The early stages of age-related macular degeneration (AMD), characterized by fundus features that include drusen and pigment changes, present a window of opportunity in which to intervene before vision is threatened by the development of advanced AMD, in the form of atrophy and/or neovascularization. However, a major challenge to the evaluation of novel interventions in these early stages is the lack of sensitive biomarkers of disease severity and progression. The standard measurement of best-corrected visual acuity (BCVA) is inadequate at these earlier time points in AMD. Novel biomarkers could potentially provide surrogate endpoints to monitor when evaluating novel interventions.

Both objective and subjective methods for determining retinal function have been extensively explored in the early stages of AMD, and they have the potential to act as robust biomarkers of disease severity and progression. A measurement that has been of interest is the multifocal electroretinogram (mfERG), which allows an objective assessment of retinal function. However, reports of mfERG findings in AMD have been inconsistent; some studies have reported significant functional deficits in the early stages of the disease, whereas others have not. This lack of consistency in findings may be attributed to the nonuniformity of disease severity and variation in the age of participants in different studies or differences in the techniques used to record the mfERG.

Although objective measurements of retinal function have conventionally been considered more favorable than subjective measurements, improvements in technology have allowed the development of more precise subjective measurements. One such subjective measurement that has shown to be effective in the early stages of AMD is determination of retinal sensitivity by using fundus-tracked perimetry (or microperimetry). Functional deficits subjectively measured with microperimetry have been shown to correlate with structural changes in high-resolution optical coherence tomography (OCT) in the early stages of AMD. In eyes with AMD, we have previously found the point-wise coefficient of repeatability of microperimetry (representing the location where 95% of the test–retest differences are expected to lie) to be $\pm 4.37$ dB or less. Subjective testing using microperimetry may therefore be as useful as an objective test of retinal function, as a biomarker of disease severity and progression.

### Methods

Sixty AMD participants underwent multifocal electroretinography (mfERG) and microperimetry testing in one eye, and 44 control participants were included to provide normative values for each test. Thirteen hexagons in the central three rings of a 103-hexagon stimulus grid for mfERG and retinotopically matched points on microperimetry were chosen and converted into standard deviations (SDs) away from that of normal participants ($Z$-score) to represent the magnitude of measured functional deficit and to allow a comparison of the two measures.

### Results

Comparing multifocal electroretinography (mfERG) with microperimetry in intermediate age-related macular degeneration (AMD). The measured functional deficit with microperimetry was greater than mfERG parameters in eyes with intermediate AMD. The absence of correlations between these two measures suggests that mfERG may be capturing unique aspects of retinal dysfunction. These findings are important when considering the use of these functional measures in intermediate AMD.

### Conclusions

The measured functional deficit with microperimetry was greater than mfERG parameters in eyes with intermediate AMD. The absence of correlations between these two measures suggests that mfERG may be capturing unique aspects of retinal dysfunction. These findings are important when considering the use of these functional measures in intermediate AMD.
Multifocal ERG and Microperimetry in AMD

Although one study had previously used mfERG and microperimetry to examine retinal function in the early stages of AMD, it was conducted in a small cohort of participants and the mfERG P1 implicit time, which is an important measurement of retinal function in the early stages of AMD, was not examined.16 Given the fact that both measurements offer different advantages and disadvantages, the aim of this study was to correlate and compare the magnitude of measured functional deficits obtained by these two methods in eyes with intermediate AMD.

**METHODS**

This study was approved by the Human Ethics Committee of the Royal Victorian Eye and Ear Hospital (RVEEH) and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants.

**Participants**

Participants were recruited from the medical retina clinic at RVEEH and private ophthalmology clinics. Inclusion criteria for all participants were age 50 years and older, a BCVA of better than 20/40 (or 0.30 logarithm of the minimum angle of resolution [logMAR]) in the study eye, and a refractive error of no greater than ±6.00 diopter (D; spherical equivalent). For AMD participants, the inclusion criterion was intermediate AMD in the study eye, defined as having drusen of >125 μm, with or without pigmentary abnormalities.50 Only one eye was included in this study, and when both eyes met the inclusion criteria, the eye with the better visual acuity was chosen as the study eye. Control participants were recruited from spouses or friends of the AMD participants who were of a similar age, and study eye. Control participants were recruited from spouses or friends of the AMD participants who were of a similar age, and study eye. Inclusion criteria for all participants were age 50 years and older, a BCVA of better than 20/40 (or 0.30 logMAR) in the study eye and a refractive error of no greater than ±6.00 diopter (D; spherical equivalent). For AMD participants, inclusion criterion was intermediate AMD in the study eye, defined as having drusen of >125 μm, with or without pigmentary abnormalities.30 Only one eye was included in this study, and when both eyes met the inclusion criteria, the eye with the better visual acuity was chosen as the study eye. Control participants were recruited from spouses or friends of the AMD participants who were of a similar age, and were required to have no signs of AMD in either eye (including reticular pseudodrusen), although drusen < 63 μm were allowed (normal aging changes)30, when both eyes met the inclusion criteria, the eye with the better visual acuity was also chosen as the study eye. The exclusion criteria for all participants included the presence of geographic atrophy, choroidal neovascularization, significant cataracts, glaucoma, amблиопия, and any corneal pathology that could compromise vision. Participants were also excluded if they had diabetes, any neurological or systemic disease affecting vision, were taking any medication known to affect retinal function (e.g., hydroxychloroquine), any physical and/or mental impairment preventing them from participating in this study, or were unable to sign a consent form. All participants underwent visual acuity testing and microperimetry examinations followed by mfERG testing prior to fundus imaging or examination that would potentially bleach the photoreceptors.

**Visual Acuity Measurements**

Monocular BCVA measurements were made after a standardized refraction protocol was performed, using an Early Treatment of Diabetic Retinopathy Study (ETDRS) refraction chart at 4 meters. All participants were required to guess all the letters in a given line, and visual acuity measurements were recorded as the number of letters read and logMAR value, with each letter given a score of 0.02 logMAR.

**Microperimetry Examination**

All participants were examined using the Macular Integrity Assessment (MAIA; CenterVue, Padova, Italy) microperimeter as described previously.29 Briefly, microperimetry examinations were performed following pupillary dilation using 1 drop of 1% tropicamide and 1 drop of 2.5% phenylephrine to achieve a pupil diameter of at least 6 mm. All participants were given identical verbal instructions regarding performance of the microperimetry examination.

The MAIA performs fundus tracking using a line-scanning laser ophthalmoscope with a superluminescent diode illumination that has a central wavelength of 850 nm. It uses the entire fundus as a reference when performing fundus tracking, capturing the fundus images at 25 frames per second. With fundus tracking, test stimuli can be presented at the same retinal location throughout the test, independent of shifts in fixation. A red circular fixation target of 1° diameter was used in this study, and Goldman III stimuli were presented for 200 milliseconds against a background of 1.27 cd/m² using a 4-2 threshold strategy. The maximum stimulus luminance was 318 cd²/m², creating a dynamic range of 36 dB. A customized stimulus grid (AMD 6° grid; Centre for Eye Research Australia, Victoria, Australia) consisting of 37 points located at 0°, 1°, 2.33°, 4°, and 6° (referred to as rings R1, R2, R3, R4, and R5, respectively) from fixation was used in this study, designed by our group specifically for the assessment of the macular region in AMD (Fig. 1).

In this study, two consecutive examinations of the study eye were performed for all participants, with approximately 3 minutes of rest given between each test to minimize the effect of fatigue. Although a small number of participants had previously undergone microperimetry testing, the first examination was then discarded from the analyses to avoid the influence of an intrasession learning effect, as previously reported.29 Test reliability for the second examination was assessed by the frequency of false-positive responses, measured by presentation of suprathreshold stimuli to the physiological blind spot, which was manually located on the MAIA before the presentation of the first stimuli. Any test result with false-positive responses of >25% was considered unreliable and the test was repeated. This cutoff value was chosen because the MAIA presents a false-positive stimulus approximately every 1 minute, and there are typically only 4 to 5 false-positive stimuli presented in each test, given the short duration of the examinations.

**Multifocal Electoretinography**

The recording protocol for the mfERG was performed as described previously.12,31 In short, the visual evoked response imaging system (VERIS Science 6; ElectroDiagnostic Imaging, Inc., Redwood City, CA, USA) and Dawson-Trick-Litzkow (DTL) thread electrodes were used in this study. The pupils were dilated using drops of medication applied prior to microperimetry (see above) but were measured again prior to mfERG testing to ensure that they were at least 7 mm in diameter; further pupillary dilation was performed if they were not. The test stimulus that consisted of 103 retinal-scaled hexagons (Fig. 1) was delivered using a fixation monitoring system using a pseudorandom m-sequence (m = 15) at a rate of 75 Hz. The luminance of the white hexagons was set at 5.33 cd/m², and the contrast between the white and black hexagons was approximately 99%; the background luminance was set at 200 cd/m². The fixation target was a cross that was 3° in diameter and 0.5° in thickness (20% of the fixation target diameter). This setting was chosen so that the fixation target was easily visible while maintaining minimal coverage of the central hexagon. The recorded signal was filtered using a band pass filter between 10 and 100 Hz and was amplified 100,000 times (model 12; Grass NeuroData, Quincy, MA, USA). Segments that were contaminated with blinks or eye movements were discarded and were re-recorded prior to completing the entire recording.
Figure 1. Overlay of 103 hexagon multifocal electroretinography (mfERG) and customized microperimetry stimulus grid, where overlapping areas of mfERG and the microperimetry grid are shaded gray and are represented by solid circles, respectively (A). Open circles represent microperimetry data points that were not analyzed. The eccentricities of the stimuli for microperimetry (B) and assignment of point numbers on the mfERG stimulus array (C) are presented. N, nasal; T, temporal.

Imaging
Multimodal fundus imaging was performed in all participants by using color fundus photography (CR6-45NM unit; Canon, Saitama, Japan), near-infrared reflectance, fundus autofluorescence, and spectral-domain OCT (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany) to ensure no signs of advanced AMD were missed. A senior retinal specialist (RHG) performed all clinical examinations and severity grading.

Statistical Analysis
For mfERG, the responses from the central three rings were used, where rings R1, R2, and R3 covered areas approximately 0° to 1°, 1° to 4°, and 4° to 8°, respectively from the fovea. No spatial averaging was applied to the mfERG in order to provide localized responses. An unpaired t-test was used to compare the mean BCVA of AMD subjects with that of control participants. The t-tests were also used to compare the mean N1-P1 response amplitude and P1 implicit time on mfERG with the mean retinal sensitivity in microperimetry individually at each stimulus point between the two groups of participants.

In order to allow retinotopically matched comparisons between mfERG and microperimetry, we selected mfERG hexagons that had more than 1 point on the corresponding microperimetry stimulus for analysis, ensuring that the nasal and temporal orientations were matched between the two eyes. Thirteen hexagons and their corresponding microperimetry stimuli were identified and are shown in Figure 1. Retinal sensitivity for all points within each hexagon was averaged.

Normative values for mfERG parameters and retinal sensitivity at each of these retinotopically matched locations and BCVA were determined by using linear regression analyses of the measured values relative to age; the slope, intercept of the line of best fit, and standard deviation (SD) of the residuals from the line of best fit were then obtained. The mfERG parameters, retinal sensitivity, and BCVA were then converted into Z-scores by using the age-estimated mean and SD of the residuals from the control group.

Pearson correlations were performed between mfERG parameters and retinal sensitivity at each individual location. To compare the magnitude of measured functional deficit measured by each test, a linear mixed effects model was used to account for the multiple measurements from each subject.

The different test parameters (microperimetric retinal sensitivity, mfERG response amplitude, and mfERG implicit time) were considered fixed effects, and points nested within patients were random effects.

BCVA was compared with the central hexagon in mfERG (which is aligned with the fovea), by using a Pearson correlation to examine the relationship between the two parameters, and a paired-sample t-test was used to compare the magnitude of the measured functional deficit.

RESULTS
There were 66 participants with intermediate AMD who were eligible for this study, and all participants in this study had microperimetry results on the second examination that were considered reliable on the basis of false-positive responses (see Methods). However, there were 6 participants who were unable to successfully complete the mfERG recordings and were, thus, excluded from the entire analysis. A total of 60 participants (69.6 ± 7.5 years of age, ranging from 51–89 years old) with intermediate AMD were included in this study. Twenty-two control participants (65.8 ± 7.7 years of age, ranging from 53–79 years old) were included to determine the normative range for visual acuity and microperimetry; ages of the control participants for both tests were not significantly different from those of the AMD participants (P = 0.051 and P = 0.064, respectively).

Comparison of Functional Measurements Between Groups
The mean BCVA was significantly worse for the AMD participants (0.02 ± 0.10 logMAR) than for the control participants (−0.13 ± 0.10 logMAR, P < 0.001), although both groups had relatively good central vision. For mfERG, the mean implicit time for AMD participants was significantly increased at all points compared to that in control participants (P ≤ 0.043), except at 1 point (P = 0.061) (Fig. 2A). The mean response amplitudes were significantly reduced by only 6 points for AMD participants (P ≤ 0.030; 4 of which were within the central 2 rings) but were not significantly different
from the control participants for the other 13 points ($P \geq 0.051$) (Fig. 2B). The mean retinal sensitivity was also significantly lower in AMD participants at all points than in control participants ($P \leq 0.026$), except at 1 point ($P = 0.051$) (Fig. 2C). Note that the retinal sensitivity at the first point (located at the fovea [$0^\circ$]) was the lowest, most likely because it was measured inside the fixation ring, where small fixations shifts may result in the fundus-tracked microperimeter presenting this foveal stimuli overlying the fixation target, thus making it more difficult to detect. All three functional parameters displayed regional variation (Fig. 2). When considering the average of all test points, both response amplitudes ($24.6 \pm 0.8$ nanovolt [nV] and $29.2 \pm 1.6$ nV/degree$^2$, respectively, $P = 0.013$) and retinal sensitivity were significantly lower ($26.6 \pm 0.5$ dB and $29.0 \pm 0.2$ dB, respectively, $P < 0.001$), and implicit time was significantly slower ($31.6 \pm 0.2$ ms and $29.4 \pm 0.3$ ms, respectively, $P < 0.001$) in AMD participants than in control participants.

**Multifocal Electroretinography Compared With Microperimetry**

Results of the mfERG and microperimetry were converted into Z-scores, representing the number of SDs away from the control group. Overall, linear regression analyses did not reveal a significant correlation between average Z-scores of retinal sensitivity with the average Z-scores of mfERG implicit time (correlation coefficient $R = 0.254$, $P = 0.051$) (Fig. 3A) or response amplitudes ($R = 0.006$, $P = 0.965$) (Fig. 3B) at corresponding points. Pearson correlation coefficients are shown for each individual point in Figure 3. Although there were 3 points that exhibited a weak, positive correlation between the Z-scores of implicit time and retinal sensitivity (Fig. 3C), there were no points that showed a significant correlation between the Z-scores of response amplitude and retinal sensitivity (Fig. 3D).

Results of the linear mixed-effects model using the Z-scores of the functional tests showed that the magnitude of measured functional deficit was significantly greater with microperimetry than with both mfERG response amplitude and implicit time for corresponding points at all three rings ($P < 0.001$) (Fig. 4).

Examples of mfERG and microperimetry findings of two AMD participants are shown in Figure 5, illustrating the differences between mfERG and microperimetry findings in eyes with intermediate AMD (Figs. 5B, 5C) compared to those in a normal participant (Fig. 5A). These examples illustrate cases where microperimetric retinal sensitivity in the AMD participants were reduced outside the normal limits ($\leq -2$ SDs away from normal), either focally (near the fovea in Fig. 5B) or over a larger area (Fig. 5C), whereas mfERG responses were all

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**FIGURE 2.** Mean implicit time (A) and response amplitude of multifocal electroretinography (mfERG [B]) and microperimetric retinal sensitivity (C) for all 22 control participants (black) and 60 participants with age-related macular degeneration (AMD [gray]); asterisks indicate statistically significant differences between the two groups ($P < 0.05$), and the number of each ring is indicated inside the circles (R1, R2, R3, R4, and R5). The mfERG implicit time was significantly slower and retinal sensitivity was significantly lower at all but 1 point for AMD participants compared to those for control participants, but only significantly lower in 6 (mainly central two rings) of 19 points for mfERG response amplitudes. Error bars represent 95% confidence intervals of each point.
within normal limits, except for the implicit time value at the fovea in a second example (Fig. 5C).

**Multifocal Electroretinography Compared With Best-Corrected Visual Acuity**

BCVA values were also converted into Z-scores and compared with mfERG parameters, and no significant correlation was found between the Z-scores of BCVA and implicit time ($R = 0.056, P = 0.673$) or response amplitude ($R = 0.090, P = 0.492$) (Fig. 6). Paired-sample $t$-tests when considering Z-scores showed that the measured functional deficit in mfERG implicit time was not greater than BCVA ($P = 0.153$), and the measured functional deficit of mfERG response amplitude was significantly less than BCVA ($P < 0.001$) (Fig. 4).

**DISCUSSION**

The measurement of retinal function using mfERG has been suggested to be useful in the assessment of disease in the early stages of AMD, as some studies have reported that functional deficits can be effectively detected using this objective technique. In addition, we recently reported that mfERG implicit time changes correlated with the relative intensity of the second hyper-reflective band of the outer retina with high-resolution OCT, which is reduced in eyes at early stages of AMD compared to control eyes. Because these findings suggest a potential role for mfERG as a functional biomarker in early stages of AMD, this study sought to compare the effectiveness of mfERG to that of microperimetry, a subjective functional measurement that has also been shown to have value in the detection of early stages of AMD.
In this study, we found that both mfERG parameters and microperimetric retinal sensitivity significantly declined in intermediate AMD, in agreement with previous studies of each measurement alone.\textsuperscript{12–17,22–27} We also did not find a significant correlation between the measured functional deficit of microperimetric retinal sensitivity and mfERG implicit time nor response amplitude.

The lack of correlation between microperimetric retinal sensitivity and mfERG parameters was not unexpected, as they represent different information about retinal function and are measured under different conditions. The mfERG is an objective measure of suprathreshold responses at photopic adaptation levels and the first-order kernel responses originate from the cone photoreceptors and bipolar cells,\textsuperscript{33} whereas microperimetry is a subjective measurement of retinal sensitivity at mesopic adaptation levels, which may be mediated by both rod and cone photoreceptors. Measurements of retinal sensitivity are also not solely influenced by the physiological condition of the retina, unlike mfERG, but by the entire visual pathway. However, retinal sensitivity measured by microperimetry has been found to correlate strongly with the integrity of the photoreceptor band on high-resolution OCT imaging in AMD.\textsuperscript{26,27} Thus, the different functional changes in mfERG and microperimetry observed in this study are likely to be consistent with damage primarily to the outer retina\textsuperscript{54} but may not be correlated due to the differences in the method of measurement.

Although there were no correlations between the two measurements, the greater degree of functional change within the central retinal region captured by microperimetry observed in this study could also be attributed to other factors, including differences in retinal adaptation levels or fixation stability, which influences how accurately the mfERG responses are recorded, especially at the fovea when using smaller stimulus hexagons.\textsuperscript{35–37} For the 103-hexagon stimulus pattern used in this study, fixation errors (which are more profound in eyes at early stages of AMD than in eyes of control participants\textsuperscript{24}) can further limit the ability to sensitively and topographically assess functional changes. Although patient fixation was monitored using the fixation monitoring system in this study, fixation drifts still could not be corrected, unlike fundus tracking used in microperimetry.

Although both the mfERG and microperimetry results may be affected in intermediate AMD, comparison of the magnitude of their measured deficits allows a better evaluation of their effectiveness as a functional measurement. In this study, we found that the measured functional deficit was consistently greater with microperimetry than with both mfERG implicit time and response amplitude. In order to ensure that the differences were not attributed to the different scales used to represent the data, we also transformed the mfERG parameters to a logarithmic scale. However, we did not find that this improved the relationship between the mfERG parameters and microperimetric retinal sensitivity, nor did mfERG parameters show a greater magnitude of measured functional deficits (data not shown). These findings are in agreement with those of recent studies in other diseases involving the outer retina, where microperimetry has been shown to be more sensitive at detecting early functional changes\textsuperscript{38} and changes in response to treatment.\textsuperscript{59} In addition, the measured functional deficit at the fovea was not greater for both of the mfERG parameters compared to BCVA. It should be noted that the magnitude of measured functional deficit expressed as Z-scores in this study is in part influenced by the test-retest repeatability of each test. Thus, we can only infer from these findings that microperimetry is able to detect subtle differences in retinal function better than mfERG, but we are not able to determine the implications of the measured functional deficits.

These findings have significant implications when considering mfERG as a functional measurement in intermediate AMD. The finding that the measured functional deficit was less with mfERG parameters may reduce the ability of mfERG to detect small changes in retinal function that could occur with disease progression and in response to treatment. In addition, although mfERG may be a useful, objective functional biomarker, its findings do not correlate with the more clinically relevant subjective functional changes\textsuperscript{40} of BCVA and microperimetry in this study.

Microperimetry has several advantages over mfERG as a clinically applicable functional measurement in the early stages of AMD. First, the large area of the mfERG hexagons do not allow localized defects to be captured within the small macular region in AMD, with the central 5 points on microperimetry covering less than one-third of the area of the central hexagon in this study. Although microperimetry may also fail to sample a localized defect if the sampling density is insufficient, the option of manually selecting a region of interest to sample is available. Second, microperimetry is a noninvasive and rapid test, with the total procedural time for each test in our study lasting for approximately 6 to 7 minutes. This contrasts with the total procedural time of at least 15 to 20 minutes for mfERG recording of one eye, assuming optimal patient compliance and recording by an experienced examiner, although the need for re-recording segments of suboptimal quality (e.g., contaminated by blinks) and inclusion of rest between segments during this demanding procedure frequently resulted in a total procedural time of approximately 20 to 30 minutes for the AMD participants in this study.

Despite these advantages, both mfERG and microperimetry may provide unique information about the risk of progression to advanced stages of AMD. Changes in both mfERG implicit time\textsuperscript{41} and retinal sensitivity with flicker perimetry\textsuperscript{19} have been shown to play a role in predicting the development of advanced AMD. Therefore, microperimetry and mfERG may together act as useful functional biomarkers in early stages of
Examples of findings for microperimetry (top) and multifocal electroretinography (mfERG) (bottom) in a normal participant (A) and in two participants with intermediate age-related macular degeneration (AMD) (B, C). mfERG findings are shown as individual responses, with the P1 implicit time value shown on top of each response. Microperimetric retinal sensitivity was reduced outside normal limits (≤ -2 standard deviations from normal), either focally (near the fovea [B]) or over a larger area (C). mfERG responses of the AMD participants (B, C), however, were within normal limits, except for the implicit time value at the fovea in (C).

**FIGURE 5.** Examples of findings for microperimetry (top) and multifocal electroretinography (mfERG) (bottom) in a normal participant (A) and in two participants with intermediate age-related macular degeneration (AMD) (B, C). mfERG findings are shown as individual responses, with the P1 implicit time value shown on top of each response. Microperimetric retinal sensitivity was reduced outside normal limits (≤ -2 standard deviations from normal), either focally (near the fovea [B]) or over a larger area (C). mfERG responses of the AMD participants (B, C), however, were within normal limits, except for the implicit time value at the fovea in (C).

**FIGURE 6.** Best-corrected visual acuity (BCVA) plotted against multifocal electroretinography (mfERG) implicit time (A) and response amplitude (B). Values at each point are represented by standard deviations (SDs) away from normal participants (Z-scores). Pearson correlation coefficients are shown, and there were no significant correlations between Z-scores of BCVA and either of the mfERG parameters.
Multifocal ERG and Microperimetry in AMD

In summary, although mfERG may capture unique aspects of retinal dysfunction in the intermediate AMD, the magnitude of measured functional deficit was greater with microperimetry than with mfERG parameters. These findings are important when considering these two techniques for measuring retinal function in the early stages of AMD.

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