise noted.

### RESULTS

#### Ocular Anatomy

Representative high-resolution images from young and aged C57BL/6 and DBA/2J mice are presented in Figure 1. The overall 15% increase in ocular perimeter in the aged DBA/2J mice is readily visualized compared to a 3% increase for the C57BL/6 eye. Quantitative analysis, summarized in Table 1, revealed other significant changes between young and aged C57BL/6 mice. Specifically, total retinal thickness decreased by 8% ($P < 0.05$), while iridocorneal angle increased by 15% ($P < 0.05$). From the combined young and aged C57BL/6 data set, only ocular perimeter and inner retinal thickness correlated ($r = -0.65, P < 0.05$). In contrast, for DBA/2J mice, a relatively greater constellation of significant decreases was noted with aging ($P < 0.05$): total (7%), inner retinal thickness (8%), ciliary body area (37%), and iridocorneal angle (66%); optic nerve head width increased (38%). In young and aged animals combined, strong correlations with ocular perimeter were found for all anatomic parameters measured in the DBA/2J ($r > 0.7, P < 0.05$). Ocular perimeter and inner retinal thickness also correlated (Fig. 2) in both the DBA/2J and the C57BL/6 groups.

#### Ion Regulation

As expected, in light-adapted mice (Roberts R, et al. IOVS 2008;49:ARVO E-Abstract 4926), significant differences ($P < 0.05$) between inner and outer retinal uptake were measured for both 3 and 10 months. C57BL/6 mice (Fig. 3), with uptake for the inner retina approximately 15% greater in each case. Comparison of 3 and 10 months C57BL/6 mice also revealed a significant ($P < 0.05$) 18% and 24% increase with age in inner and outer retinal uptake of manganese, respectively (Fig. 3). In contrast, in DBA/2J mice, despite being light adapted, a difference in inner and outer retinal uptake signal was noted only in the 3-month mice and not in the 11-month animals (Fig. 3). This difference appears to be due to the inner retina, since it had the largest change (6%; Fig. 3).

### Axon Counts

The density of axons in the proximal optic nerve was not significantly different between any of the groups ($P > 0.05$, Table 2), although the aged DBA/2J group had an average axon density 18% less than that of the young group, as expected. The lack of significance in this difference reflects the large variability within each group, as also noted in the literature, the relatively small sample size for this first study, and the relatively modest elevations in IOP for the aged animals we
choose (see the Methods section). These data are consistent with our previous work showing that some aged DBA/2J can demonstrate a complete complement of axons. However, a plot of axon density against MEMRI-measured optic nerve head width in the same animals revealed a significant negative correlation (r = −0.62, P = 0.02, Fig. 4). If 10-month C57BL/6 data are included on this plot, no change in the degree of correlation was noted (r = −0.60, P = 0.01, data not shown). Thus, as has been reported with IOP, changes in axon density accompany MEMRI-measured changes in optic nerve head width.

**DISCUSSION**

In this study, MEMRI data of C57BL/6 and DBA/2J mice were used to identify, for the first time, unique phenotypes of differential age-related changes in ocular anatomy and retinal physiology. Like other neurodegenerative conditions, age is the greatest risk factor for glaucoma, with the likelihood of development of the disease increasing nearly sevenfold after 55 years of age. Thus, in deciphering the pathogenesis of retinal ganglion cell degeneration in glaucoma, it is critical to discriminate contributing factors associated with aging. The unique ability of MEMRI to simultaneously measure colocalized retinal structure and function as well as other ocular anatomic parameters raises the possibility that it may become a standard procedure for future ocular studies in mice. These doubtless will include critical time points in mid-disease progression (roughly, 5–8 months). The present studies provide a baseline of noninvasive MEMRI-detectable changes that anchor both an early (2–3 months) and later (10–11 months) phase of disease progression in the DBA/2J against which to evaluate future therapeutic success longitudinally.

The dose of MnCl₂ used in the present study (66 mg/kg) is not expected to affect retinal function or anatomy adversely, especially in the 4 hours between injection and examination. A similar dose has been used in other mouse MEMRI studies, without any reported adverse affects or neurotoxicity. In initial studies, we administered a dose that has worked well in rats (44 mg/kg, IP) but did not find reliable contrast changes in the mouse retina, possibly due to the relatively higher overall metabolic rate in the mouse (data not shown). Based on these considerations and our previous work with a nontoxic dose of MnCl₂ (44 mg/kg), it is reasonable to consider the 66 mg/kg dose as nontoxic and the intraretinal uptake of manganese as a quantitative biomarker of ion activity regulation in vivo.

In C57BL/6 mice, aging was linked with increased ocular perimeter which correlated with increased iridocorneal angle and decreased total retinal thickness. Comparisons of these structural changes with the literature are limited, as the in vivo ocular perimeter and iridocorneal angle in mice have been examined in few studies. We note that previously, MEMRI has been validated as an accurate measure of retinal thickness without choroidal contamination in the rat and has been applied to mouse eyes but at far lower spatial resolution than in the present study and after intravitreous delivery of manganese.

In the present study, in young control mice, MEMRI-based total retinal thickness measure (224 ± 2 μm) was in reasonable agreement with published murine thickness values from in vivo optical coherence tomography (220–250 μm) and ex vivo literature. One caveat is that the thickness changes found were smaller than the pixel size, and so some caution is needed when interpreting subtle differences. Nonetheless, the decrease in retinal thickness that we found in aged C57BL/6 mice was as expected, based on the literature. Since inner retinal thicknesses did not change over time in this study, it is reasonable to speculate that changes in the outer retina that occurred were most likely related to photoreceptor dysfunction.

Changes in intraretinal ion regulation with aging in C57BL/6 mice were also found. Previously, we documented a supernormal manganese uptake in the inner and outer retina before histopathology after an insult to the photoreceptor–retinal pigment epithelium complex in rats. These data, together with the above considerations that outer retina appeared damaged in the aged C57BL/6 mouse, raise the possibility that the supernormal uptake of manganese reflects outer retinal damage. We have previously demonstrated that, in the rat, intraretinal manganese uptake patterns robustly correlated with normal retinal physiology responses associated with light and dark visual processing. Of interest, the photoreceptor damage ability of the outer retina did not seem affected by age, since the expected differences in inner and outer retina uptake in light-
adapted conditions were found (Fig. 2). We did not find any correlation between structural and physiologic parameters. This finding may not be surprising, given the different types of information provided by each measurement (i.e., snapshot of intraretinal ion regulation versus chronic changes in anatomy). These structural and functional changes help define a baseline aging phenotype that can be measured with MEMRI.

Aged DBA/2J mice exhibited structural and physiologic differences relative to those measured in the C57BL/6 mice. Large increases in ocular perimeter, decreased ciliary body area and chamber angle, expansion of the region of low manganese uptake in the optic nerve head region, and thinning of the inner retina are all consistent with the development of glaucoma in this model, including neurodegeneration. These anatomic phenotypes appeared linked since perimeter or nerve head changes were well correlated with the other changes—for example, axon density (Fig. 4).33 MEMRI is clearly sensitive to the effects of glaucoma, and more work is now needed to gain a better understanding of the temporal evolution of structural and functional changes in experimental glaucoma. In any event, noninvasive MEMR-detectable changes accompanying the development of glaucoma are expected to be useful in future studies designed for longitudinal evaluation of novel neuroprotective drug therapies.

In summary, we have demonstrated the utility of MEMRI for in vivo studies of age-related differences and those due to glaucoma in the mouse. Despite its somewhat low relative resolution, relative to OCT, for example, the information content in each MEMRI data set is relatively much higher. These observations support the use MEMRI for phenotyping glaucomatous eyes in vivo.

### References


![Figure 3](image1.png)

**Figure 3.** MEMRI signal intensity (arbitrary units) changes in central inner retina (IR) and outer retina (OR) in light-adapted C57BL/6 mice (top) and DBA/2J mice (bottom) measured 4 hours after intraperitoneal systemic administration of MnCl₂. *(Significantly different (P < 0.05) from the 3-month inner retinal value; **significantly different from the 10-month inner retinal value. Note that the minimum value (50) of the y-axis is set to the signal intensity measured in the absence of manganese exposure. Error bars, SEM; numbers above the bars, number of animals studied.

![Figure 4](image2.png)

**Figure 4.** Plot of mean axon density in the proximal optic nerve measured histologically versus MEMRI-derived optic nerve head (r = -0.60, P = 0.02). Data points are combined from 3- and 11-month-old DBA/2J mice.

**Table 2. Summary of Axon Density Counts**

<table>
<thead>
<tr>
<th>Group</th>
<th>Density (Axons/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young C57BL/6 (n = 5)</td>
<td>186,223 ± 44,019</td>
</tr>
<tr>
<td>Aged C57BL/6 (n = 6)</td>
<td>219,069 ± 22,213</td>
</tr>
<tr>
<td>Young DBA/2J (n = 9)</td>
<td>232,567 ± 18,265</td>
</tr>
<tr>
<td>Aged DBA/2J (n = 5)</td>
<td>191,603 ± 48,006</td>
</tr>
</tbody>
</table>

Data are the mean ± SEM.


