Special Issue

Determining Optimal Test Parameters for Assessing Dark Adaptation in People With Intermediate Age-Related Macular Degeneration

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Purpose. The primary aim was to determine optimal test conditions for evaluating dark adaptation in intermediate age-related macular degeneration (iAMD) in order to minimize test time while maintaining diagnostic sensitivity.

Methods. People with AMD and age-similar controls were recruited (aged >55 years). Rod intercept time (RIT) was assessed after a 76%, 70%, and 65% rhodopsin bleach at 5° eccentricity and 76% and 70% bleach at 12°. Test order was randomized and a 30-minute washout period added between tests. Results were compared between control and iAMD groups and receiver operating characteristics (ROC) curves were constructed.

Results. A total of 26 participants with variable grades of macular health attended for two visits. There was a statistically significant difference in average RIT between the control and iAMD groups at 5° (median, IQR controls = 5.8 minutes, 3.8–7.5; iAMD = 20.6 minutes, 11.1–30.0; Mann-Whitney, $P = 0.01$) and at 12° (mean, controls: 4.54 minutes ± 2.12 SD, iAMD = 7.72 minutes ± 3.37 SD; independent samples *t*-test, $P = 0.03$) following a 76% bleach. Area under the ROC curves was 0.83 (confidence interval [CI]: 0.64–1.0) and 0.79 (CI: 0.59–0.99) for these two test conditions, respectively. Five participants (45%) in the iAMD group had RITs >20 minutes for 76% bleach at 5°, but none for any other test condition.

Conclusions. Nearly half of the participants with iAMD produced unacceptably long recovery times (>20 minutes) using a 76% bleach at 5° eccentricity. The 76% bleach at 12° provided almost equivalent separation between AMD and controls but recovery was achieved within 20 minutes.

Keywords: dark adaptation, age-related macular degeneration, rod intercept time

AMD remains the leading cause of blindness in developed countries, despite the advent of anti-vascular endothelial growth factor therapy to treat the neovascular form of the condition.[1–4] The advanced forms of AMD are associated with a debilitating loss of central vision, impacting on ability to perform activities of daily living,[5,6] quality of life,[5,7–9] well-being and mood,[5,10,11] social participation,[12] and risk of falls.[13–15] A key aim of future interventions must be to prevent the progression to this end stage in people with intermediate AMD. Sensitive biomarkers of disease progression have the potential to facilitate cost-effective evaluation of new interventions.[16,17]

The time taken for rods to dark adapt, assessed using an adaptometer (AdaptDX; Maculogix, Middletown, PA, USA), has been shown to progressively increase with increasing severity of AMD, suggesting that it has potential as a biomarker for disease progression.[18–21] However, substantial longitudinal data are required to confirm the clinical utility of the test in this capacity. The test records the so-called 'rod intercept time' (RIT; i.e., time taken to reach a threshold located within the second component of rod recovery).[19] Previous studies have mainly aimed to develop a diagnostic test using the device, whereby it is appropriate to designate individuals as 'abnormal' if RIT exceeds a criterion time.[19,22–24] However, for longitudinal evaluation, it is necessary to obtain an actual value for the rate of rod adaptation, to allow assessment of progression.

Longitudinal studies are expensive to conduct, requiring a large cohort of participants to be followed over a prolonged period. As such, optimization of the dark adaptation test protocol is necessary to ensure that maximum value is obtained from future cohort studies. The optimal testing protocol would obtain a valid RIT result within a clinically viable timeframe, while maintaining sensitivity to disease progression. This may be achieved by modifying test location or bleach intensity. Previous studies have employed a 5° or 11°/12° test locus in the inferior field, with either an 82%, 83%, or 76% equivalent photopigment bleach.[18–29] Published data suggest that a 76% bleach of rod photopigment is sufficient to highlight the AMD-related deficit.[19] One of the key issues is demonstrated by the findings of Owsley et al.[20] When recovery was recorded at a location 11° in the inferior field of 30 individuals with iAMD after a 76% equivalent rhodopsin bleach, 11 participants (37%) had a RIT exceeding 20 minutes at baseline. A test protocol which could reliably be completed within 20 minutes would be preferable, given the restricted time available within clinical environments. Given the known preferential damage of the parafoveal retina in the earliest stages of AMD,[30,31] the test location of 5° is likely to provide greater sensitivity to the earliest manifestation of the disease than the 12° location but at

Table 1. Test Parameters Used in Each Condition

Test	Equivalent Rhodopsin Bleach, %[34,43]	Bleach Luminance (scot cd/m^{2}s)	Location
1	76	1.8×10^4	5° inferiorly
2	70	5.8×10^3	5° inferiorly
3	65	2.4×10^3	5° inferiorly
4	76	1.8×10^4	12° inferiorly
5	70	5.8×10^3	12° inferiorly

Test order was randomized.

the expense of a longer testing time in those with more advanced changes.

The aim of this study was to determine the optimal bleach intensity and retinal location combination for the dark adaptation assessment of people with iAMD.

Methods

People with early AMD, iAMD, non-central geographic atrophy and age-similar controls were recruited through advertisements in the Macular Society Sideview magazine and by contacting a database of research volunteers at City, University of London. Additional control participants were recruited through an advertisement posted in the International Glaucoma Association magazine. Inclusion criteria included age >55 years, sufficiently clear ocular media and adequate pupillary dilation and fixation to permit quality fundus imaging (Grade ≤2 on the LOCS III scale[32]), signs of AMD or healthy ageing (controls) in test eye, best corrected visual acuity of 6/30 (logMAR 0.7) or better in test eye. Participants were excluded if they had received any previous treatment for AMD, any other retinal or optic nerve pathology in the test eye (such as glaucoma), significant systemic disease known to affect visual function, or a history of medication known to affect visual function. Participants were also excluded if they were unable to perform the test. The participant information sheet was provided to all participants at least 24 hours before the first study visit, and each participant was given the opportunity to ask questions before signing a consent form. Institutional research ethical approval was obtained (School of Health Sciences, City, University of London), and all procedures adhered to the tenets of the Declaration of Helsinki.

Rod Intercept Time was assessed under five test conditions over two visits (see Table 1). Test order was randomized. At visit 1, a spectral domain optical coherence tomography (SD-OCT) image was captured using the Spectralis device (30° × 20° volume scan, Heidelberg Engineering, Heidelberg, Germany) in order to determine the AMD status of each eye before selecting an eye for testing, without exposing the eyes to a bright adapting light. A randomization schedule was used to select the study eye if both were eligible for inclusion. After structured history and symptoms, a brief objective and subjective refraction took place and best corrected visual acuity (BCVA) was measured on a letter-by-letter basis and recorded as a recorded as a logMAR score for each eye using the Early Treatment of Diabetic Retinopathy Study (ETDRS) chart at 4 m.

After baseline data had been collected, the participant was seated in a dark room for 30 minutes, during which time the procedure was explained. The adaptometer (Maculogix) measures and adjusts for pupil diameter, hence pupil dilation was not required prior to testing. The RIT was then tested using one of the test conditions (see Table 1), selected according to the randomization schedule. When data collection for the first test was complete, a 30-minute washout and rest period was provided, before conducting the second test. Fundus photography was conducted on both eyes at the end of the visit (30° field centered on the fovea; Topcon 3D OCT; Topcon, Japan) to minimize the impact on the state of retinal adaptation. If pupils were too small to allow adequate imaging of the retina, pupils were dilated using tropicamide 1.0%. Fundus photographs were graded for AMD severity by an Optometrist grader (DT or LE) according to the Beckman initiative severity scale,[33] and OCT images were used to confirm the presence/absence of features of advanced AMD. The Beckman initiative scale comprises five stages; no apparent aging changes, normal aging changes, early AMD, intermediate AMD, and late AMD. Early AMD is characterized by medium sized drusen (>63 μm and ≤125 μm in the absence of pigmentary abnormalities), while intermediate AMD features drusen > 125 μm and/or pigmentary abnormalities and late AMD comprises either geographic atrophy or neovascular AMD.

At the second visit, visual acuities were rechecked and the participant questioned about any change in their ocular status. Following an initial 30-minute period of dark adaptation, the remaining three test conditions were assessed, again with a 30-minute washout period between each.

Adaptometry Procedure

Before dark adaptation commenced, a spherical trial lens was inserted into the lens holder of the adaptometer (+3.00 DS + spherical distance prescription). The participant was instructed to place their head on the chinrest of the adaptometer, and to focus on a 635-nm fixation light. An infrared camera displayed the eye on a monitor. The examiner adjusted the chin-rest position in order to achieve the correct centration of the participant's eye relative to the fixation target.

The pupil diameter was automatically measured prior to the delivery of a 505-nm photoflash (~80 ms duration, equivalent bleach described in Table 1). The luminance of the flash was modified by the device to account for baseline pupil diameter in producing a consistent retinal illuminance. The flash subtended 4° and was centered at either 5 or 12° on the inferior vertical meridian. The test stimulus was presented at the same location as the bleach. Threshold was measured for a 2° diameter, 505 nm circular target, beginning 15 seconds after the bleaching flash. The participant was instructed to maintain fixation on the red fixation light and to press a response button when a flashing target first became visible within the bleached area. Threshold was estimated using a 3-down/1-up modified staircase estimate procedure and continued at 30-second intervals for 30 minutes or until the rod intercept was reached. There was a 15-second rest period after each threshold was set. If the RIT was not reached within the test, it was set at the maximum test duration (30 minutes).

The device records the percentage of threshold points which are suggestive of a fixation error. In this study, as in previous reports,[19] if fixation errors exceeded 30%, the test was deemed unreliable. An additional potential source of error was the bleach not being presented to the correct retinal location, or while the eye was fully open. Participants were invited to return for a third visit to repeat any unreliable tests. If the results were not valid on repetition, then data were not supplied for this test condition, but the remaining test results were included in the analysis.

Analysis

Statistical analysis was carried out in a statistical environment (IBM SPSS, version 25; IBM Corp., Armonk, NY, USA), and

graphs were constructed in spreadsheet software (Excel 2016; Microsoft Corp., Redmond, WA, USA) and using the *ggplot2* and *pROC* packages in R 3.4.3 (http://www.r-project.org/) under R Studio, version 1.1.383 (RStudio, Boston, MA, USA). The Shapiro-Wilk test was used to assess normality of data distribution. Results were compared between those with and without AMD (independent samples *t*-test or Mann-Whitney *U* test for nonnormally distributed data), and ROC curves were constructed in order to assess the sensitivity of the test conditions to disease-related adaptational dysfunction. A 1-way ANOVA test with post-hoc Bonferroni testing (or Kruskal Wallis with post-hoc Mann-Whitney) was used to determine the differences in RIT between test conditions within each group. The proportion of participants failing to reach the RIT within a 20-minute window was also evaluated.

Results

Twenty-six participants with variable grades of macular health participated in the study ($n = 10$ controls, $n = 2$ early AMD, $n = 10$ iAMD, $n = 4$ geographic atrophy). The main analysis consisted of a comparison between control and iAMD groups. Early AMD and geographic atrophy data from individuals are presented for comparison. Six other volunteers (four AMD, two controls) were excluded due to inability to perform the test reliably (repeated testing yielded fixation errors exceeding 30% or inability to calculate the RIT). A further two individuals with AMD withdrew after one visit, after finding the repeated testing protocol too arduous. There was no significant difference in age (mean controls: 69 years ± 8 SD; mean iAMD: 71 years ± 8 SD, independent samples *t*-test, $P = 0.73$), or in BCVA (mean controls: 0.15 ± 0.15 SD, mean AMD: 0.17 ± 0.19 SD, independent samples *t*-test, $P = 0.22$) between control participants and those with AMD. Details of each participant are given in Table 2.

Figure 1 shows sample recovery data from three control participants and three participants with iAMD for each test condition. Recovery tended to be slower for participants with iAMD, particularly for the 76% bleach condition. It was not possible to obtain an accurate RIT from all participants for all test conditions (see Table 2 for individual data). For the 65% bleach condition, rod intercept could not be calculated for 14/26 (54%) of tests as threshold recovered too rapidly. In contrast, only 2/26 (8%) of tests were invalid at the 5° location following a 76% bleach, and only 4/26 (15%) of tests were invalid in the 12° location for the same bleach. With respect to testing time, for five individuals with iAMD (45%) and one control the RIT exceeded 20 minutes following a 76% bleach at 5° eccentricity. The RIT did not exceed 20 minutes for any individual for any of the other test conditions.

The distributions of RIT for participants with iAMD and controls are shown in Figure 2. Data from the individuals with early AMD and geographic atrophy are shown for comparison. Average recovery times were longer for participants with AMD under all test conditions, with the greatest difference between groups at the 5° location, following the 76% bleach. There was a statistically significant difference in average RIT between the controls and iAMD group for the 5° location following a 76% bleach (median, IQR controls = 5.8 minutes, 3.8–7.5; iAMD = 20.6 minutes, 11.1–30.0; Mann-Whitney *U* test, $P = 0.01$) and for the same bleach at the 12° location (mean, controls: 4.54 minutes ± 2.12 SD, iAMD = 7.72 minutes ± 3.37 SD; independent samples *t*-test, $P = 0.03$). As expected, average recovery times increased in each group with an increasing proportion of photopigment bleached (Fig. 2). Recovery was more rapid for the 12° location than the 5° location for any given bleach intensity, especially for the AMD participants. This was statistically significant for the 5° location, where the 76% bleach produced significantly longer RIT than any other test condition (Kruskal Wallis, $P < 0.001$). The average recovery time for the 5° location following a 76% bleach was more than twice that at the 12° location following the same magnitude of bleach for iAMD participants.

Receiver operating characteristics (ROC) curves were constructed for those tests which differed significantly between groups in the univariate analysis to determine the ability of each test to differentiate between controls and people with iAMD (see Fig. 3). The best separation between groups was exhibited by the 76% bleach at 5° (area under the curve [AUC] = 0.83, CI: 0.64–1.0), and the 76% bleach at 12° (AUC = 0.79, 0.59–0.99 CI). For the 76% at 5° test condition, using the published diagnostic cutoff for the RIT of 6.5 minutes,[19] five out of the eight control participants for whom valid data were collected were correctly identified (63% specificity), while all 10 iAMD participants were correctly identified as abnormal (100% sensitivity). For the 12° location, specificity remained at 63% (5/8 controls correctly identified) while sensitivity fell to 89% (8/9 AMD for whom data were available) for a 5.5-minute optimal cutoff.

Discussion

Participants with iAMD were of key interest in this exploratory study. A control comparator group was included to ensure that sensitivity to the disease related deficit was maintained. Dark adaptation assessed using a 76% equivalent bleach at a location 5° in the inferior field provided optimal separation between people with AMD, predominantly of the intermediate stage, and healthy controls, but at the expense of a long recording duration. An alternative test location at 12° in the inferior field was still able to distinguish between groups in our sample of data, but recording time was reduced by more than 50%.

Mean recovery time for people with iAMD in this study for the 76% bleach at 5° was 19.9 minutes ± 10.6 SD. This was broadly in alignment with the 16.6 minutes ± 5.2 SD reported by Jackson et al. in their larger sample of 72 participants with iAMD using the same test parameters.[19] Owsley et al.[20] reported a substantially longer recovery time in their cohort of 30 participants with iAMD, assessed using a 76% bleach at 11° eccentricity (20.3 minutes ± 14.3 SD vs. 7.7 minutes ± 3.3 SD for the participants with iAMD in the current study). This is likely to be due to the difference in severity grading between studies. Owsley required multiple, large confluent drusen, with or without focal pigmentary changes. In contrast, the Beckman scale employed in the current study classifies a patient as having iAMD if only one large druse is present.[33] Furthermore, 21/30 (70%) of the participants in Owsley's study had advanced AMD in the fellow eye, compared to 3/10 (30%) of participants with iAMD in the current study. The presence of nAMD in the fellow eye has been reported to be associated with an increased delay in RIT,[21] so the increased proportion of people with advanced disease in the fellow eye is likely to have resulted in an increase in RIT in the previous study.

As reported in previous studies,[19–21,23] we found a substantial variation in the recovery times between people with comparable retinal appearance. It has been reported that the presence of any structural abnormality in the retinal testing spot is associated with a significantly prolonged RIT during dark adaptation assessment.[26] Thus, the variability is likely to reflect the topographical heterogeneity of the disease process in AMD, and the focal nature of the test stimulus used in this device. This variability in results was also high for the control group. This is in alignment with the findings of Owsley et al.,[23] who found that one quarter of 381 apparently macular healthy

Table 2. Clinical Characteristics of all Participants

Px ID	LogMAR Test Eye	AMD Status Test Eye	AMD Status Fellow Eye	Rod Intercept Time/Min				
				5° at 65%	5° at 70%	5° at 76%	12° at 70%	12° at 76%
RR0013	0.16	1	1	1.6	3.5	6.5	2.9	7.5
ET0007	0.34	1	1	1.4	1.4	5.1	2.3	NC
JE0008	0.00	1	1	1.7	6.0	4.1	UD	6.3
KG0020	0.46	1	1	NC	3.3	UD	2.2	NC
JC0032	0.16	1	1	1.2	2.7	3.0	2.1	5.2
GM0035	−0.04	1	1	NC	2.6	12.8	2.2	1.8
BW0037	0.00	1	1	NC	13.1	3.1	1.9	5.0
MI0033	0.16	1	1	NC	1.3	21.5†	NC	2.7
SF0034	0.10	1	1	NC	NC	UD	0.9	2.0
FJ0038	0.16	1	1	NC	1.3	7.5	3.6	5.8
AG0002	0.20	2	2	NC	7.1	12.3	NC	NC
KM0003	0.16	2	2	NC	NC	14.5	NC	3.6
DH0005	0.44	3	3	1.7	1.5	10.8	2.6	2.6
MM0006	0.20	3	3	1.4	11.1	7.6	NC	6.7
GE0010	0.00	3	3	NC	3.5	12.0	2.9	6.8
PS0012	0.20	3	3	NC	4.2	30.0*	UD	6.9
GD0014	−0.04	3	3	NC	UD	29.0†	1.5	6.0
VC0015	0.02	3	4	NC	2.1	30.0*	5.6	10.2
BB0016	0.42	3	4	0.9	1.3	30.0*	NC	NC
PN0009	0.06	3	4	1.2	11.2	7.3	2.0	5.9
JB0018	0.00	3	3	1.2	UD	12.3	3.9	10.1
WP0032	0.40	3	3	1.7	9.4	30.0*	2.2	14.4
JG0027	0.20	4	4	NC	10.0	4.6	NC	12.3
EC0011	0.44	4	4	1.8	1.5	10.6	2.1	8.7
AF0028	0.50	4	4	2.6	1.9	14.0	1.7	3.2
PF0031	0.12	4	4	NC	8.9	3.8	2.2	2.3

AMD status refers to the Beckman severity scale, where 1 = no changes or normal aging changes, 2 = early AMD, 3 = iAMD, and 4 = late AMD.[33] NC indicates that rod intercept could not be calculated due to threshold dropping too rapidly to below 3.0 log units of stimulus attenuation (due to insufficient bleach intensity or bleach ineffectively delivered), UD indicates unreliable data (fixation errors exceeding 30%).

* RIT was not reached within the maximum test duration, and a value of 30 minutes was ascribed.

† RIT exceeded the 20-minute optimal maximum test duration.

individuals had abnormally long dark adaptation times.[23] This was associated with known risk factors for AMD onset. The possibility that the control group in the current study was heterogenous is also indicated by the lower than expected specificity of the 76% bleach at 5° test condition (63% vs. 90% in a previous report[19]). It is possible that those whose adaptation times extended beyond the 6.5-minute cutoff may be found in future to have been at a sub-clinical stage of AMD onset.

As in previous reports,[20] we also found some individuals to show a very rapid rate of recovery. In some cases, this resulted in the inability of the device to calculate the RIT (see Table 2). This is likely to reflect a loss in fixation or a blink of the participant during the presentation of the bleach, resulting in a failure to present the photoflash to the correct area of the retina. As the bleaching flash subtends only 4°, and the test stimulus is 2° in diameter, a small eye movement of more than 1° degree in either direction during the presentation of the bleach may result in the test stimulus being presented to an unbleached area of the retina during the test. However, the high number of cases of failures to calculate the rod intercept for the lowest bleach (54% of tests were invalid for the 65% equivalent bleaching flash), including otherwise compliant participants, suggests that for this intensity even a correctly aligned bleaching flash produced a recovery which was too rapid to evaluate using this technology.

It is important to note that the aim of the current study was to determine optimal testing conditions for evaluating people with iAMD for longitudinal evaluation. As such, the inter-participant variability and diagnostic accuracy is not of prime importance as each individual will act as their own comparator in longitudinal evaluation. However, some evidence that the test is challenging the macula sufficiently to reveal AMD-related deficits is required. This is why a comparison of average data between AMD and control groups was carried out, as well as the ROC analysis. On the basis of this analysis, which showed very little separation between iAMD and control groups for the 70% and 65% bleach conditions, the lower bleach conditions evaluated in this study would not be recommended for longitudinal evaluation.

The earliest AMD related damage appears in the parafoveal region, from 2 to 4° from fixation, decreasing steadily with increasing eccentricity.[30,31] On this basis, the 5° retinal eccentricity would seem a more logical choice for longitudinal evaluation of AMD than the 12° retinal location. However, this study and others[19,21,26] have demonstrated that the time taken for people with iAMD to reach the rod intercept in the 5° location is substantial and may exceed 20 minutes in a quarter or more of participants. Threshold measurement continues throughout this time, which is fatiguing for patients (a factor known to reduce performance in psychophysical tests[35]), and is inconvenient and costly in terms of clinic time. Although the 12° location showed a slightly reduced separation between groups in this study, the area under the ROC curve for our sample of data remained high, suggesting that there is sensitivity to disease-related dysfunction at this location, at least for a cohort of people with iAMD. This is in accordance with evidence that AMD related abnormalities extend beyond the parafovea, especially as the disease progresses.[20,30,36–40] For example, Owsley et al.[40] demonstrated a significant deficit

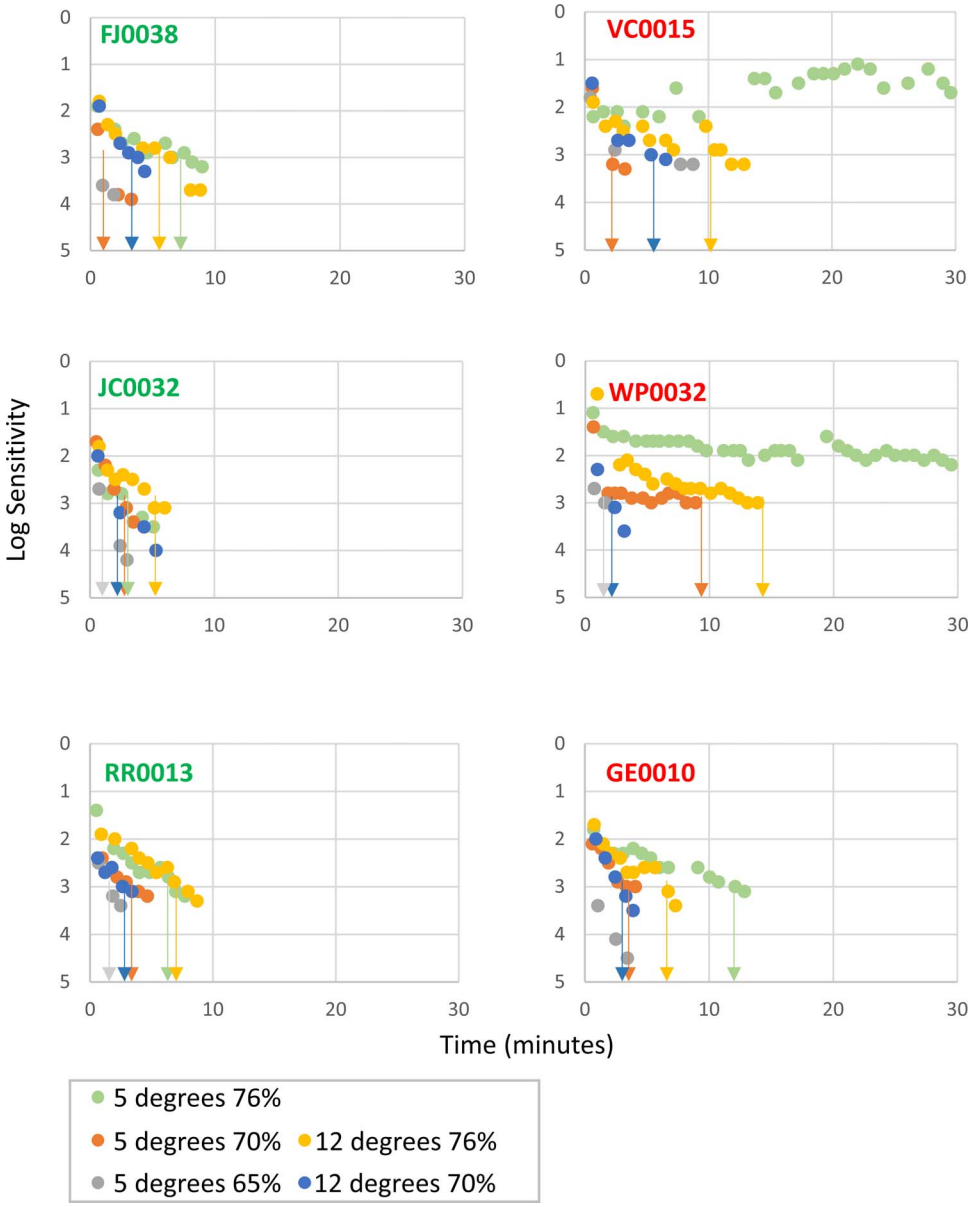

FIGURE 1. Graph showing sample adaptometer (Maculogix) data for three controls (RR0013, JC0032, FJ0038) and three participants with iAMD (WP0032, GE0010, VC0015). *Arrows* indicate the RIT for each participant, where it occurred within the 30-minute testing window. For example, for participant GE0010 the rod intercept was too fast to evaluate using the 5° at 65% condition while the RIT for the 70% bleach at 5° was 3.5 minutes, the 76% bleach at 5° was 12 minutes, the 70% bleach at 12° was 2.9 minutes, and the 76% bleach at 12° was 6.8 minutes. The recovery times for the participants with AMD tended to be longer, especially for the 76% bleach.

in rod adaptation parameters in people with early AMD and near normal VA at a locus 12 degrees on the vertical meridian. Nguyen et al.[38] in a study of retinotopic rod function showed that, while the effect of the disease was greatest in the central macula, as expected, there was a significant difference in RIT between controls and people with iAMD at the 12° location. Owsley et al.[20] reported that, when RIT was evaluated at 11° eccentricity, 22 of 30 eyes (73.3%) with iAMD had an increase in RIT exceeding 1 minute over a follow-up period of 24 months, and 17 of 30 eyes (56.7%) had an increase exceeding 3 minutes. This suggests that the 12° location is likely to be sensitive to disease progression in people with iAMD. However, this requires further longitudinal evaluation, and it is still likely to be more appropriate to adopt the 5° location when evaluating a cohort of individuals with early AMD who are likely to proceed more rapidly with dark adaptation than those with iAMD.[19]

The strength of this study is that, for the first time, a comparison is made of the bleach recovery characteristics of people with iAMD using different test protocols of the adaptometer (Maculogix). This will assist future longitudinal study design. One limitation of this study was that a relatively high proportion of patients was unable to complete all test conditions. It should be noted, however, that under clinical trial conditions it would not be recommended to carry out more than one trial in a session. Fatigue may have affected performance in the current study, in which three consecutive trials were recorded in a single visit, resulting in an increased number of missing datasets. The sample size in this exploratory study was, consequently, small. An additional limitation is that, to minimize the participant burden in this study, a limited

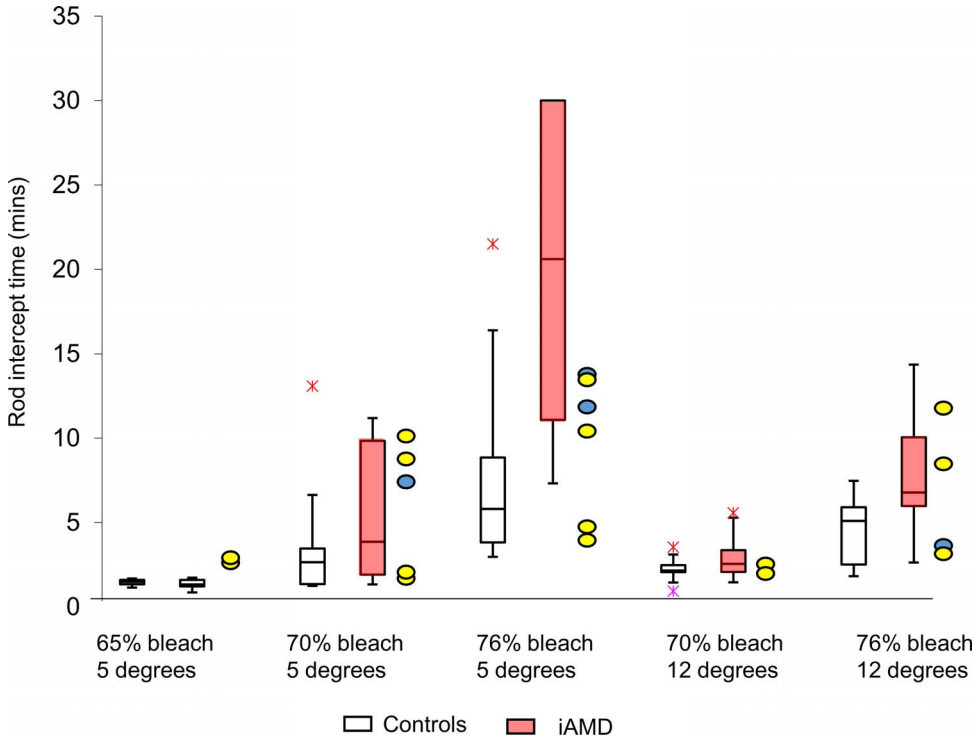

Figure 2. *Box-and-whisker* plots showing distribution of rod intercept time data for all participants for all test conditions. The ends of the *whiskers* are set at ×1.5 the IQR above the third quartile and below the first quartile. Values outside this range are shown as outliers (*red* and *pink stars*). The "65%," "70%" and "76%" bleach refer to the equivalent rhodopsin bleach applied before testing began, while the "5°" and "12°" refer to the location in the inferior field at which the stimulus was presented (see Table 1 for test conditions). The *blue* and *yellow circles* present data from individuals with early AMD and geographic atrophy, respectively, for comparison. There is no evidence from these individuals of a marked prolongation in recovery time associated with GA, and there is a substantial spread of recovery times in these individuals, which is in keeping with the variability of results in the main comparison groups.

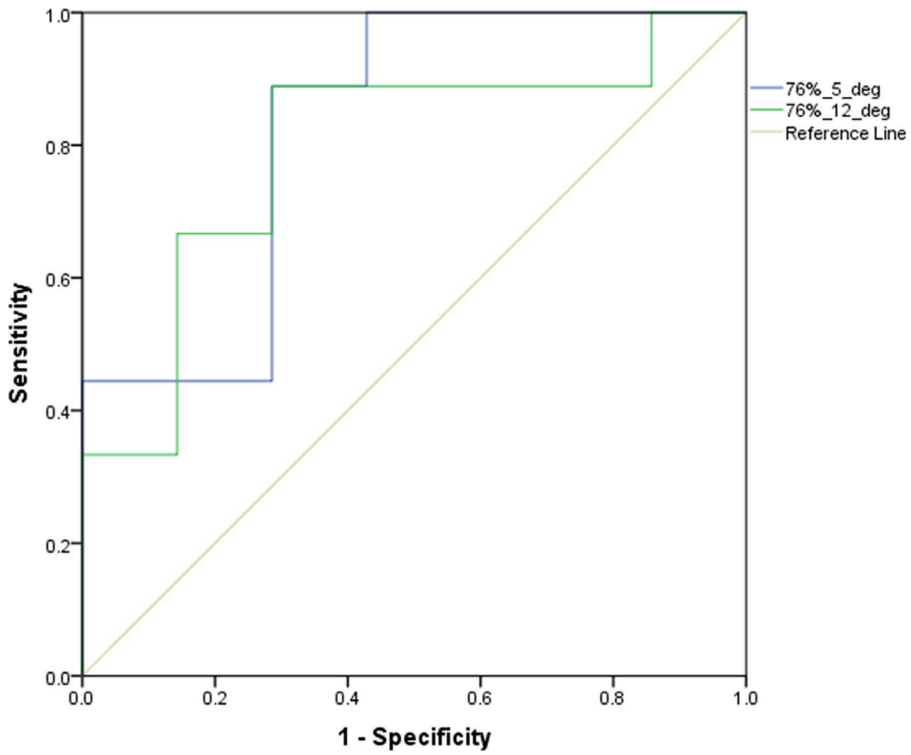

Figure 3. ROC curves and AUC for test conditions which showed a significant difference in univariate analysis, showing ability to discriminate between iAMD and controls for all RIT cutoff points.

range of bleach intensities was explored. The range was chosen after preliminary scoping studies which showed that recovery following equivalent bleaches of less than 65% was too rapid, and did not allow the reliable assessment of the RIT. While studies using other methodologies have reported using much lower bleaches to effectively assess individuals with AMD,[41,42] the psychophysical methods used have differed from those employed by the adaptometer (Maculogix). Furthermore, the bleaching levels calculated by the adaptometer (Maculogix) are based on the equivalent bleach concept, consistent with arguments first given by Pugh.[34,43] This accounts for the fact that it is not possible to bleach 100% of rhodopsin with a photoflash, and that threshold recovery proceeds in a similar fashion after a scotopic-energy equivalent photoflash and long duration bleach, even though a substantially smaller proportion of photopigment is bleached by the photoflash. The lower proportional bleaches cited in some other studies,[41,42] would actually be higher when expressed as an equivalent bleach.

A final limitation of this study related to the lack of grading for the presence or absence of subretinal drusenoid deposits (SDD). This means that the iAMD group is likely to have included a mixture of participants with and without this clinical feature. Prevalence estimates for SDD vary depending on the imaging techniques used, and the classification of AMD. For example, in the Alienor study, using multimodal imaging, the prevalence of SDD in people with early/intermediate AMD was reported as ranging from 13% to 62.6% (in the absence and presence of focal pigmentary changes, respectively).[44] Analysis of color fundus photographs and near-infrared images from the Rotterdam study cohort revealed a prevalence of 35% for individuals with soft drusen OR pigmentary changes, and 14.5% for those with soft drusen AND pigmentary changes.[45] In the current study, as there is substantial evidence to suggest that those with SDD will take longer to reach the rod intercept,[21,26] the composition of the group with respect to SDD status is likely to have had an impact on the performance characteristics under each test condition. Future studies employing a higher proportion of participants with SDD may find that the test conditions identified as optimal here require an unacceptably long recovery time, while the 5° location may provide a higher sensitivity to progression of disease-related changes in a group which has a lower proportion of participants with SDD than the current study. Grading scales and most clinical trials of iAMD currently do not include SDD as a grading or inclusion criterion. For example, NCT02848313 is a trial of subcutaneous elamipretide for iAMD, in which iAMD is defined by the presence of large/medium sized drusen with or without non-central geographic atrophy. Similarly, trials NCT00776451 and NCT03349801 also make no mention of SDD in their disease groupings for iAMD individuals. As the aim of this study was specifically to determine the most appropriate test for the longitudinal assessment of a heterogeneous group of individuals with iAMD, such as that which might be recruited into such a trial, subdividing according to their SDD status fell outside of the remit of the current analysis. On the basis of the imaging carried out, it was not possible to carry out accurate retrospective SDD grading for included participants, however it would be of value for future studies to compare the dark adaptation performance of people with and without SDD using the test parameters identified in this study.

To summarize, this exploratory study has demonstrated that the RIT, when assessed at 12° in the inferior field after a 76% equivalent bleach of rod photopigment, shows promise as a clinical test for iAMD. This test combination was able to provide valid results for most participants within a 20-minute test window, while maintaining sensitivity to the disease-related functional deficit.

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References

1. Augood CA, Vingerling JR, De Jong PTVM, et al. Prevalence of age-related maculopathy in older Europeans: The European Eye Study (EUREYE). *Arch Ophthalmol*. 2006;124:529–535.
2. Klein R, Chou CF, Klein BEK, Zhang X, Meuer SM, Saaddine JB. Prevalence of age-related macular degeneration in the US population. *Arch Ophthalmol*. 2011;129:75–80.
3. Jonas JB. Global prevalence of age-related macular degeneration. *Lancet Glob Heal*. 2014;2:e106–e116.
4. Friedman DS, O'Colmain BJ, Muñoz B, et al. Prevalence of age-related macular degeneration in the United States. *Arch Ophthalmol*. 2004;122:564–572.
5. Mathew RS, Delbaere K, Lord SR, Beaumont P, Vaegan, Madigan MC. Depressive symptoms and quality of life in people with age- related macular degeneration. *Ophthalmic Physiol Opt*. 2011;31:375–380.
6. Gopinath B, Liew G, Burlutsky G, Mitchell P. Age-related macular degeneration and 5-year incidence of impaired activities of daily living. *Maturitas*. 2014;77:263–266.
7. Taylor DJ, Edwards LA, Binns AM, Crabb DP. Seeing it differently: Self-reported description of vision loss in dry age-related macular degeneration. *Ophthalmic Physiol Opt*. 2017;38:98–105.
8. Taylor DJ, Hobby AE, Binns AM, Crabb DP. How does age-related macular degeneration affect real-world visual ability and quality of life? A systematic review. *BMJ Open*. 2016;6:e011504.
9. Hassell JB, Lamoureux EL, Keeffe JE. Impact of age related macular degeneration on quality of life. *Br J Ophthalmol*. 2006;90:593–596.
10. Dawson SR, Mallen CD, Gouldstone MB, Yarham R, Mansell G. The prevalence of anxiety and depression in people with age-related macular degeneration: a systematic review of observational study data. *BMC Ophthalmol*. 2014;14:78.
11. Casten RJ, Rovner BW. Update on depression and age-related macular degeneration. *Curr Opin Ophthalmol*. 2013;24:239–243.
12. Cimarolli VR, Boerner K, Reinhardt JP, et al. A population study of correlates of social participation in older adults with age-related vision loss. *Clin Rehabil*. 2017;31:115–125.
13. Wood JM, Lacherez P, Black AA, Cole MH, Boon MY, Kerr GK. Risk of falls, injurious falls, and other injuries resulting from visual impairment among older adults with age-related macular degeneration. *Investig Ophthalmol Vis Sci*. 2011;52:5088–5092.
14. Van Landingham SW, Massof RW, Chan E, Friedman DS, Ramulu PY. Fear of falling in age-related macular degeneration. *BMC Ophthalmol*. 2014;14:1–9.
15. Cruess A, Zlateva G, Xu X, Rochon S. Burden of illness of neovascular age-related macular degeneration in Canada. *Can J Ophthalmol*. 2007;42:836–843.

16. Freidlin B, McShane LM, Korn EL. Randomized clinical trials with biomarkers: Design issues. *J Natl Cancer Inst*. 2010;102: 152–160.
17. Buyse M. Toward validation of statistically reliable biomarkers. *Eur J Cancer Suppl*. 2007;5:89–95.
18. Jackson GR, Clark ME, Scott IU, Walter LE, Quillen DA, Brigell MG. Twelve-month natural history of dark adaptation in patients with AMD. *Optom Vis Sci*. 2014;91:925–931.
19. Jackson GR, Scott IU, Kim IK, Quillen DA, Iannaccone A, Edwards JG. Diagnostic sensitivity and specificity of dark adaptometry for detection of age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2014;55:1427–1431.
20. Owsley C, Clark ME, McGwin G. Natural history of rod-mediated dark adaptation over 2 years in intermediate age-related macular degeneration. *Trans Vis Sci Tech*. 2017;6(3):15.
21. Flamendorf J, Agrón E, Wong WT, et al. Impairments in dark adaptation are associated with age-related macular degeneration severity and subretinal drusenoid deposits. *Ophthalmology*. 2015;122:2053–2062.
22. Jackson GR, Edwards JG. A short-duration dark adaptation protocol for assessment of age-related maculopathy. *J Ocul Biol Dis Infor*. 2008;1:7–11.
23. Owsley C, Huisingh C, Jackson GR, et al. Associations between abnormal rod-mediated dark adaptation and health and functioning in older adults with normal macular health. *Investig Ophthalmol Vis Sci*. 2014;55:4776–4789.
24. Owsley C, McGwin G, Clark ME, et al. Delayed rod-mediated dark adaptation is a functional biomarker for incident early age-related macular degeneration. *Ophthalmology*. 2016;123: 344–351.
25. Owsley C, Huisingh C, Clark ME, Jackson GR, McGwin G. Comparison of visual function in older eyes in the earliest stages of age-related macular degeneration to those in normal macular health. *Curr Eye Res*. 2016;41:266–272.
26. Laíns I, Miller JB, Park DH, et al. Structural changes associated with delayed dark adaptation in age-related macular degeneration. *Ophthalmology*. 2017;124:1340–1352.
27. Munch IC, Altuntas C, Li XQ, Jackson GR, Klefter ON, Larsen M. Dark adaptation in relation to choroidal thickness in healthy young subjects: a cross-sectional, observational study. *BMC Ophthalmol*. 2016;16:1–7.
28. Sevilla MB, McGwin G, Lad EM, et al. Relating retinal morphology and function in aging and early to intermediate age-related macular degeneration subjects. *Am J Ophthalmol*. 2016;165:65–77.
29. Yazdanie M, Alvarez J, Agrón E, et al. Decreased visual function scores on a low luminance questionnaire is associated with impaired dark adaptation. *Ophthalmology*. 2017;124:1332–1339.
30. Owsley C, Jackson GR, Cideciyan AV, et al. Psychophysical evidence for rod vulnerability in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2000;41:267–273.
31. Curcio CA, Medeiros NE, Millican CL. Photoreceptor loss in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 1996;37:1236–1249.
32. Chylack LT, Wolfe JK, Singer DM, et al. The lens opacities classification system III. *Arch Ophthalmol*. 1993;111:831–836.
33. Ferris FL, Wilkinson CP, Bird A, et al. Clinical classification of age-related macular degeneration. *Ophthalmology*. 2013;120: 844–851.
34. Pugh EN. Rushton's paradox: rod dark adaptation after flash photolysis. *J Physiol*. 1975;248:413–431.
35. Hudson C, Wild JM, Neill ECO. Fatigue effects during a single session of automated static threshold perimetry. *Invest Ophthalmol Vis Sci*. 1994;35:268–280.
36. Lengyel I, Csutak A, Florea D, et al. A population-based ultra-widefield digital image grading study for age-related macular degeneration-like lesions at the peripheral retina. *Ophthalmology*. 2015;122:1340–1347.
37. Walter P, Widder RA, Lüke C, Königsfeld P, Brunner R. Electrophysiological abnormalities in age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol*. 1999; 237:962–968.
38. Nguyen CT, Fraser RG, Tan R, et al. Longitudinal changes in retinotopic rod function in intermediate age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2018;59:AMD19–AMD24.
39. Gaffney AJ, Binns AM, Margrain TH. Topography of cone dark adaptation deficits in age-related maculopathy. *Optom Vis Sci*. 2011;88:1080–1087.
40. Owsley C, Jackson GR, White M, Feist R, Edwards D. Delays in rod-mediated dark adaptation in early age-related maculopathy. *Ophthalmology*. 2001;108,1196–1202.
41. Dimitrov PN, Guymer RH, Zele AJ, Anderson AJ, Vingrys AJ. Measuring rod and cone dynamics in age-related maculopathy. *Invest Ophthalmol Vis Sci*. 2008;49:55–65.
42. Fraser RG, Tan R, Ayton LN, Guymer RH, Luu CD. Assessment of retinotopic rod photoreceptor function using a dark-adapted chromatic perimeter in intermediate age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2016;57: 5436–5442.
43. Pugh EN. Rhodopin flash photolysis in man. *J Physiol*. 1975; 248:393–412.
44. Chan H, Cougnard-Gregoire A, Delyfer MN, et al. Multimodal imaging of reticular pseudodrusen in a population-based setting: the Alienor Study. *Invest Ophthalmol Vis Sci*. 2016; 57:3058–3065.
45. Buitendijk GH, Hooghart AJ, Brussee C, et al. Epidemiology of reticular pseudodrusen in age-related macular degeneration: the Rotterdam Study. *Invest Ophthalmol Vis Sci*. 2016;57: 5593–5601.

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