Longitudinal Assessment of Rod Function in Intermediate Age-Related Macular Degeneration With and Without Reticular Pseudodrusen

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Purpose. To evaluate rod function longitudinally in intermediate age-related macular degeneration subjects with reticular pseudodrusen (RPD) and without RPD (AMD).

Methods. Retinal sensitivities (505 and 625 nm) during dark adaptation, at 14 locations within the central 12° macula were obtained after photobleaching at baseline and 12-month visits. Pointwise sensitivity differences between both stimuli were used to assess static rod function, while rod intercept time (RIT) and rod recovery rate (RRR) were used to evaluate dynamic function. Changes in function over time were compared between groups.

Results. A total of 23 controls, 12 AMD, and 13 RPD cases were followed-up. At baseline, the RPD group had significantly worst static and dynamic rod function compared to AMD and control groups. Static function in AMD was similar to controls. Static and dynamic function across the central 12° was consistent in controls; however, it was most impaired at 4° compared to 12° eccentricity in disease groups. Over 12 months, no AMD cases progressed clinically and static function in AMD improved (P ≤ 0.04), but remained unchanged in control and RPD groups (P ≥ 0.17). The RRR for control and RPD groups remained stable, while the AMD group deteriorated, but only at 12° (P = 0.02). The RIT was stable in AMD (P = 0.75) and RPD (P = 0.71) groups but improved in the control group (P = 0.002).

Conclusions. A decrease in RRR was detected over 12 months at 12° eccentricity in the AMD group. Evaluating changes in rod function requires testing at multiple locations including the peripheral macula.

Keywords: rod function, age-related macular degeneration, reticular pseudodrusen

A major impediment to our ability to conduct clinical trials of new interventions in the early stages of AMD is the lack of a robust biomarker that is abnormal early in the disease and can be followed, showing decline with the natural history of disease progression. It is considered highly desirable to have a functional biomarker that could identify change over a reasonably short period of time, ideally over 1 to 2 years that could potentially be used in intervention trials as a functional outcome to determine the potential efficacy of a new treatment.

Rod function has been shown to be a promising biomarker to evaluate disease severity¹⁻² and progression³⁻⁷ in the early stages of AMD. Many cross-sectional studies have demonstrated that rod function is abnormal early in AMD⁵⁻¹⁰ and more recently it has been found that AMD cases with the additional phenotype of reticular pseudodrusen (RPD, also known as subretinal drusenoid deposits) have particularly poor rod function.²¹⁻¹⁵ This finding is particularly significant as RPD are very common in AMD¹⁶⁻¹⁷ and are highly correlated with disease progression to late AMD; geographic atrophy and neovascular AMD.¹⁸⁻²⁵ In addition, RPD are more likely distributed at superior perifoveal retina, while the common drusen in AMD are more at central fovea.²⁶⁻²⁸ Therefore, understanding the nature of the rod function in AMD and its relationship with the presence of RPD is currently of interest as new instruments have now made it more practical to measure rod function at different locations within the retina.

Different instrument and techniques have been developed to measure both static and dynamic rod function across the macula and to detect rod dysfunction in disease.²⁹⁻³² Many research groups use a dynamic measure of rod intercept time (RIT)³⁻⁵,³³ or rod recovery rate (RRR)²⁻⁹ as potentially the most clinically useful indicators of poor rod function. However, there are only a few reports on the longitudinal natural history of rod decline in the early stages of AMD, especially ones using modern multimodal imaging techniques to identify the presence of RPD and analyze the data of group with and without RPD separately.⁴⁻⁷ A study by Jackson et al.⁴ reported an average worsening of 6.2 minutes of RIT in 19% of AMD patients in 12 months when both visual acuity and stage of intermediate AMD (iAMD) did not change. However, the study did not report on the presence of RPD in the study subjects. Owseley et al.⁵ found an average of 3 minutes worsening of RIT in 43.5% of the AMD subjects, with varying severity, over 12 months and 56.7% over 24 months. However, this study did not determine the presence of RPD at baseline and 12 months, only at 24-month follow-up where RPD were present in 83.3% of AMD cases. Chen et al.⁷ found that RIT was more prolonged in
the group that developed RPD than the group that did not have RPD over 48 months follow-up. However, these three studies did not evaluate AMD cases with RPD separately to those AMD cases without RPD at the same stage of AMD. In addition, in all studies, rod function was assessed at only one retinal location either at 5° or 11° superior retina.4,5,7

Our group has previously investigated rod function in AMD and we have reported worsening of RIT in intermediate AMD over 12 months within the central 6° of the macula.6 However, our initial protocol only followed recovery over 20 minutes and we found that approximately 36% of test points did not reach the rod intercept level at baseline and thus we were unable to observe a worsening in function at these points. Therefore, we wished to further evaluate rod function over a 12-month period, allowing a longer 30 minutes recovery time and within the central 12° of the fovea, in a well characterized, clinically stable cohort, all with bilateral large drusen, subgrouped on the presence or absence of RPD, to further our understanding of the impact of RPD on longitudinal changes in rod function in eyes with intermediate AMD.

METHODS

This prospective observational study was approved by the Human Research Ethics Committee of the Royal Victorian Eye and Ear Hospital (RVEEH) and conducted in the Macular Research Unit at the Centre for Eye Research Australia (CERA) in adherence with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants after the study had been explained.

Participants

In the macular research unit (MRU) at CERA, cases of AMD and normal control participants are enrolled in a natural history study where they are followed with imaging and functional testing over time. For this dark-adapted chromatic perimetry (DACP) substudy, participants were invited to participate in a 12-month longitudinal study of rod function if they fitted the inclusion criteria of being at least 50 years of age and having a best-corrected visual acuity (BCVA) of 20/60 or better. Control participants were required to have no drusen, no pigmentary abnormalities nor RPD in either eye and AMD participants were required to have drusen >125 μm with or without any AMD pigmentary abnormalities in both eyes, satisfying the classification of intermediate AMD based upon the Beckman classification.54

Exclusion criteria included a myopic refractive error of greater than −6 diopters (D) and a hyperopic refractive error greater than +4D. Other exclusions were a cataract grade >2 (WHO grading system)55 and other significant ocular media opacity that could obscure the fundus examination or multimodal imaging examination, diabetic retinopathy, glaucoma, severe neck, and spinal problems preventing the performance of the perimeter tests and having medications that might affect the retinal function.

For the longitudinal follow-up, we included only cases who remained with the same AMD stage as at baseline. Any cases with clinical signs of progression during the study period were excluded. This allowed us to determine whether it is possible to detect rod function changes prior to any clinical signs of AMD progression. Participants who developed advanced AMD, significant ocular media opacity, diabetic retinopathy, glaucoma or severe illness that could possibly affect to retinal function during the review period were excluded from this study. Participants who could not finish performing the DACP test or who did the test poorly were also excluded.

Procedures

All participants underwent a comprehensive eye examination, including assessment of BCVA with a modified version of the Early Treatment of Diabetic Retinopathy Study (EDTRS) chart at 4 meters, and low luminance visual acuity (LLVA) following a placement of a neutral density filter (2.0 ND).56 DACP, multimodal imaging, and clinical eye examination of the anterior and posterior segments at substudy baseline and 12-month follow-up. An interview for eye history, current symptoms of disease, and medication was also undertaken. Only one eye, the study eye, which was the eye with the better BCVA underwent the DACP examination.

Assessment of Rod Function

Rod function was assessed using a dark-adapted chromatic perimeter (DACP; Medmont Pty Ltd, Nunawading, Victoria, Australia). The pupils were dilated to at least 6 mm with tropicamide 0.5% (Mydriacyl; Alcon Laboratories (Australia) Pty Ltd, French Forest, New South Wales, Australia). Sphero-cylindrical lens for distance correction was inserted into a lens holder at a viewing distance of 30 cm. A stimulus size of Goldmann V (1.75°) was used. All participants were given the same verbal instructions regarding the procedure of performing DACP tests. The study eye was then bleached, approximately 20%, with a customized Ganzfeld flash while the fellow eye was patched. The intensity of the flash was approximately 2.45 × 10^6 scotopic cd/m². Retinal sensitivity was assessed regularly for 30 minutes after photobleaching using the 505 nm stimuli at 14 testing location within the central 12° of the macula (see Fig. 1). Once retinal sensitivity measurements for the 505-nm stimulus were completed, a single perimetric examination using the 625-nm stimuli was performed as part of the two-color perimetry (2CP) procedure. The changes in retinal sensitivity obtained with the 505-nm stimulus over 30 minutes of DA were used to determine the RIT and rod recovery rate. Rod function was also assessed with 2CP technique, by calculating the sensitivity difference between the 505- and 625-nm stimuli at 30 minutes after DA.11,37,38 The sensitivity difference was used for determining static rod function and may not be necessary an absolute rod threshold. All participants performed the same testing protocol for the baseline and 12-month visit.

Multimodal Imaging

Multimodal imaging was performed after the DACP testing was complete in order to avoid additional bleaching of the retina. All participants underwent near-infrared reflectance (NIR), short-wavelength fundus autofluorescence (SW-FAF), optical coherence tomography (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg Germany), and color fundus photography (Canon CR6-i5NM; Canon, Saitama, Japan). We obtained 49 B-scans within the central 20° × 20° of the retina and averaged 25 frames for every single OCT scan. All multimodal images were graded to confirm AMD classification and phenotypes by two graders, masked to the perimetry data.

Clinical Grading of AMD and RPD

The control and AMD grading were based on the Beckman Classification and Grading System.54 We included individuals with no apparent aging changes (no drusen and no AMD pigmentary abnormalities), and no RPD in the control group. Cases with intermediate AMD (drusen > 125 μm with or without any AMD pigmentary abnormalities) were subgrouped into those with and without RPD, where only those graded
with definite RPD being included in this RPD cohort and only definitely absent in the no RPD group. We excluded anyone who did not have either definitely present or absent RPD in both eyes. The diagnosis of RPD on SD-OCT has been prescribed in our previous study. Briefly, definitely present RPD required the presence of at least five clear round or cone-shaped subretinal deposits between external limiting membrane (ELM) and RPE on SD-OCT in more than one B-scan and in at least one en face modality (CFP, FAF, NIR) or RPD present on two en face modalities in the absence of SD-OCT findings (including outside the SD-OCT grid).

**Statistical Analysis**

For this study investigating changes in rod function over time, we included only those participants whose clinical disease remained stable. We therefore excluded from the analysis any participant whose AMD status changed over the course of 12 months.

We analyzed the data with our participants in three groups based upon their clinical phenotype: control group, AMD without RPD (AMD group), and AMD with RPD (RPD group) in both eyes. ANOVA and a post hoc analysis using Bonferroni correction were used to compare the age, BCVA, LLVA, and the difference of BCVA and LLVA (low-luminance deficit, LLD) between the study groups at baseline. We used the paired t-test to determine if BCVA, LLVA, and LLD changed between the baseline and 12-month follow-up.

A rod sensitivity plot for the 505-nm stimulus for every test point was generated and fitted into the dark adaptation curve using an exponential decay function following bleach out to 30 minutes. Two dark adaptation parameters were evaluated through this plot: rod intercept time (RIT) and the rod recovery rate. RIT was defined as the time for the stimuli to recover to a criterion level at −3.0 log units of stimulus intensity (i.e., 0.001 cd/m²) after an exposure to the photobleach. Rod recovery rate was defined as the slope that may represent the second component of dark adaptation and is derived from the modelling of dynamic recovery of rod.

The static rod function was calculated as the point wise sensitivity difference (PWSD) of 505- and 625-nm stimuli at the end of the 30-minute test. The smaller the PWSD, the worse the rod function. This has been described in our previous paper.

To examine the extent of rod dysfunction as a function of eccentricity, the average PWSD between 505- and 625-nm stimuli, the RIT and the rod recovery rate for each concentric ring were calculated and compared between the study groups using 1-way ANOVA test. For RIT analysis, test points which did not recover to the criterion threshold after 30 minutes of DA were arbitrarily assigned a RIT of 30 minutes. RIT was also compared between the central 4° ring and the peripheral 12° ring using a paired t-test. To compare the average PWSD and rod recovery rate at four different ring eccentricities, linear mixed effects analysis was used. Study groups, ring eccentricities and time points were considered as fixed effects and age was treated as an adjusted covariate.

**RESULTS**

A total of 54 eyes of 54 participants (27 normal control, and 27 AMD with bilateral large drusen) participated in our DACP substudy at baseline. Of the 27 eyes with AMD, 14 eyes had RPD and 13 eyes did not have RPD. One participant in the control group was excluded due to progression from no drusen at baseline to “normal aging changes” stage (developed a small druse of <65 μm without AMD pigmented abnormalities) during the 12-month review period. All AMD cases remained with a stable grade classification and none of the participants progressed to late AMD during the 12-month review period. All AMD cases remained with a stable grade classification and none of the participants progressed to late AMD. There were five participants who were unable to complete their 12-month review, four participants (two control, one AMD, and one RPD participants) due to illness, and 1 control participant due to work commitments. Therefore, there were 48 participants (48 eyes) who were included in the final analysis: 23 normal eyes (control group), 12 AMD without RPD (AMD group), and 13 AMD with RPD eyes (RPD group).

The clinical characteristics of the participants who completed the longitudinal study and their visual function at baseline are presented in Table 1. At baseline, the RPD group was significantly older (P = 0.01) and had worse BCVA (P = 0.03) and LLVA (P = 0.04) but not LLD (P = 0.34) when compared to the control group. There was no significant difference between the control and the AMD group nor

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**Table 1. Baseline Characteristics of Participants**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 23)</th>
<th>AMD (n = 13)</th>
<th>RPD (n = 12)</th>
<th>Control vs. AMD P Value</th>
<th>Control vs. RPD P Value</th>
<th>AMD vs. RPD P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD, y</td>
<td>66.1 ± 9.0</td>
<td>71.3 ± 8.7</td>
<td>75.0 ± 7.2</td>
<td>0.25</td>
<td>0.01</td>
<td>0.82</td>
</tr>
<tr>
<td>BCVA, mean ± SD, letters</td>
<td>89.7 ± 5.1</td>
<td>84.9 ± 6.5</td>
<td>84.0 ± 6.7</td>
<td>0.06</td>
<td>0.03</td>
<td>1</td>
</tr>
<tr>
<td>LLVA, mean ± SD, letters</td>
<td>78.8 ± 7.6</td>
<td>71.0 ± 12.3</td>
<td>70.1 ± 9.1</td>
<td>0.06</td>
<td>0.04</td>
<td>1</td>
</tr>
<tr>
<td>LLD, mean ± SD, letters</td>
<td>11.0 ± 3.6</td>
<td>13.8 ± 7.2</td>
<td>13.9 ± 5.1</td>
<td>0.3</td>
<td>0.34</td>
<td>1</td>
</tr>
</tbody>
</table>

Obtained by Bonferroni post-hoc test after 1-way ANOVA.
between AMD and RPD groups for age, BCVA, LLVA, and LLD at baseline (Table 1).

There was no significant change in BCVA, LLVA, and LLD between baseline and 12-month follow-up in control nor the AMD group ($P \geq 0.28$; Table 2). However, in the RPD group, BCVA was significantly worse at the 12-month follow-up (−2.8 ± 0.5 letters, $P = 0.01$), but not the LLVA ($P = 0.11$) nor LLD ($P = 0.59$; Table 2) compared to the baseline.

### Dark Adaptation Parameters

#### Sensitivity Difference Between 505- and 625-nm Stimuli

The average point wise sensitivity differences (PWSD) between 505- and 625-nm wavelengths at various retinal eccentricities are shown in Table 3. The smaller the PWSD indicates the worse rod function. At baseline, the worst rod function was detected at the 4° ring in both AMD and RPD groups. A significant difference in the PWSD was found between RPD and control groups and between RPD and AMD groups, at 4°, 6°, and 8° eccentricities ($P < 0.001$) but not at the 12° ring (Table 3). There was no difference in average PWSD between control and AMD groups at any eccentricity ($P \geq 0.26$; Table 3).

Over 12-month follow-up period, there were no significant changes in PWSD ($P \geq 0.08$) in any of the eccentric rings for both the control and RPD groups (Fig. 2). In the AMD group, the average PWSD improved over a 12-month follow-up at 4° (1 dB, $P = 0.01$), 8° (1.1 dB, $P = 0.04$), and 12° (2.2 dB, $P = 0.03$) rings.

#### Rod Intercept Time (RIT)

RIT could be derived at all locations in all control participants at baseline and 12 months visits. However, there were 5 of 182 (3%) test points in the AMD group and 56 of 168 (33%) test points in the RPD group that did not reach the criterion level after 30 minutes of DA at baseline. After 12 months' follow-up, the proportion of test points that did not reach the rod criterion level for the AMD and RPD groups was 2% and 36%, respectively, with some change in their location. The failed points were located more within the central 4° ring than in the peripheral ring (12° ring) in both disease groups. These points were arbitrarily assigned a maximum RIT value of 30 minutes, hence the average RIT values of these groups was likely underestimated. We found that the average RIT was significantly different between control and AMD groups ($P < 0.001$), AMD and RPD groups ($P < 0.001$), and control and RPD groups ($P < 0.001$) both at baseline or 12 months follow-up. The average RIT in control, AMD, and RPD groups was 7.3 (95% CI: 7.1, 7.5), 11.8 (95% CI: 11.0, 12.7), and 19.5 (95% CI: 18.2, 20.8) minutes, respectively, at baseline and 6.7 (95% CI: 6.5, 7.0), 11.7 (95% CI: 10.9, 12.4), and 19.1 (95% CI: 17.8, 20.5) minutes, respectively, at 12 months' follow-up (Fig. 3A). During this review period, the RIT improved significantly in control group (0.6 ± 0.2 minutes, $P = 0.002$), with the improvement mainly noted at 12° ring (0.9 ± 0.4 minutes, $P = 0.013$). The average RIT change in AMD (0.1 ± 0.5 minutes, $P = 0.75$) and RPD (0.4 ± 1.0 minutes, $P = 0.71$) groups was not different from the baseline.

To determine whether the difference in RIT among the groups varied with retinal eccentricity, we evaluated the RIT by ring eccentricities (Fig. 3B). In the control group, the average RIT at baseline was 7.4 minutes (95% CI: 6.9, 7.9) at 4° eccentricity and the mean RIT did not vary with eccentricity ($P = 0.45$). At the 12-month visit, the average RIT was 6.9 minutes (95% CI: 6.3, 7.5), and the average RIT was also not different with eccentricity ($P = 0.42$). In the AMD and RPD groups, the average RIT was greatest at the 4° ring, 14.7 minutes (95% CI: 12.7, 16.6) in the AMD group and 21.6 minutes (95% CI: 19.3, 23.8) in the RPD group, respectively, and the least at the 12° ring, 8.6 minutes (95% CI: 7.4, 9.8) in the AMD group and 15.3 minutes (95% CI: 11.4, 19.2) in the RPD group, respectively, at baseline. The greatest deviation from normal control eyes was at the 4° ring. After 12 months' follow-up, the RIT remained longest at 4° ring in both AMD and RPD groups, AMD group 13.7 minutes (95% CI: 12.2, 15.2) with the RPD group recording the longest RIT 21.4 minutes (95% CI: 18.8, 24.0). There remained a significant difference between the 12° and the 4° ring in AMD ($P < 0.001$) and RPD ($P = 0.002$) groups, but not in control group ($P = 0.42$). Over time, there was no RIT change at any eccentricities in AMD ($P \geq 0.45$) and RPD ($P \geq 0.45$) groups.

In a separate analysis, when we excluded the points that did not reach the criterion level, the average RIT remained significantly different between all study groups both at baseline ($P < 0.001$) or 12 months' follow-up ($P \leq 0.002$). There were no significant changes in RIT over the 12-month follow up at any eccentricities for both the AMD ($P \geq 0.11$) and RPD groups ($P \geq 0.45$).

#### Rod Recovery Rate

At baseline, the rod recovery rate of each ring eccentricity was significantly different between all study groups ($P \leq 0.012$). The recovery was slowest at the 4° ring and was faster with the increasing eccentricities in both AMD and RPD groups but was relatively consistent in control group at all ring eccentricities (Table 4). The RRR at the 4°

### Table 2. Change in Visual Acuity Between Baseline and 12 Months Follow-up

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>AMD</th>
<th>RPD</th>
<th>Control vs. AMD</th>
<th>Control vs. RPD</th>
<th>AMD vs. RPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCVA, mean ± SD, letters</td>
<td>88.8 ± 6.3</td>
<td>85.1 ± 5.4</td>
<td>81.7 ± 6.2</td>
<td>−0.9 ± 4.1</td>
<td>0.2 ± 4.5</td>
<td>0.29</td>
</tr>
<tr>
<td>LLVA, mean ± SD, letters</td>
<td>77.4 ± 7.9</td>
<td>70.1 ± 14.1</td>
<td>66.8 ± 8.3</td>
<td>−1.4 ± 5.8</td>
<td>−0.6 ± 5.0</td>
<td>0.28</td>
</tr>
<tr>
<td>LLD, mean ± SD, letters</td>
<td>11.4 ± 4.2</td>
<td>14.7 ± 9.8</td>
<td>14.8 ± 5.3</td>
<td>0.4 ± 4.1</td>
<td>0.8 ± 4.9</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Obtained by paired t-test.

### Table 3. Baseline Average Point Wise Sensitivity Differences (PWSD) in the 3 Study Groups at Different Eccentricities

<table>
<thead>
<tr>
<th>Ring, °</th>
<th>Control</th>
<th>AMD</th>
<th>RPD</th>
<th>Control vs. AMD</th>
<th>Control vs. RPD</th>
<th>AMD vs. RPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>20.5 ± 2.6</td>
<td>19.1 ± 3.4</td>
<td>13.5 ± 8.0</td>
<td>0.26</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6</td>
<td>21.3 ± 2.9</td>
<td>20.4 ± 4.0</td>
<td>14.7 ± 7.2</td>
<td>0.77</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>8</td>
<td>21.6 ± 2.6</td>
<td>21.2 ± 2.8</td>
<td>17.2 ± 6.6</td>
<td>1</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>12</td>
<td>21.9 ± 3.7</td>
<td>21.3 ± 4.7</td>
<td>20.2 ± 5.7</td>
<td>1</td>
<td>0.42</td>
<td>1</td>
</tr>
</tbody>
</table>

Obtained by Bonferroni post-hoc after 1-way ANOVA.
rings was slowest in the RPD group (0.11 ± 0.07) compared to the AMD and control groups (0.15 ± 0.07 and 0.26 ± 0.08 log units/minute respectively). At 12 months’ follow-up, there was no significant change in RRR over time at each ring eccentricity in the control and RPD groups between the visits (P > 0.08). In the AMD group however, there was a significant slowing of the RRR in the 12° ring (P = 0.02) with the average slope approaching the toward the level of the RPD group (Fig. 4).

**DISCUSSION**

This longitudinal study evaluated rod functional changes over 12 months in a cohort of participants all with intermediate AMD with and without the presence of RPD using a dark-adapted chromatic perimeter. We found that the greatest rod dysfunction was within the central 8° of the macula in the RPD group in the agreement with a previous report. However, of the rod functional parameters evaluated, only the rod recovery rate at 12° from the fovea in the AMD group showed significant worsen over time.

Rod recovery rate is an important parameter to evaluate rod function as it is not affected by the levels of photobleach (if >20% is used) and always returns with a value for every location. Our findings on the rod recovery rate for normal eyes were similar to those reported in other studies. Rod recovery was slowest in the RPD group and also reduced in the AMD group that did not reach the rod intercept level after 30 minutes of dark adaptation. While slightly smaller than in our previous protocol where testing was only for 20 minutes (46%), we still had about a third of all points in the AMD group not reaching the rod intercept level, and as such was uninformative to follow over time. Previously we have reported that RPD patients could take many hours to recover after bleach, with many test points not recovering after 24 hours of dark adaptation. We designed a 30-minute testing protocol so that it could be used clinically but it may be that a longer testing duration is required to be able to accurately measure the true extent of rod dysfunction and capture change over time. Thus, RIT may not be a clinically useful parameter for monitoring functional changes in RPD eyes due to the long testing time required.

Static rod function did not deteriorate over time in any study groups and in fact improved in the AMD group at most eccentricity. This is most likely a result of a learning effect in the AMD group, where the control group performed well and may have reached a ceiling response, while the RPD group performed poorly with little ability to improve through learning. A slight variation in bleach levels between the two visits might explain a variation, this is not likely to have arbitrarily affected one disease group more than another. Furthermore, although we found a 1- to 2.2-dB improvement in the iAMD group over time, this is well within the intersession test retest coefficient of repeatability of approximately ± 5 dB. Hence, the improvement in static rod function observed in this study unlikely to yield a clinical significant.

Besides measuring rod function, we also evaluate visual function changes in this study. Neither BCVA, LLVA, nor LLD did change significantly over the 12 months’ follow-up in either of the AMD nor control groups. BCVA, but not LLVA nor LLD did decline in the RPD group over a 12-month follow-up. However, none of these acuity tests were particularly good to differentiate changes between groups. The worsening of BCVA in RPD eyes suggests that it was not only rod function that is impaired, but cone function is also being affected. This finding is consistent with observation using adaptive optics imaging showing that cone density over reticular pseudodrusen was significantly decreased compared to that over conventional drusen.

As we included only subjects who did not have clinical disease progression in the study, it was not our intention to address the issue of whether changes in rod function observed, in clinically stable eyes are indications of future AMD progression. However, our results suggest that even within
clinically stable eyes, it may be possible to use a functional biomarker to identify those cases most likely to progress to vision loss. Since we were able to detect a small change in rod function within a period of 12 months, the next step will be conducting prospective studies with a longer follow-up to examine the relationship between rod function changes and clinical AMD progression.

The strength of our study was that we evaluated multiple testing points (14 points) within one eye and even with the small number of participants the amount of individual test point data is large. This allowed us to report on the different sensitivities at different eccentricities. Another strength of the study was that our AMD cohort had participants at the same stage of disease (intermediate stage with large drusen size

![Figure 3](image_url)

**Figure 3.** RITs for all test points are plotted for baseline and 12 months’ follow-up. (A) The overall RIT and (B) The RIT at various ring eccentricities. For points not reaching the criterion level, they are plotted at the top of the graph arbitrarily assigned as 30 minutes, but no RIT was able to be derived for these points. At both baseline and 12 months’ visits, the overall RIT was significantly longer in both AMD and RPD groups compared to control group (A) and RITs in the central retina were worse compared to peripheral macula (B). The control group had very consistent RIT across the 4 rings within 12° of the central macula. The 8° ring returned the longest RIT in both the AMD group and RPD group (B). Error bars represent mean ± 95% CI.

### Table 4. Baseline Rod Recovery Rate in 3 Study Groups at Different Eccentricities

<table>
<thead>
<tr>
<th>Ring (°)</th>
<th>Control</th>
<th>AMD</th>
<th>RPD</th>
<th>Control vs. AMD</th>
<th>Control vs. RPD</th>
<th>AMD vs. RPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.26 ± 0.08</td>
<td>0.15 ± 0.07</td>
<td>0.11 ± 0.07</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>6</td>
<td>0.25 ± 0.07</td>
<td>0.16 ± 0.06</td>
<td>0.10 ± 0.07</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>8</td>
<td>0.26 ± 0.06</td>
<td>0.17 ± 0.05</td>
<td>0.12 ± 0.07</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>12</td>
<td>0.26 ± 0.06</td>
<td>0.22 ± 0.05</td>
<td>0.15 ± 0.09</td>
<td>0.012</td>
<td>&lt;0.001</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Obtained by Bonferroni post hoc after 1-way ANOVA.
A declining of RRR was noted in the AMD group, but only at the 12 month visit was not changed after 12 months’ follow-up in all study groups. The RPD group had the slowest approach to evaluate progression of AMD over time.

Severity, testing the more peripheral locations could be a useful way to evaluate the disease. Within the macula were important to evaluate the disease functional changes over time. While the central 8 points follow up was detected in eyes with AMD but only at the 12 month RIT, thus losing the actual valuable point wise data, especially in RPD cases.

In conclusion, worsening of rod function over a 12-month follow up was detected in eyes with AMD but only at the 12" test points. Since the degree of rod dysfunction is related to AMD phenotypes and retinal eccentricities, evaluating rod function at multiple locations may improve the detection of functional changes over time. While the central 8 points within the macula were important to evaluate the disease severity, testing the more peripheral locations could be a useful approach to evaluate progression of AMD over time.

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References


