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Focal electroretinogram and microperimetry testing of photoreceptor-retinal pigment epithelium function in intermediate age-related macular degeneration

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ABSTRACT.

Purpose: **To compare the performance of focal electroretinogram (FERG) and fast mesopic microperimetry in evaluating macular function of intermediate age-related macular degeneration (iAMD) subjects with preserved visual acuity.**

Methods: **Cross-sectional, observational study. Participants with drusen >125 µm and VA ≥80 ETDRS letters and age- and sex-comparable healthy subjects were consecutively enrolled in the study. Three photopic FERG recordings of the central 9° of the macula with luminance modulated stimuli flickering at 42.5 Hz and a fast mesopic microperimetry with a custom pattern of 3 central (CS) and 3 paracentral (pCS) stimuli at 1.2° and 6° from fixation were acquired.**

Results: **Overall, 112 eyes of 77 participants (age 73.0 ± 7.1 years, 47 iAMD eyes) were analysed. Mean FERG amplitude, CS and pCS (all $p < 0.05$) were lower in the iAMD group. A significant association was observed between FERG amplitude and iAMD (OR 9.58, $p < 0.001$) in multiple logistic regression analysis. *Z*-scores of FERG were lower than microperimetry in iAMD ($p = 0.002$) but not for healthy participants. AUC of the ROC curve was greater for FERG than microperimetry (0.895 versus 0.644 and 0.675, both $p < 0.05$).**

Conclusion: **Focal ERG objectively measures a cumulative response originating from the photoreceptor-RPE complex of the central 9° of the macula and demonstrated high accuracy in identifying decreased central macular function in iAMD patients with preserved visual acuity, performing better than fast mesopic microperimetry. Focal ERG should be considered a reliable technique for measuring retinal sensitivity of iAMD patients.**

Key words: focal electroretinogram – intermediate AMD – large drusen – macular sensitivity – microperimetry

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Introduction

Age-related macular degeneration (AMD) is the most common cause of progressive central vision loss in older adults in developed countries (Wong et al. 2008, 2014). Intermediate AMD (iAMD) is characterized by the presence of large drusen and/or pigmentary changes (Coleman et al. 2008; Vujosevic et al. 2011; Ferris et al. 2013; Mitchell et al. 2018), which have been demonstrated to meaningfully correlate with the development of late-stage AMD (either macular atrophy or neovascular AMD) (Sarks et al. 1988). Large drusen in particular are defined as mound-shaped, moderately hyperreflective elevations of the RPE on optical coherence tomography (OCT) (Khan et al. 2016), measuring more than 125 µm in diameter. Histologically, they consist of neutral lipids, esterified and non-esterified cholesterol, carbohydrates and proteins originating from incomplete degradation of membrane material and waste products of photoreceptors and RPE cells (Pauleikhoff et al. 1992; Haimovici et al. 2001; Curcio et al. 2005; Crabb 2014).

Decrease in macular function has been described with both subjective and objective testing in patients affected by early or intermediate AMD with preserved best-corrected visual acuity (BCVA) (Seiple et al. 1986; Falsini et al. 2000; Wu et al. 2014; Hirooka et al. 2016; Steinberg et al. 2017). Best-corrected visual

acuity (BCVA) is in fact often unaffected until features of late AMD develop, highlighting the need for a more precocious and reliable marker of structure–function correlation and of disease progression (Cheng & Vingrys 1993).

Microperimetry (MP) is a fundus controlled, subjective, full-threshold testing of macular sensitivity, capable of evaluating its functional integrity (Molina-Martín et al. 2017). The main advantage of MP, compared with conventional perimetry, is the ability to visualize the patient's retina in real time. As with traditional perimetry, retinal sensitivity tends to decrease with age, as well as stability of fixation (Denniss & Astle 2016). Studies on early and intermediate AMD have been conducted with MP (Vujosevic et al. 2011), and its performance has been compared with conventional perimetry (Hirooka et al. 2016).

With regards to objective, electrophysiological studies, several papers have described the decline in multifocal ERG (mfERG) amplitude in different stages of AMD, demonstrating localized retinal dysfunction reflected by delayed implicit times (Huang et al. 2000; Li et al. 2001), even if no clear correlation with morphologic changes was determined (Gerth et al. 2003, 2006). Other works identify a decrease in mfERG amplitude in different stages of AMD either with (Kader 2017) or without correlation with OCT characteristics of the disease (Parisi et al. 2020).

On the other hand, few studies have explored the performance of focal macular ERG (FERG) in iAMD (Falsini et al. 2000, 2007). FERG recording is a potentially quick to perform, objective procedure which focuses on central macular function (Seiple et al. 1986). A correlation between FERG amplitude reduction and central macular alterations induced by early and intermediate AMD is known to exist in an eccentricity-dependent fashion (Seiple et al. 1986; Falsini et al. 1999, 2000, 2007).

Furthermore, a paper by Wu et al. (2014) suggested that microperimetry could be considered a more sensitive method than mfERG for detecting decrease in macular function in iAMD patients, but no study has ever compared MP to focal macular ERG.

Aim of this study was to evaluate macular function in patients with large drusen and preserved visual acuity, with both subjective and objective methods (microperimetry and FERG, respectively) in a group of iAMD patients and a cohort of healthy subjects, and to compare the results of the two modalities.

Methods

Participants

The study was designed as a cross-sectional, observational and comparative case series. The investigation was approved by the Institutional Review Board of the Luigi Sacco Hospital (Milan, Italy), and all procedures followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from each subject.

Participants were recruited between March and November 2020 at the Eye Clinic of Luigi Sacco Hospital among patients of our medical retina service for the intermediate AMD group, while healthy participants were recruited from subjects undergoing routine examinations at our clinic.

Inclusion criteria for the iAMD group were BCVA equal to or better than 80 ETDRS letters and presence of large drusen (>125 μm) in the macular region; both eyes of the same patient could be included in the study. Inclusion criteria for the healthy subjects group were BCVA equal to or better than 80 ETDRS letters, similar age to the iAMD group age range (61-85) and no sign of AMD in either eye; for all but one of the healthy participants, both eyes were included in the study.

Exclusion criteria for all participants included (1) refractive errors >3 diopters of spherical equivalent and >1.5 diopters of astigmatism, (2) the presence of large drusenoid RPE detachments, (3) any sign of late AMD (macular atrophy, choroidal neovascularization), (4) significant medium opacity, (5) amblyopia, (6) glaucomatous optic nerve head damage and (7) any other retinal (e.g. retinal vein occlusion, diabetic retinopathy) or corneal pathology that could compromise vision. All participants had no history of significant systemic illnesses.

Each participant underwent a complete eye examination, including measurement of visual acuity with the ETDRS chart at 4 m, slit-lamp biomicroscopy of the anterior segment and of the fundus oculi, fundus imaging on a Spectralis instrument (Spectralis HRA+OCT, Heidelberg Engineering, Heidelberg, Germany), microperimetry examination (Macular integrity assessment (MAIA), CenterVue, Padua, Italy) and FERG recording (BM 6011, Biomedica Mangoni, Pisa, Italy).

Drusen imaging

Large drusen were identified by means of slit-lamp indirect ophthalmoscopy and defined through SD-OCT as mound-shaped, moderately hyperreflective elevations of the RPE measuring more than 125 μm in length (Khan et al. 2016); linear dimension was calculated through the built-in software (Heidelberg Eye Explorer Version 1.9.13.0, Heidelberg Engineering).

Fundus-controlled microperimetry

Participants underwent fundus-controlled mesopic microperimetry with the MAIA device, following adaptation to room illumination, with examination modalities already described in other works (Wu et al. 2014; Hirooka et al. 2016; Molina-Martín et al. 2017; Steinberg et al. 2017).

Briefly, a custom pattern of 6 stimuli (Goldmann size III, 200 ms, full threshold 4-2 strategy, background luminance 1.27 cd/m^2) was projected on the upper half of the macula at 1.2° (Central Sensitivity – CS) and 6° (Paracentral Sensitivity – pCS) from the preferred retinal locus (PRL). The slight offset of the central stimuli accounts for artefactual reductions in central sensitivity caused by fixation targets. Any examination with false-positive responses greater than 10% was discarded and repeated until test reliability was less than or equal to 10%.

Focal (1F) ERG methodology

After pupil dilation of at least 7 mm and correction of refractive errors, silver chloride skin electrodes were applied on the forehead (ground electrode) and on the inferior eyelids (active electrode for the study eye; reference electrode for the fellow eye);

FERG responses were recorded monocularly.

A CRT monitor was set 57 cm apart from the study eye and covered by a white panel with a 9 × 9 cm square opening through which the stimuli were presented to the central 9° of the visual field; the panel was uniformly illuminated by a halogen lamp with a luminance slightly higher (55 cd/m^2) than the mean luminance of the stimulus (52 cd/m^2) in order to minimize the influence of stray light (Messenio et al. 2013).

A stimulus flickering sinusoidally with luminance modulation at a frequency of 42.5 Hz was employed; the amplification was set at 50 000 dB, band-pass filtered (1–200 Hz), with automatic rejection of artefacts enabled. For each study, eye three series of 200 signal recordings were averaged. Steady-state Fourier analysis was performed to obtain a measure of the amplitude of the major harmonic.

In this particular setting, the major harmonic wave is the first harmonic, and the recording is thus referred to as '1F' focal ERG (Messenio et al. 2013), corresponding to responses elicited primarily from the outer macular layers (i.e. RPE and photoreceptor complex) (Baron et al. 1979; Choshi et al. 2003).

Light calibrations of the stimulus, room luminance and luminance at the white panel were handled by a Starlite photometer (Gossen, Nürnberg, Germany); fixation was monitored by a fundus camera operating in visible and IR wavelength. Focal macular ERGs were recorded using a BM 6011 (Biomedica Mangoni, Pisa, Italy) acquisition system. Exemplifying FERG recordings of both an iAMD patient and a healthy subject are shown in Fig. 1.

Statistical analysis

Descriptive statistics for demographic data and main clinical features are presented as mean ± standard deviation, mean and 95% confidence interval (CI) or as frequencies (%) where appropriate.

The distribution of all variables in the two groups was assessed for normality with the Shapiro–Wilk test and confronted accordingly by means of either the Student's t-test or the Kruskal–Wallis rank-sum test.

Multivariate logistic regression was carried out to estimate the influence of the various parameters that have been measured on the presence or absence of large drusen; results are reported as odds ratio (OR) and 95% CI.

In order to compare the degree of deviation in our study population, the mean values of FERG, CS and pCS were standardized into *Z*-scores (i.e. $Z = x - \mu/\sigma$) by subtracting from each value (x) of FERG, CS and pCS the mean (μ) of the values recorded in the respective modality and dividing by their standard deviation (σ), thus representing the number of standard deviations away from the mean.

The correlation of the, therefore, normalized *Z*-scores between the two examination modalities was calculated as a Pearson correlation coefficient. The FERG *Z*-scores of the iAMD group and the comparison group were then confronted with the respective retinal sensitivity *Z*-scores by means of a paired Student's *t*-test.

Finally, the receiver operating characteristic (ROC) curves for the two examination modalities were drawn as a function of true positive rate (i.e. sensitivity) against false-positive rate (i.e. 1 – specificity) in detecting macular function abnormality for each modality. The respective areas under the curve (AUC) were calculated and confronted by means of DeLong's test for two correlated ROC curves.

All calculations were performed with the open-access software R version 4.0.0 (R Project – The R Foundation for Statistical Computing, Vienna, Austria). The chosen level of statistical significance was $p < 0.05$.

Results

A total of 47 eyes from 44 patients with macular drusen and 65 eyes from 33 healthy subjects met inclusion criteria and were consecutively included in the study; characteristics of the two groups are summarized in Table 1.

Mean age of iAMD patients was higher than that of healthy subjects ($p = 0.006$). Although both groups had very good central visual acuity, the

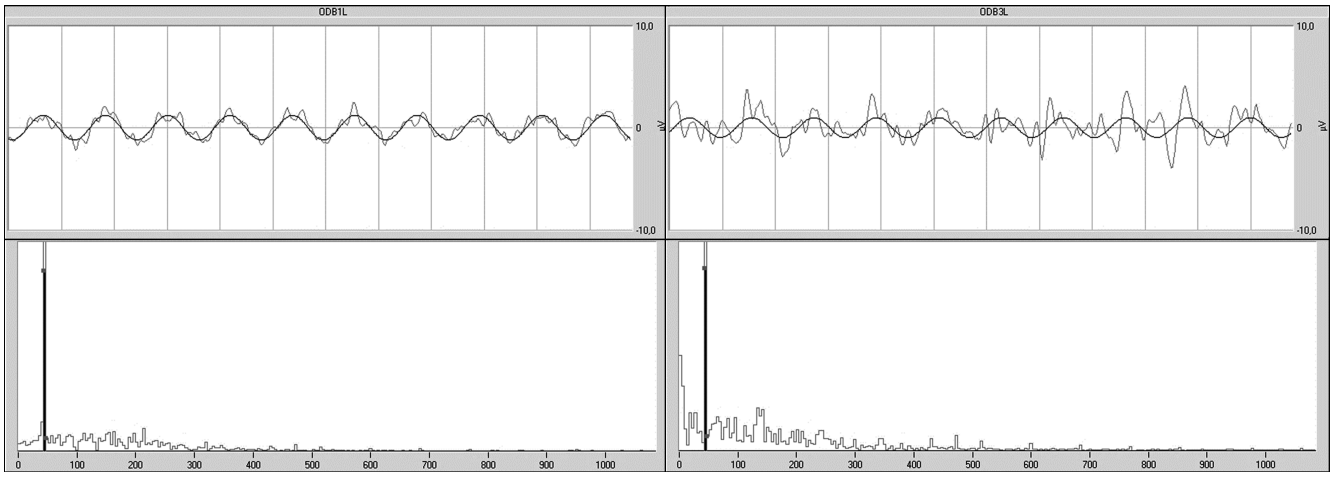

Fig. 1. An example of focal electroretinogram recording in a healthy participant (left) and an iAMD patient (right). Top row: the raw inscribed sinusoidal wave from which the amplitude of the focal electroretinogram is computed. Bottom row: the Fourier analysis of the raw wave. In this setting, the first harmonic (1F) at 42.5 Hz represents the major component of the discrete Fourier series, and it is considered representative of the FERG recording since almost all of the signal energy is comprised in this harmonic. In these recording, the peak-to-peak amplitude is 2.7 µV for the healthy subject and 1.4 µV for the iAMD patient.

Table 1. Summary of participants' characteristics. Categorical variables are reported as percentages, continuous variables as mean (standard deviation).

	Total	iAMD	Healthy subjects	p-Value
Eyes, *n*	112	47	65	
Patients, *n*	77	44	33	
Females, %	59.74%	56.82%	63.64%	0.712*
Age, years – mean (SD)	73 (7.02)	74.91 (6.48)	70.45 (7)	0.006†
BCVA, ETDRS letters – mean (SD)	83.94 (2.13)	82.89 (2.65)	84.69 (1.21)	<0.001†
fERG amplitude, μV – mean (SD)	1.82 (0.9)	1.15 (0.55)	2.32 (0.77)	<0.001‡
Central sensitivity, dB – mean (SD)	26.56 (4.03)	25.8 (4.67)	27.76 (2.31)	0.036‡
Paracentral sensitivity, dB – mean (SD)	24.27 (3.64)	23.46 (3.99)	25.59 (2.53)	0.011‡

*Pearson's chi-squared test.
†Welch two sample *t*-test.
‡Kruskal–Wallis rank sum test.

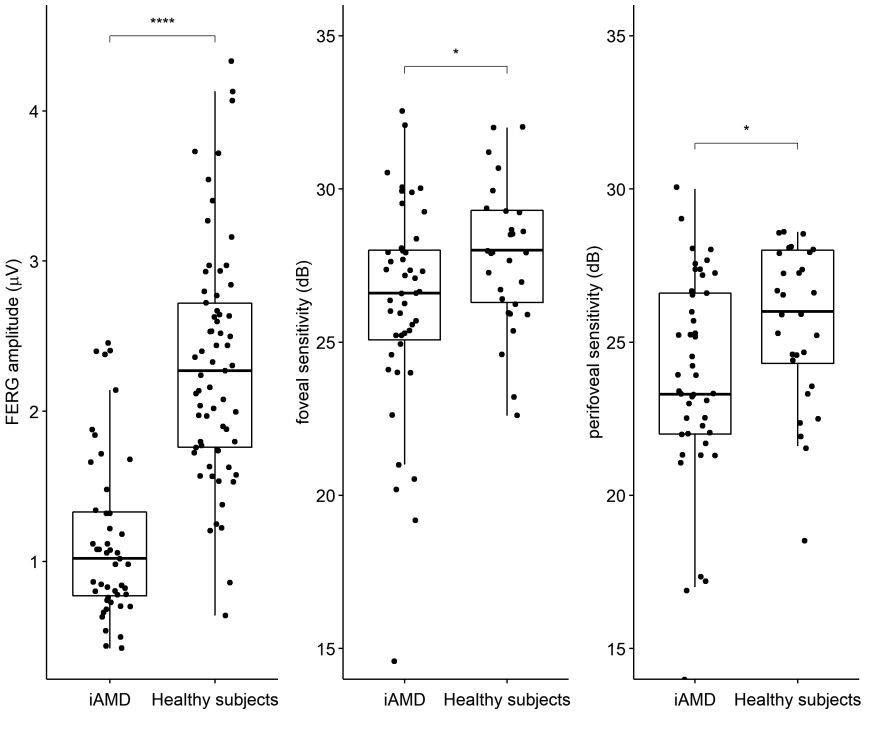

Fig. 2. Boxplots displaying the distribution of mean FERG amplitude, CS and pCS in iAMD patients and healthy subjects. The arms of the boxplots extend to the 25th and 75th quartiles plus or minus the interquartile range. Each dot represents an individual eye.

Table 2. Summary of multiple logistic regression analysis. The resulting coefficients are reported as odds ratio and respective 95% Confidence Interval. Only FERG amplitude showed a significant influence on the odds of belonging to the iAMD group, with an inverse relationship: in our study population, an increase in FERG amplitude decreases the odds of being affected by iAMD.

	Odds ratio	95% Confidence Interval		p-Value
Age	1.062	0.963	1.183	0.244
Sex, male	0.451	0.115	1.610	0.230
fERG amplitude	9.579	3.428	33.977	<0.001
Central sensitivity	0.998	0.760	1.347	0.990
Paracentral sensitivity	1.093	0.828	1.511	0.560

mean BCVA of the iAMD subjects was significantly worse than that of the control group ($p < 0.001$).

A statistically significant difference in mean FERG amplitude between the two groups was found, as mean FERG was significantly lower in the iAMD group (1.15 ± 0.55 μV versus 2.32 ± 0.78 μV, $p < 0.001$); similarly, lower values for the iAMD group were recorded using microperimetry, as both mean central ($p = 0.03$) and mean paracentral sensitivity ($p = 0.01$) showed a small, albeit significant, reduction. These results are summarized in Fig. 2.

Multiple logistic regression revealed that, of all the variables measured, only mean FERG amplitude was statistically significant when independent variables were considered altogether to compare the two groups (OR = 9.579, $p < 0.001$). A summary of multiple logistic regression is shown in Table 2.

The relationship between the two modalities was confirmed through Pearson correlation coefficients, which showed a significant positive correlation between mean FERG amplitude *Z*-scores and CS, pCS *Z*-scores ($R = 0.38$ and $R = 0.44$ respectively, both $p < 0.001$; see Fig. 3.).

To evaluate the performance of the two test modalities in detecting macular sensitivity decrease a paired-sample Student's t-test was conducted on the *Z*-scores of the iAMD group and healthy participants: Results showed significantly lower scores for mean FERG values compared with mean central and paracentral sensitivity for the iAMD group respective difference (−0.56 and −0.53, both $p = 0.002$) compared with healthy subjects (−0.0025 and −0.066, $p = 0.985$ and $p = 0.622$). These findings are summarized in Table 3.

Finally, the measured AUC of the ROC curve was greater for FERG than for CS and pCS ($AUC_{FERG} = 0.851$, $AUC_{CS} = 0.644$, $AUC_{pCS} = 0.675$); the ROC curves and respective confidence intervals are represented in Fig. 4.

Accordingly, our population study had an 89.5% chance of being correctly identified as either having a reduced or normal macular function by means of FERG recording, against a 64.4% and a 67.5% chance by means of CS and pCS microperimetric testing.

The difference between FERG's ROC curve versus CS and pCS was statistically significant when applying DeLong's test for two correlated ROC curves ($p = 0.001$ and $p = 0.007$, respectively; see Table 4).

Discussion

In this study, we objectively measured macular sensitivity by means of FERG

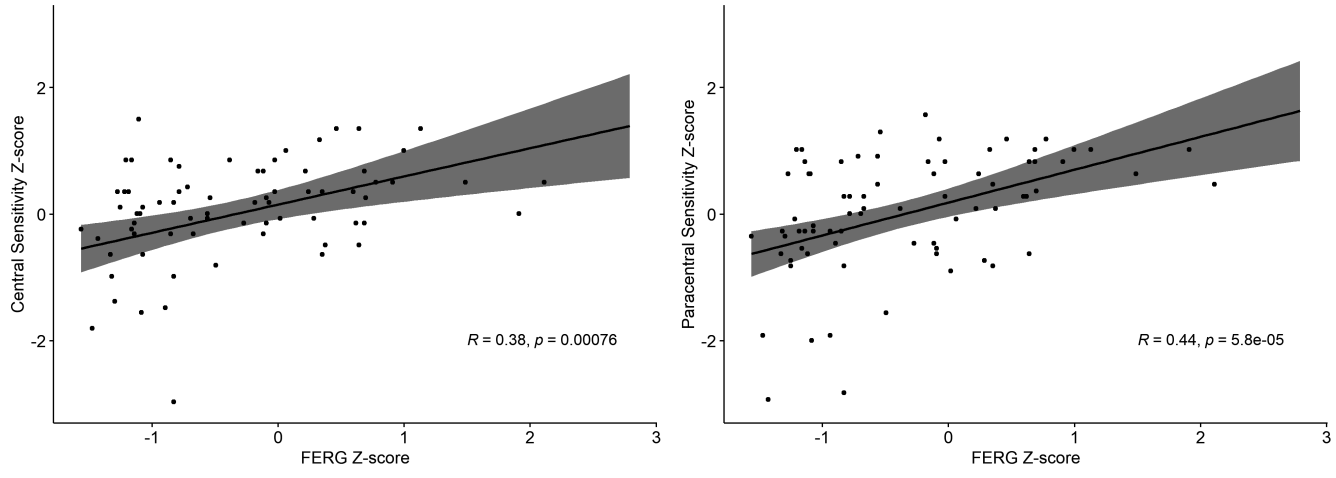

Fig. 3. Scatter plots illustrating the Pearson's linear correlation between focal electroretinogram *Z*-scores and CS, pCS *Z*-scores; the positive correlation of the two modalities implies that for each unitary increase in FERG *Z*-score the CS and pCS *Z*-scores increased of an average of 0.38 and 0.44, respectively.

Table 3. Summary of the distribution of *Z*-scores of the three modalities. *Z*-score are a normalized value representing the number of standard deviations away from the mean. The intragroup differences between FERG and CS, pCS are also reported.

Group	iAMD	Healthy subjects
FERG	−0.76 (−0.94, −0.58)	0.55 (0.33, 0.76)
Central sensitivity	−0.19 (−0.53, 0.16)	0.3 (0.08, 0.52)
Paracentral sensitivity	−0.22 (−0.55, 0.098)	0.36 (0.098, 0.63)
FERG – CS	−0.56 (−0.9, −0.21)	−0.0025 (−0.28, 0.27)
p-value*	0.002	0.985
FERG – pCS	−0.53 (−0.85, −0.21)	−0.066 (−0.34, 0.21)
p-value*	0.002	0.622

*Paired *t*-test.

recording of the central 9 degrees of the macular region in iAMD patients and in healthy subjects with preserved visual acuity, and we compared it with measurements of central and paracentral macular sensitivity as evaluated by the MAIA microperimeter.

We found that both FERG recordings and microperimetry were significantly reduced in iAMD patients when compared to healthy subjects, in agreement with previous works (Seiple et al. 1986; Midena et al. 2007). Nevertheless, in the multiple regression analysis, we performed only mean FERG amplitude was significantly associated with the iAMD group, with an inverse relationship: In our model, increasing values of FERG amplitude substantially decrease the odds of belonging to the iAMD group, while microperimetry measurements did not reach levels of significance.

We, therefore, set out to investigate the magnitude of alterations for FERG and microperimetry in our population: When measured as deviation from the mean (*Z*-scores) and tested with a paired t-test, FERG scores were significantly lower than microperimetry only for the iAMD group. A similar approach was used by Wu et al. in a study comparing mfERG and microperimetry in intermediate AMD, where microperimetry demonstrated a greater magnitude of macular functional deficit (Wu et al. 2014).

The findings of our study are corroborated by the greater AUC of the ROC curves we calculated for the two examinations, highlighting the stronger accuracy of FERG in detecting altered macular function in our study population.

The patients enrolled in our study had a BCVA of 20/25 or higher: This criterion was implemented because central retinal sensitivity is known to decrease in the initial stages of AMD even in the presence of preserved best-corrected visual acuity (Midena et al. 2007; Vujosevic et al. 2011; Steinberg et al. 2017). High-contrast VA testing is, therefore, an unreliable marker of disease progression, and other visual function tests available to clinicians could be employed to detect initial decline of retinal sensitivity (Cheng & Vingrys 1993; Steinberg et al. 2017). Among these, low-luminance visual acuity, microperimetry and dark adaptometry have been shown to be significantly affected in the early stages of AMD, while other functional tests such as contrast sensitivity and cone-specific contrast have not shown the same degree of correlation (Jackson & Edwards 2008; Chandramohan et al. 2016).

Microperimetry allows for fundus controlled, subjective testing of macular function (Midena et al. 2007); mesopic microperimetry, such as the one recorded in this work, is thought to target more specifically the cone population (Crossland et al. 2012). It is a relatively quick examination to perform, ranging from about 1 to 5–10 min per eye, depending on the number of stimuli and the threshold strategy chosen, but it is highly dependent on patient's fixation and adequate responsiveness to the presented stimuli. While it is demonstrated that retinal sensitivity, measured through both traditional perimetry and microperimetry, tends to decrease with age (Vujosevic et al. 2011; Molina-Martín et al. 2017), as well as stability of fixation (Denniss & Astle 2016), a reduction in mesopic sensitivity has also been demonstrated

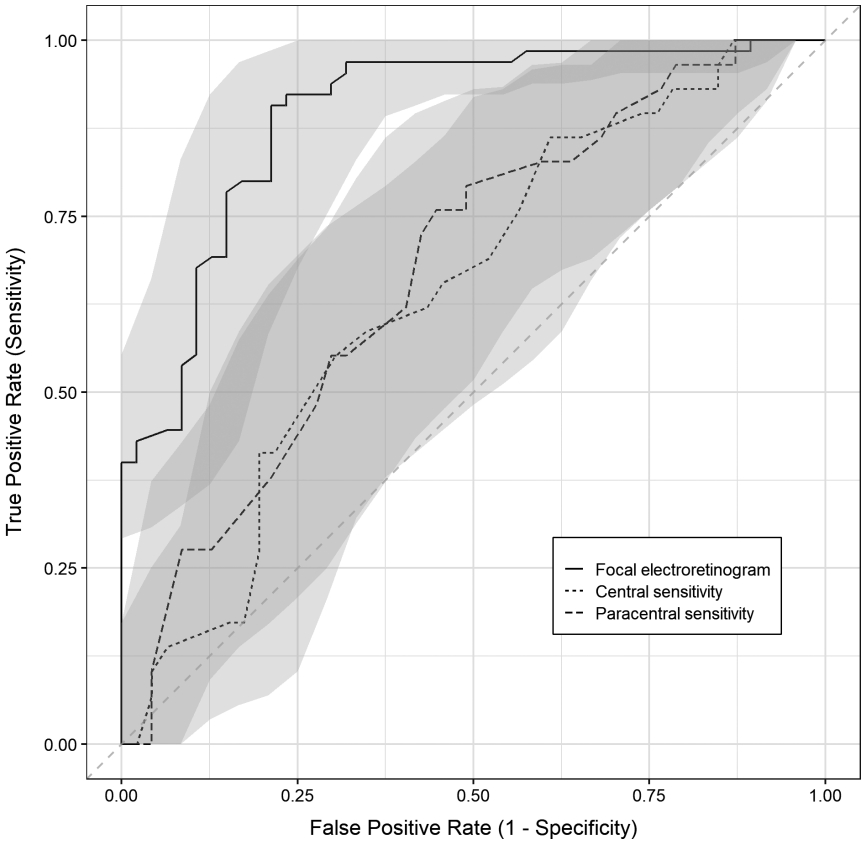

Fig. 4. ROC curve analysis and respective 95% Confidence Interval comparing the accuracy of the three modalities in study (see also Table 4 for a comparison of the respective AUC).

Table 4. Measured area under the curve (AUC) of the receiver operating characteristic (ROC) curves for the three modalities with the respective 95% Confidence Interval. The AUC relative to focal electroretinogram resulted significantly greater than those relative to central sensitivity and paracentral sensitivity in this study.

	AUC	95% Confidence Interval	p-Value*
fERG	0.851	(0.835, 0.956)	
Central sensitivity	0.644	(0.517, 0.771)	0.001
Paracentral sensitivity	0.675	(0.552, 0.797)	0.007

*DeLong's test for two correlated ROC curves (i.e. fERG versus CS, fERG versus pCS).

in early and intermediate AMD (Midena et al. 2007; Vujosevic et al. 2011; Steinberg et al. 2017).

Among electrophysiological studies, full-field electroretinography (ffERG) has been demonstrated to show a linear decrease in amplitude with age (Birch & Anderson 1992), along with a reduction in scotopic and photopic b-waves and a marked reduction in rod a-wave (Jackson et al. 2006).

Multifocal electroretinogram (mfERG) instead topographically evaluates the retinal photopic response of the 24 central retinal degrees: A significant reduction in amplitude has been observed in early AMD eyes, particularly that of the central ring (within the central 5 degrees) along with an increase in mean mfERG implicit time in the central 12 degrees (Gin et al. 2011; Parisi et al. 2020). It is unclear whether these findings correlate with anatomical modifications of AMD, as different works do not reach an agreement on the relationship between mfERG alterations and OCT signs of AMD (Kader 2017). A prospective study by Gerth et al. (2006) demonstrated a worsening of the implicit time during a follow-up of three years, despite an apparent stability of visual acuity and without correlation between drusen location and mfERG parameters: This might represent a predictive factor for drusen regression and RPE atrophy.

Few studies have instead explored the performance of focal electroretinogram (FERG) in early and intermediate AMD (Seiple et al. 1986; Falsini et al. 2000, 2007; Minnella et al. 2020; Savastano et al. 2020), and none has compared it to macular function testing through microperimetry.

FERG objectively measures a functional response originating from the cone photoreceptors and bipolar cells of the macular area under photopic conditions (Baron et al. 1979; Seiple et al. 1986). It is relatively quick and straightforward to perform and unlike perimetric testing, it does not rely on patients' compliance for its execution. Recording of three consecutive series of 200 waves, which yields results with an adequate signal-to-noise ratio, takes about 5 min per eye. The examiner must be careful to minimize possible artefacts, ensuring sufficient pupil dilation, correction of refractive error and alignment of the eye with the stimulus panel, while the cooperation required from the patient is minimal, provided adequate fixation.

Correlations between the amplitude of FERG and ageing (Messenio et al. 2013) as well as intermediate AMD have been reported (Falsini et al. 1999, 2000, 2007); two recent works have correlated decline in FERG amplitude with subjective deterioration of vision quality (Minnella et al. 2020) and to development of incomplete RPE and outer retinal atrophy (iRORA, Wu et al., 2021) in early and intermediate AMD patients (Savastano et al. 2020). Interestingly, mean FERG amplitude of iAMD patients in Savastano et al. was extremely close to that measured in our patients (mean amplitude: 1.1 μV versus 1.15 μV, respectively), possibly attesting for the reproducibility of FERG recording results.

Multifocal and full-field ERG (ffERG) are more frequently employed in the clinical setting than focal electroretinography; unlike FERG though, ffERG elicits a cumulative, mass response from the retina, not specifically a macular one, therefore, having an intrinsic lower accuracy for macular function. In fact, FERG was specifically created in order to evaluate macular function. Likewise, mfERG evaluates the entire photopic field: In

order to isolate and analyse exclusively the macular region, the operator must select only the responses originating from the central 9 degrees and/or magnify the signal (i.e. increase the density of the tested points), with a consequent reduction in the signal-to-noise ratio and, therefore, a drop in the sensitivity of the test.

Unlike other electrophysiological studies (e.g. full-field and multifocal ERG), the use of focal ERG in clinical and research environments is limited by the absence of a standardized methodology and the need of extrapolating normative data for each laboratory.

Supported by the data reported in this study, focal ERG could be considered a reliable, objective technique for quantitatively measuring macular sensitivity of intermediate AMD patients, and a valid alternative to subjective methods such as microperimetry, being capable of accurately highlighting initial macular loss of sensitivity even in the presence of no reduction in visual acuity.

However, while it is true that FERG can support the early diagnosis of macular sensitivity loss as an ancillary examination, its role is less prominent in the setting of overt retinal damage such as features of late AMD. In fact, when loss of retinal function has developed, FERG recording seems less sensible in the detection of damage progression and its utility appears, therefore, limited in the follow-up of more advanced disease. To date though, no study has performed a longitudinal analysis of FERG modifications in the follow-up of early and intermediate AMD, which remains an unexplored area of clinical research.

This study has several limitations: iAMD patients and healthy subjects were not matched for potentially confounding demographic factors; for instance, there is a significant age difference in the two groups, although the age range is identical. Nevertheless, this difference is not statistically significant when analysed through multiple regression analysis. We can, therefore, state that, in our study population, age did not significantly affect the odds of belonging to either group when all variables were considered altogether.

Furthermore, the better performance of FERG in this study when compared to microperimetry could be partially attributed to the particular custom settings of our fast mesopic microperimetry testing. This pattern was designed considering that large drusen are most prevalent in the central and paracentral macula, respecting the spatial relationship between areas of decreased sensitivity and iAMD findings, as previously described by other works (Midena et al. 2007; Khan et al. 2016). Given the low number of stimuli presented to the patient, this custom microperimetry could have missed areas of decreased macular sensitivity.

Lastly, macular ERG techniques such as FERG have the main disadvantage of a small signal amplitude, coming only from the restricted macular area, in relationship to the background noise.

Despite these limitations, our study has several strengths, such as the enrolling of a fairly large number of eyes, all with preserved visual acuity (20/25 or better) and coming from a reasonably homogenous population, as well as the employment of a precise, repeatable experimental design of both FERG and MP recording.

In conclusion, our findings confirm that a reduction in macular function can exist in iAMD patients even when visual acuity is preserved, and that FERG shows a greater degree of alteration with respect to subjective testing such as fast mesopic MP, supporting the role of FERG as an ancillary examination for the diagnosis of macular function loss in iAMD patients. Future research is needed to evaluate the possible utility of FERG in the follow-up of these patients, as no longitudinal data on the modifications of FERG recordings through time have been reported.

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