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Early detection of retinal dysfunction in type 1 diabetes without retinopathy using multifocal electroretinography

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Diabetic retinopathy (DR) significantly impacts vision and quality of life in diabetic patients. This study aimed to evaluate multifocal electroretinogram (mfERG) changes in long-term type 1 diabetes mellitus (T1DM) patients without clinical signs of DR to detect early functional retinal alterations. A prospective observational study was conducted involving 46 eyes from 23 T1DM patients and 46 eyes from 23 age-matched healthy controls. mfERG was assessed using the RETI-port/scan21 following ISCEV protocols. T1DM patients exhibited significantly decreased mfERG response amplitude density (RAD) in all the retinal rings except R2 (R1, $p = 0.003$; R3, $p = 0.006$, R4, $p = 0.023$ and R5, $p = 0,027$) and in the inferior quadrants Q2 and Q3 ($p = 0.030$ and $p = 0.004$, respectively) compared to controls. Minimal differences in implicit time (IT) were observed between both groups. Age correlated positively with different ITs, and HbA1c showed a significant negative correlation with various RADs. T1DM patients show early retinal dysfunction, as indicated by reduced RAD in mfERG, even without clinical signs of DR. These findings highlight the importance of early functional testing and metabolic control in preventing DR progression. RAD may serve as a subclinical marker of bipolar cell and photoreceptor damage in long-term T1DM patients prior to the onset of clinical DR.

Keywords Type 1 diabetes, Diabetic retinopathy, Multifocal electroretinogram, Implicit time, Response amplitude density

Diabetic retinopathy (DR) is a leading cause of vision impairment among individuals of working age, significantly impacting their quality of life and resulting in substantial direct and indirect costs. Early detection, effective prevention strategies, and comprehensive management of DR are crucial, not only for preserving vision, but also for improving the overall well-being and socioeconomic outcomes of individuals with diabetes.

DR is a highly specific neurovascular complication of diabetes mellitus (DM)[1]. Evidence suggests that DR involves abnormalities in retinal vasculature as well as neural retina dysfunction[2], a perspective recognized over 60 years ago[3]. This dual aspect of DR implies that visual impairment can result from both microvascular and neural changes, with anatomical and functional manifestations progressing as the disease advances.

Neural dysfunction can precede the appearance of vascular signs, making diagnosis challenging[4–7]. Comprehensive eye examinations, including fundus examination and imaging techniques such as optical coherence tomography (OCT), are necessary; however, the assessment of neural dysfunction requires more complex functional tests.

In recent years, electrofunctional exams have gained prominence for their sensitivity in identifying disease signs even in the preclinical phase. The electroretinogram (ERG) serves as a noninvasive method to evaluate retinal function, providing valuable, objective, and quantitative data. Studies have shown that full-field ERG (ffERG) abnormalities may precede visible fundus changes, with an increase in the implicit time (IT) of oscillatory potentials (OPs) being a consistent finding[8,9]. Functional impairments have been detected in patients without retinopathy, such as delayed IT using flickering white stimuli (28.3 Hz) with no background light, as part of the RETeval DR assessment protocol, and an amplitude reduction in higher-frequency flicker responses; some

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authors reported a reduction in 30 Hz flicker parameters[10–14]. ffERG provides insights into neuronal impairment and can identify patients at risk of disease progression, making it a valuable tool for disease management and monitoring[15].

The multifocal electroretinogram (mfERG) and pattern ERG (pERG) play complementary roles in the diagnosis and monitoring of macular disorders[16]. mfERG overcomes the limitations of ffERG in identifying localized macular and near-peripheral field abnormalities[17,18], particularly in cases with minimal changes or localized dysfunction. By recording electrical signals from multiple areas across the posterior pole under photopic conditions, mfERG effectively detects diseases of the outer retina[19,20]. The typical mfERG waveform includes an initial negative deflection (N1), followed by a positive peak (P1), and concluding with a subsequent negative deflection (N2). This waveform reflects the combined activity of overlapping cone ON- and OFF-bipolar cells, with minor contributions from cone photoreceptors[17,20]. The standard response is the first-order kernel, and the parameters for assessing amplitude and timing are the amplitude from the N1 trough to the P1 peak, and the peak time of P1[17,20,21].

The mfERG is highly suitable for assessing neural function in diabetes, as the initial vascular lesions primarily affect the macular region[8]. Studies on mfERG abnormalities in DR[22,23] consistently show abnormal findings in diabetic individuals, both with and without DR. This highlights mfERG's potential in predicting the location of future vascular abnormalities or the progression of DR[22,24–33]. Compared to healthy individuals, DM patients show reduced amplitudes and prolonged IT in mfERG, with these changes worsening with poor glycemic control or the appearance of vascular signs of DR. Most published studies on this topic include both type 1 (T1DM) and type 2 diabetic patients (T2DM), presenting diverse ocular manifestations of the disease and with varying durations of diabetes.

Our study aimed to evaluate changes in the mfERG in long-term type T1DM patients who do not exhibit vascular signs of DR. Detecting early functional alterations could help identify patients at risk of developing DR or experiencing disease progression, thereby facilitating timely intervention and management to prevent vision loss in DM patients.

Materials and methods

The study adhered to the principles outlined in the Declaration of Helsinki and received approval from the Ethics Committee for Clinical Research of Aragon (CEICA PI22/587). Written informed consent was obtained from each participant before any examinations were conducted.

We conducted a prospective observational study on visual function involving 46 eyes from 23 T1DM patients and 46 eyes from 23 age-matched normal subjects from October 2022 to May 2023 using mfERG. All patients were evaluated at the Neurophysiology and Ophthalmology Departments of the Lozano Blesa University Hospital (Zaragoza, Spain).

The T1DM patients were monitored by the Endocrinology Unit, and their glycosylated hemoglobin (HbA1c), lipid values, and arterial blood pressure remained well controlled. All participants underwent an ophthalmological and neurophysiological examination, which included a medical history review, assessment of best corrected visual acuity (BCVA), slit lamp examination, intraocular pressure (IOP) measurement, fundus examination, wide field retinography with Clarus 700 (Carl Zeiss Meditec, Dublin, USA), macular thickness measurement using Spectralis Spectral-Domain OCT (SD-OCT) (Heidelberg Engineering, Heidelberg, Germany), and mfERG.

Inclusion criteria for the DM group included a diagnosis of T1DM at least 15 years prior, no clinical signs of DR, BCVA superior to 20/25 on the Snellen chart, refractive errors less than 5 diopters (D) of spherical equivalent or 3D of astigmatism and signed informed consent. The control group consisted of healthy subjects of similar age who met the same inclusion criteria, except for the presence of DM. Additionally, all participants were required to have well-controlled arterial blood pressure and lipid profiles. To minimize variability related to ethnicity, all study participants were Caucasian.

Exclusion criteria for both groups included the presence of any signs of DR or other ocular diseases, IOP higher than 21 mmHg as evaluated by Goldmann tonometry, changes at the optic nerve head suggesting glaucoma, optic nerve pathology, ocular inflammation, or any previous ocular surgery.

The electroretinography examination was performed using the RETI-port/scan21 (Roland Consult, Brandenburg, Germany. Version 1021.3.0.0, released on 31 May 2021), following the protocols established by the International Society for Clinical Electrophysiology of Vision (ISCEV) updated in 2021[17,20].

Pupils were fully dilated (≥7 mm) using 1% tropicamide eye drops. After applying topical anesthesia, active sterile DTL electrodes were positioned on the bulbar conjunctiva. A gold-cup skin reference electrode was placed superotemporally to the orbital rim, and the ground electrode was positioned on the forehead. Preparation gel (Nuprep EEG & ECG gel) was used to clean the skin, minimize impedance, and optimize the recordings, maintaining a target impedance level below 5 kΩ. Patients used appropriate refractive correction during the examination, and simultaneous recordings were made for both eyes.

The viewing distance was set to 285 mm. Sixty-one hexagonal elements, scaled in size and subtending a 20–30° field of the retina, were presented. Each hexagon underwent temporal modulation between light and dark based on a predetermined pseudorandom binary sequence (m-sequence) with a base interval of approximately 16.6 ms. The luminance was 220 cd/m^2 for the white hexagons and 1 cd/m^2 for the black hexagons, resulting in a high contrast of 99%. The pulse was displayed on a 19-inch high-quality monitor at a frame rate of 60 Hz. Retinal signals were filtered between 10 and 100 Hz and amplified 100,000 times.

Peripheral dark adaptation was avoided by maintaining a similar level of light adaptation across the retina, with dim room lights on. The session lasted 6 min and 16 s, completing 8 cycles of 47 s each. Recording quality was monitored, and segments contaminated by electrical artifacts (such as significant noise or saturation), or loss of fixation were discarded and repeated. To enhance fixation stability, resting periods were allowed every 30 s.

First-order kernels were recorded (Figs. 1 and 2). The response amplitude density (RAD) between N1 and P1 was evaluated and presented in nV/deg^2. P1 IT was measured in ms. The responses were represented and analyzed in different formats:

- A trace array of 61 local retinal responses.
- A three-dimensional topographical plot.
- The average response obtained from five concentric annular rings centered on the fovea: Responses derived from 0° to 2° (ring 1, R1), 2° to 5° (ring 2, R2), 5° to 10° (ring 3, R3), 10° to 20° (ring 4, R4) and > 20° (ring 5, R5).
- Responses were also analyzed in four quadrants (Q): inferior (I), nasal (N), superior (S) and temporal (T). The same quadrants were compared between eyes, named Q1 (ST), Q2 (IT), Q3 (IN) and Q4 (SN).

To minimize variability in mfERG recordings and the challenges in interpreting results, despite adhering to ISCEV standards, we ensured clear instructions for participants, all data were analyzed by a single examiner to reduce inter-examiner variability, and periodic breaks were implemented to improve cooperation.

All data were collected in an Excel spreadsheet (Microsoft Office Excel 2023, Microsoft Corporation, Redmond, WA, USA). Statistical analysis was performed using the Statistical Package for the Social Science (SPSS) 22.0 (SPSS Inc., IBM Corporation, Sommers, NY, USA). Normal distribution was assessed using the Kolmogorov-Smirnov test. Since the data did not follow a normal distribution, nonparametric tests were conducted (Mann-Whitney U test for independent samples) to assess differences between groups. The electrophysiological data were correlated with age, duration of diabetes, and HbA1c levels using Pearson's correlation test. A p value < 0.05 was considered statistically significant.

Results

The T1DM group consisted of 46 eyes from 23 patients. The age at diagnosis was 17.96 ± 13.43 (range 2–47), and the average disease duration was 28.88 ± 8.04 years (range 18–47). Mean age was 48 ± 9.77 years (range 28–69), with a distribution of 14 females (60.8%) and 9 males (39.13%). The mean HbA1c value was 7.29 ± 0.89% (range 6.2–10), and blood pressure and lipid levels remained within normal limits. Metabolic parameters are presented in Table 1. The control group included 46 eyes from 23 healthy subjects, comprising 14 females (60.8%) and 9 males (39.13%); mean age was 51.7 ± 4.75 years (range 40–59). There were no differences in age or sex distribution between the two groups.

There was a statistically significant decrease in BCVA in the T1DM group (0.03 ± 0.06 LogMAR) compared to the control group (-0.01 ± 0.04 LogMAR) ($p = 0.001$). No differences were found in IOP (15.76 ± 2.14 and 15.75 ± 3.42 in the T1DM and control groups, respectively; $p = 0.614$).

Regarding the mfERG results, the T1DM group showed a statistically significant decrease in N1-P1 RAD recorded in the R1, R3, R4 and R5 compared to the control subjects ($p = 0.003$, $p = 0.006$, $p = 0.023$ and $p = 0.027$, respectively). The R2 RAD was the only ring with no differences (Table 2; Fig. 3). Regarding IT, no statistically significant differences were found between the T1DM group and the control group (Table 2; Fig. 4).

In the quadrant analysis, a statistically significant decrease in P1 RAD was recorded in the inferior quadrants, both Q2 (IT) and Q3 (IN), in the T1DM group compared to the control subjects ($p = 0.030$ and $p = 0.004$, respectively) (Table 2; Fig. 5). However, the control group exhibited a statistically significant shorter P1 IT in Q2 (IT) and Q4 (SN) compared to the T1DM group ($p = 0.024$ and $p = 0.041$) (Table 2; Fig. 6). No interocular differences were found in the T1DM patients.

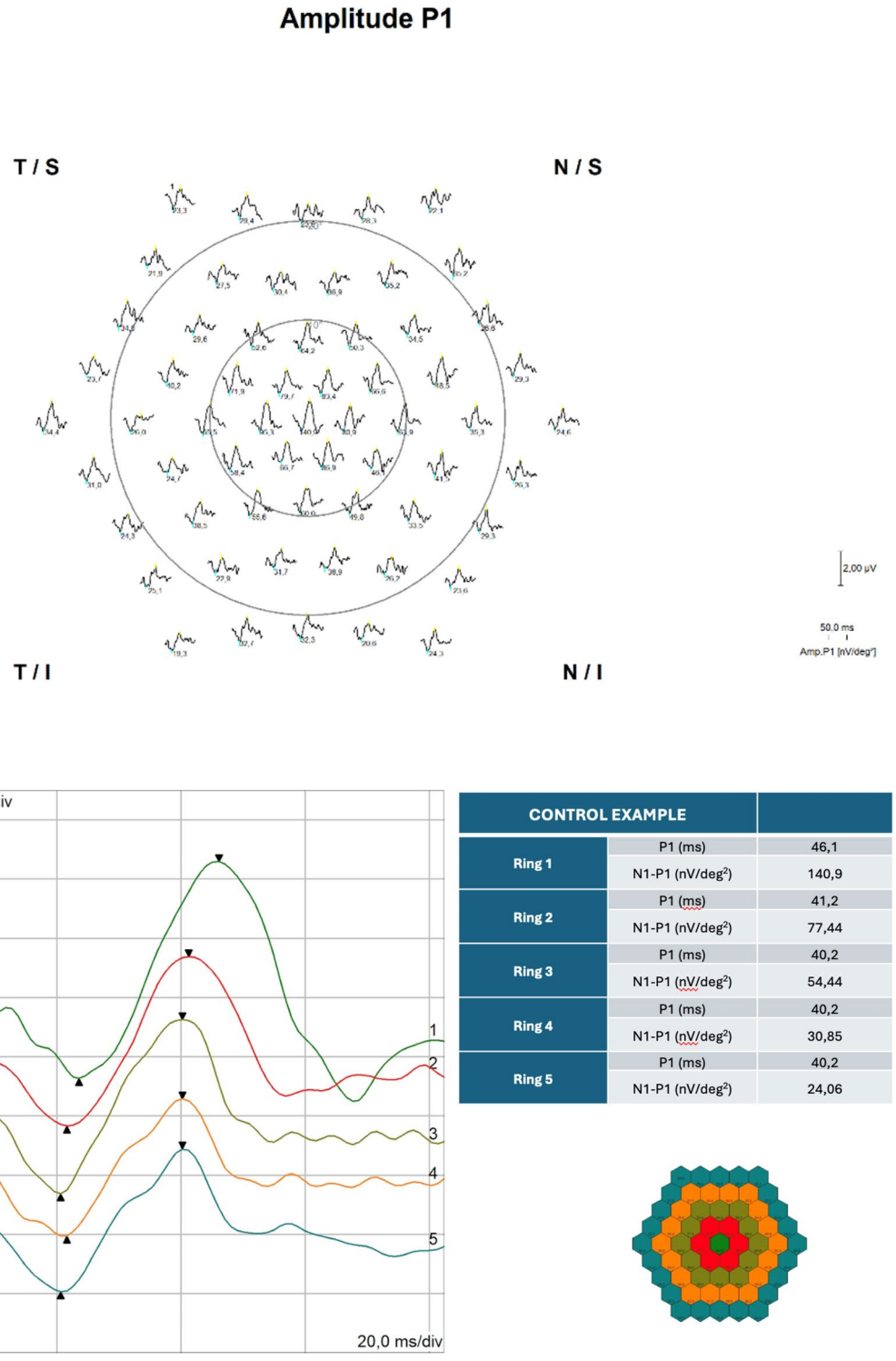
In the correlation study, age was positively correlated with different ITs: R1 P1 IT ($r = 0.390$ $p = 0.008$); R4 P1 IT ($r = 0.676$ $p < 0.001$); R5 P1 IT ($r = 0.561$ $p < 0.001$). HbA1c showed a significant negative correlation with R3 P1 IT ($r = -0.465$, $p = 0.019$), Q2 P1 RAD ($r = -0.427$, $p = 0.033$), Q3 P1 RAD ($r = -0.472$, $p = 0.017$) and Q4 P1 IT ($r = -0.400$, $p = 0.047$). No significant correlation was found between the duration of the disease and either ITs or RADs.

Discussion

Our study reveals functional alterations in individuals with long-term T1DM without vascular signs of DR. DR affects both retinal vascularization and retinal neurons, and neuronal changes can be demonstrated using functional tests such as neurophysiological assessments[20]. ERG dysfunction has been identified as a potential indicator of neuronal impairment even before the onset of DR symptoms. Neurodegeneration appears before the emergence of vascular signs and becomes more pronounced as DR progresses; both neurons and glial components are altered and interrelated by the disease. Inflammation and oxidative stress lead to cell apoptosis and reactive gliosis, which, in turn, contribute to the apoptosis of retinal neurons[34,35].

Our study focused on analyzing functional changes using mfERG in long-standing T1DM patients without DR, adhering to the ISCEV protocol and utilizing both traditional ring analysis and quadrant evaluation. Previous research has demonstrated the effectiveness of mfERG in detecting retinal function alterations in diabetic individuals[22]. The use of mfERG allows for precise correlation between functional deficits and affected areas of the central retina, offering valuable insights into disease progression. While ffERG provides a comprehensive assessment of overall retinal function, mfERG effectively overcomes this limitation[17], being particularly effective in monitoring specific localized vascular changes associated with DR progression[22,24].

mfERG studies have shown different outcomes in either amplitude or IT in T1DM patients[15,27,31,36–39]. The most common finding is the delay in P1 IT. mfERG times have been described to be delayed in areas within and outside of vascular lesions[22,29,31,40,41]. Our study did not find significant differences in mfERG IT between T1DM patients and controls in the ring analysis. Although the delay in P1 IT is frequent, it has not been reported in

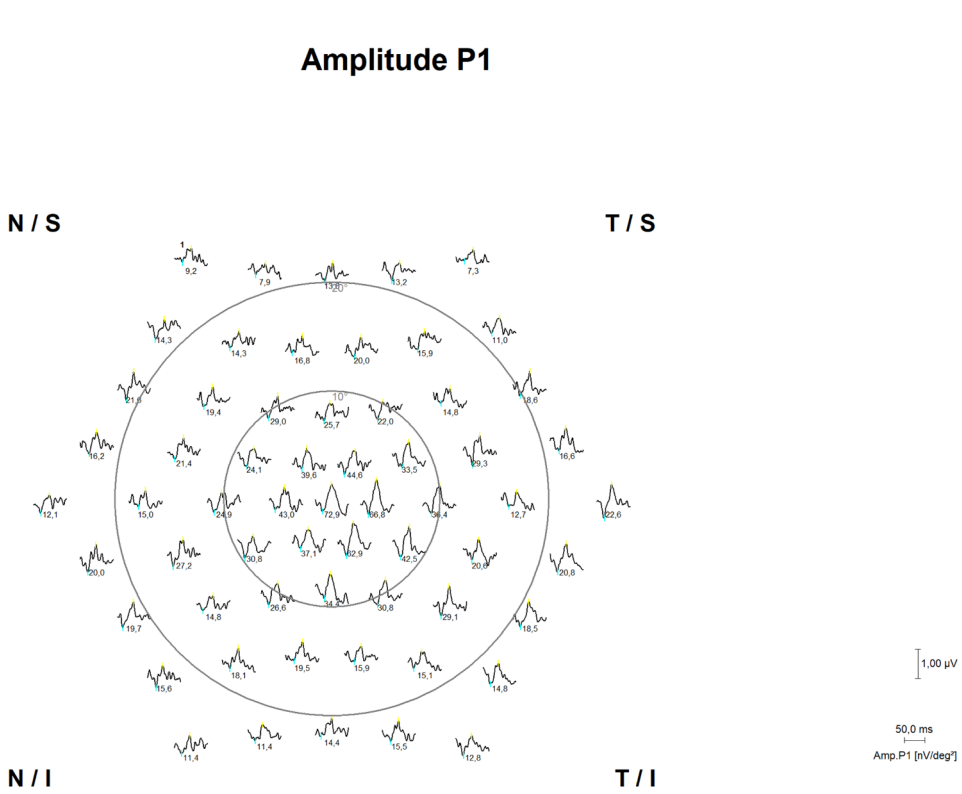


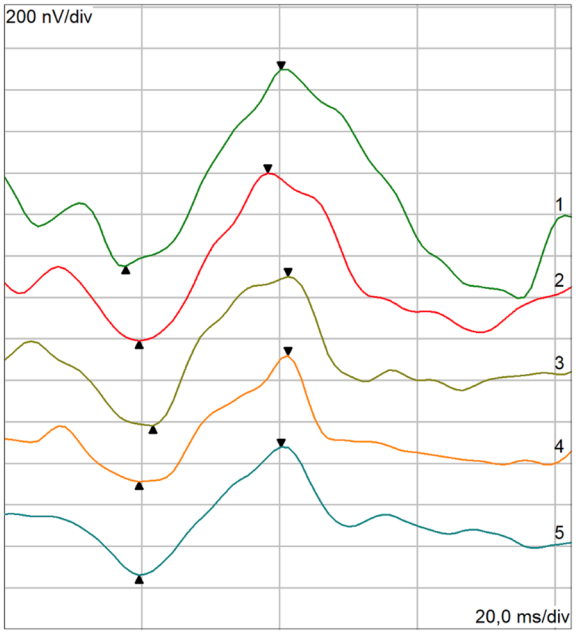
CONTROL EXAMPLE		
Ring 1	P1 (ms)	46,1
	N1-P1 (nV/deg²)	140,9
Ring 2	P1 (ms)	41,2
	N1-P1 (nV/deg²)	77,44
Ring 3	P1 (ms)	40,2
	N1-P1 (nV/deg²)	54,44
Ring 4	P1 (ms)	40,2
	N1-P1 (nV/deg²)	30,85
Ring 5	P1 (ms)	40,2
	N1-P1 (nV/deg²)	24,06

Fig. 1. Example of a control subject (left eye), displaying first-order kernels analyzed using a trace array of 61 local retinal responses. Additionally, the average response was calculated from five concentric annular rings centered on the fovea. The response amplitude density (RAD) between the N1 and P1 peaks was measured and expressed in nV/deg^2, while the P1 implicit time (IT) was recorded in milliseconds. Abbreviations: RAD, response amplitude density; IT, implicit time.

every study[42–47]. Interestingly, quadrant analysis revealed statistically significant shorter IT in Q2 and Q4 in T1DM, though the differences were minimal.

An increase in IT has also been described as a predictor of vascular abnormalities over a one-year[48] or three-year[49] period. Regression models have been used to determine whether mfERG IT could predict new sites of





T1DM EXAMPLE		
Ring 1	P1 (ms)	40,2
	N1-P1 (nV/deg²)	72,9
Ring 2	P1 (ms)	38,2
	N1-P1 (nV/deg²)	43,78
Ring 3	P1 (ms)	41,2
	N1-P1 (nV/deg²)	26,62
Ring 4	P1 (ms)	41,2
	N1-P1 (nV/deg²)	16,2
Ring 5	P1 (ms)	40,2
	N1-P1 (nV/deg²)	12,38

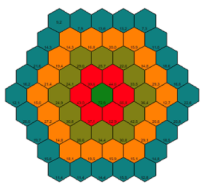


Fig. 2. First-order kernel analysis of a type 1 diabetes mellitus (T1DM) patient (right eye) presented using a trace array of 61 local retinal responses. The average response was calculated from five concentric annular rings centered on the fovea. The response amplitude density (RAD) between the N1 and P1 peaks was measured in nV/deg^2, and the P1 implicit time (IT) was recorded in milliseconds. Abbreviations: T1DM, type 1 diabetes mellitus; RAD, response amplitude density; IT, implicit time.

vascular abnormalities over a one-year period with good sensitivity (73%) and specificity (77%). Harrison et al.[50] developed a multivariate model for predicting the onset of retinopathy in adult patients with diabetes, with mfERG IT as the primary predictive risk factor. Patients with T1DM were found to be at higher risk compared to those with T2DM.

Type 1 diabetes group	Mean ± SD
HbA1c (%)	7.29 ± 0.89
Glycaemia (mg/dL)	149.00 ± 66.13
Total cholesterol (mg/dL)	190.61 ± 33.15
HDL cholesterol (mg/dL)	62.43 ± 12.38
LDL cholesterol (mg/dL)	114.30 ± 27.78
Urea (mg/dL)	34.35 ± 8.62
Creatinine (mg/dL)	0.78 ± 0.10
Albumin/creatinine ratio (mg/g Cr)	7.13 ± 10.19

Table 1. Mean and standard deviation (SD) of the metabolic characteristics of the diabetic group. Abbreviations: HbA1c, glycosylated haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation.

		Control group	T1DM group	
		n = 46	*n* = 46	
		Mean ± SD	Mean ± SD	p
Ring analysis				
Ring 1	P1 (ms)	45.25 ± 2.97	45.67 ± 4.62	0.713
	N1-P1 (nV/deg^2)	111.5 ± 31.18	90.77 ± 28.72	**0.003**
Ring 2	P1 (ms)	41.51 ± 2.24	41.08 ± 2.30	0.416
	N1-P1 (nV/deg^2)	53.5 ± 17.79	46.86 ± 13,55	0.087
Ring 3	P1 (ms)	41.42 ± 1,67	40.98 ± 1.91	0.296
	N1-P1 (nV/deg^2)	41.51 ± 10.90	27.97 ± 7.23	**0.006**
Ring 4	P1 (ms)	41.12 ± 1.71	41.32 ± 1.37	0.888
	N1-P1 (nV/deg^2)	20.91 ± 6.37	17.63 ± 4.86	**0.023**
Ring 5	P1 (ms)	41.60 ± 1.21	41.13 ± 1.27	0.111
	N1-P1 (nV/deg^2)	15.36 ± 4.89	12.95 ± 3.80	**0.027**
Quadrant analysis				
Q1	P1 (ms)	42.14 ± 1.06	41.64 ± 1.44	0.090
	N1-P1 (nV/deg^2)	20.67 ± 7.18	17.91 ± 5.50	0.084
Q2	P1 (ms)	41.65 ± 1.15	40.94 ± 1.40	**0.024**
	N1-P1 (nV/deg^2)	21.82 ± 6.24	18.78 ± 4.58	**0.030**
Q3	P1 (ms)	40.94 ± 1.73	40.97 ± 1.41	0.723
	N1-P1 (nV/deg^2)	21.33 ± 6.16	17.49 ± 3.92	**0.004**
Q4	P1 (ms)	41.95 ± 0.91	41.40 ± 1.30	**0.041**
	N1-P1 (nV/deg^2)	19.91 ± 7.26	16.72 ± 9.90	0.052

Table 2. mfERG N1-P1 response amplitude density and P1 implicit time values obtained with the Roland ERG following the ISCEV protocol in the T1DM and control groups using ring and quadrant analysis. Values are presented as mean ± standard deviation (SD). Differences that reach statistical significance are presented in red and bold ($p < 0.05$). Abbreviations: T1DM, type 1 diabetes mellitus; SD, standard deviation; Q, quadrant.

The amplitude of mfERG has been reported to be either normal or reduced in DM patients. P1 amplitude shows greater variability compared to its IT. However, our study consistently demonstrates a reduction in mfERG RAD among T1DM patients compared to controls.

Analysis by rings showed a statistically significant decrease in RAD across all rings except for R2, where the decrease did not reach statistical significance. Similarly, quadrant analysis revealed a significant decrease in RAD in both inferior quadrants, Q2 and Q3. These findings underscore RAD as a pivotal indicator of retinal dysfunction, even in the absence of visible ophthalmoscopic changes. Aroca et al.[51] compared mfERG parameters between patients with T1DM and T2DM who did not have DR. They found that mfERG amplitudes were significantly lower in T1DM patients across the three central macular rings compared to T2DM patients, whereas IT showed no significant differences between groups.

The significant decrease in RAD observed in T1DM patients suggests impaired function of bipolar cells and photoreceptors prior to the onset of clinical DR. These cells, which are primary initiators of N1–P1 RAD, are integral components of the inner retina alongside photoreceptors[52].

The EUCONDOR clinical trial (European Consortium for the Early Treatment of Diabetic Retinopathy), one of the largest studies evaluating functional findings in T2DM patients without evident DR or with mild non-proliferative DR (NPDR) ($n = 459$), provided evidence that P1 amplitude, rather than P1 IT, is the most sensitive

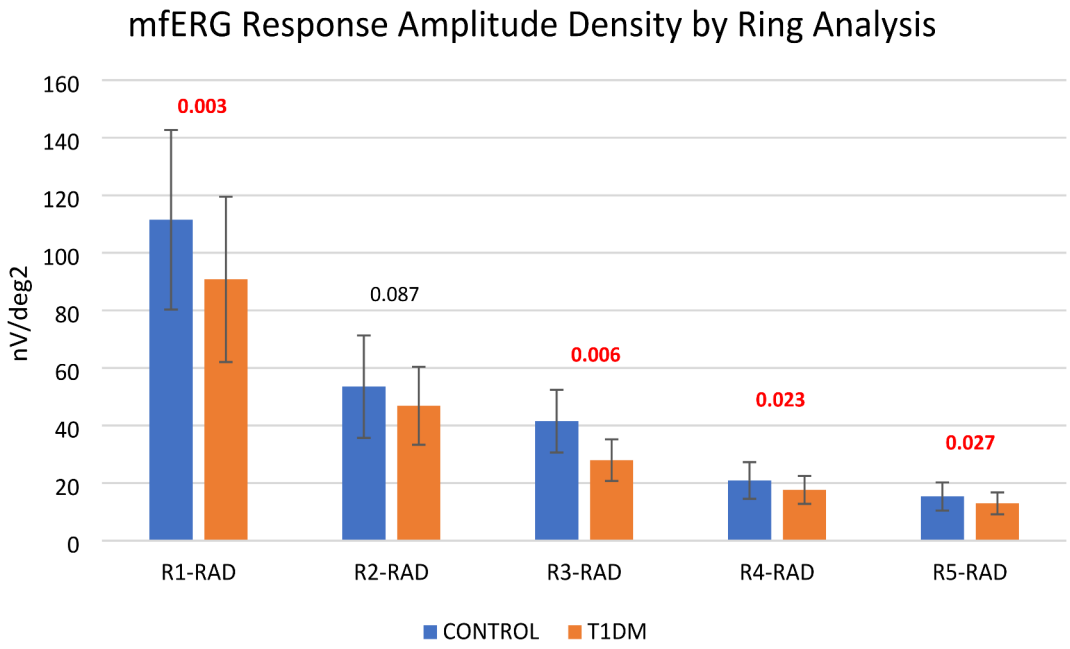


Fig. 3. mfERG response amplitude density obtained with the Roland ERG in the control (blue column) and diabetic groups (orange column) using the ring analysis. Values are expressed as mean ± standard deviation. Differences that reach statistical significance are presented in red and bold ($p < 0.05$). Abbreviations: T1DM, type 1 diabetes mellitus; RAD, response amplitude density.

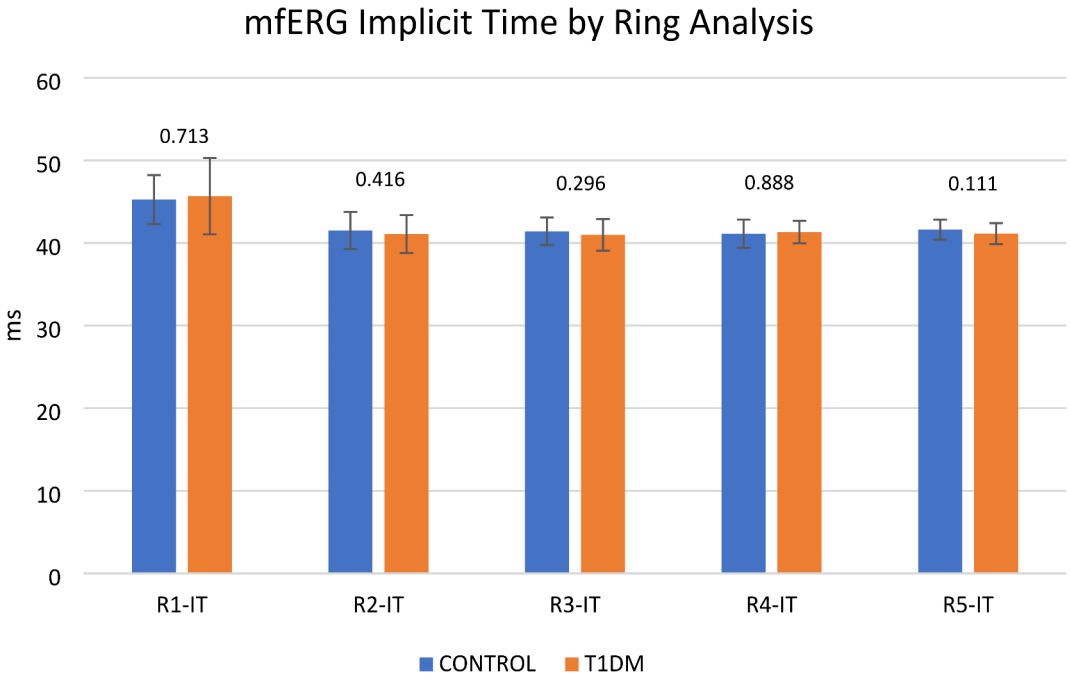


Fig. 4. mfERG implicit time obtained with the Roland ERG in the control (blue column) and diabetic groups (orange column) using the ring analysis. Values are expressed as mean ± standard deviation. Abbreviations: T1DM, type 1 diabetes mellitus; IT, implicit time.

parameter for assessing neurodegeneration[53]. 58% of patients with no DR showed changes in mfERG; 67% of patients with no DR lesions and thinner inner retina layers evaluated by OCT showed mfERG abnormalities. The decrease in P1 amplitude was more prominent from the central to the peripheral retina, suggesting possible cone impairment in these patients[54].

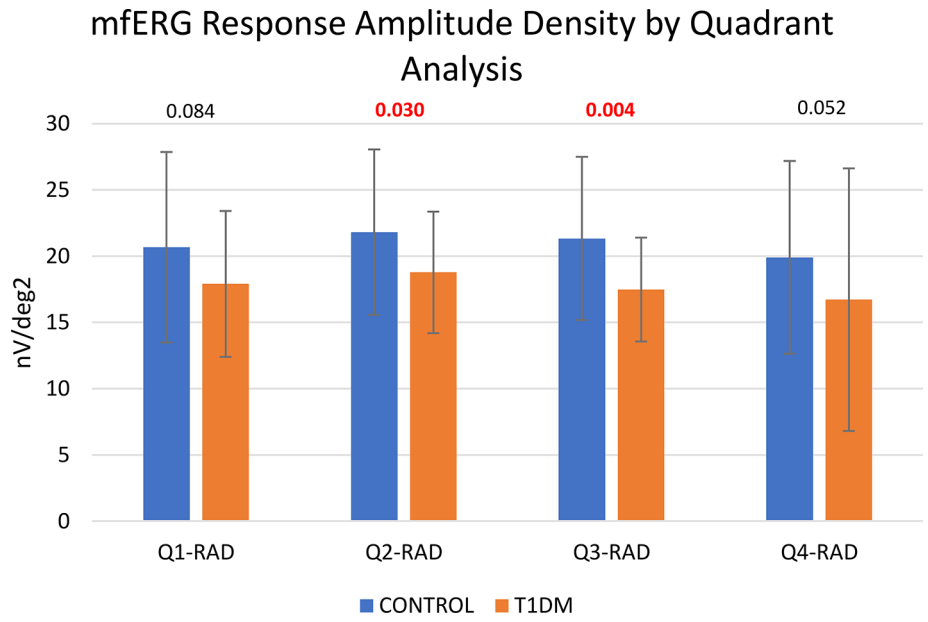


Fig. 5. mfERG response amplitude density obtained with the Roland ERG in the control (blue column) and diabetic groups (orange column) using the quadrant analysis. Values are expressed as mean ± standard deviation. Differences that reach statistical significance are presented in red and bold ($p < 0.05$). Abbreviations: T1DM, type 1 diabetes; RAD, response amplitude density; Q, quadrant.

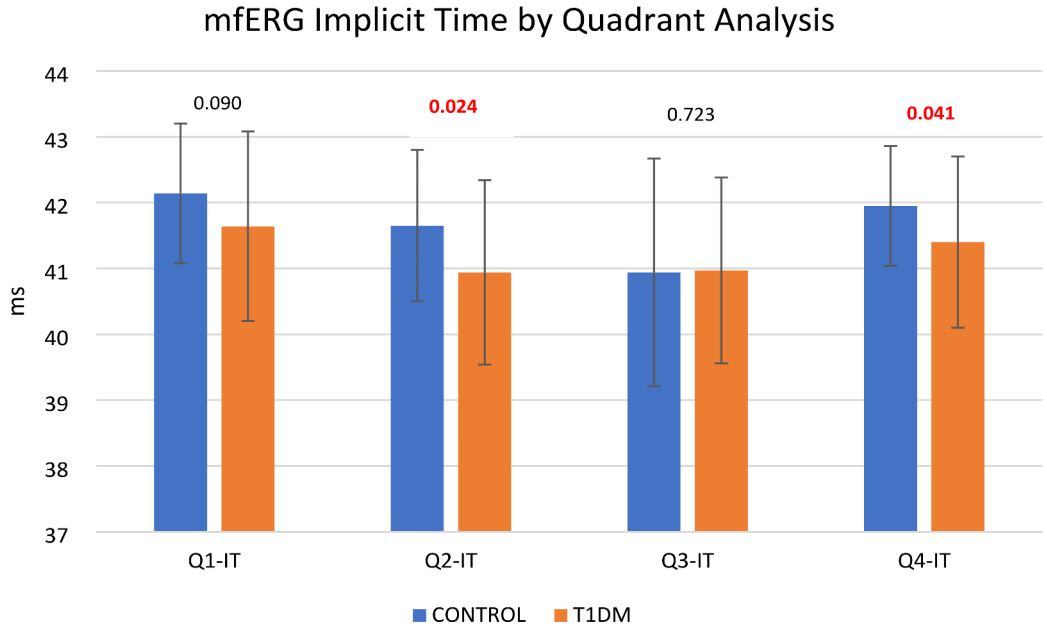


Fig. 6. mfERG implicit time response obtained with the Roland ERG in the control (blue column) and diabetic groups (orange column) using the quadrant analysis. Values are expressed as mean ± standard deviation. Abbreviations: T1DM, type 1 diabetes; IT, implicit time; Q, quadrant.

These findings suggest that the dysfunction affects not only inner retinal layers but also the outer retina[15,55], likely reflecting impairment in both vascular and neural retinal components even in the absence of visible retinopathy signs[53,56]. Animal models have shown a decrease in the inner and outer nuclear layer 10 weeks after disease onset[57]. However, ganglion cells are known to undergo apoptosis prior to the development of DR. This primary ganglion cell damage along with changes in glial cells have also been linked to impaired neurovascular coupling[35,58].

Changes in the ganglion cell layer can be detected using more specific neurophysiological tests such as pERG or visual evoked potentials, which show reductions in wave amplitudes or changes in peak times[59]. Additionally, changes in the outer retina are also present[60] and reflected in other functional tests like ffERG or mfERG. Alterations in OPs in ffERG is a common finding due to their vulnerability to changes in retinal circulation[8]. mfERG has the potential to detect OPs, but this requires optimal conditions and methodological refinements, particularly in the macular region where OPs are less concentrated[61,62]. At present, our equipment does not support the precise extraction of OPs, and standardized ISCEV protocols for incorporating OPs into mfERG are not yet available. Consequently, we were unable to assess the contribution of OPs to the mfERG signal in this study. Further research is needed to better understand the role of amacrine cells in early DR and to refine techniques for capturing OPs in mfERG.

The rod-pathway, assessed in dark-adapted ERG, is expected to be more sensitive to retinal diseases due to the oxidative stress and metabolic demands[8,10]. However, mfERG primarily evaluates the central retina, where lesions are typically located in DR, under photopic conditions mainly driven by cone bipolar cells and cone photoreceptors. A reduction in cone density associated with glycemic control has been described using adaptive optics imaging[63]. These macular changes could contribute to mfERG abnormalities.

Results from mfERG studies are different. Variability between studies may be related to other factors such as glycemic control or type of DM. For instance, Lakhani et al.[64] found that poor long-term glycemic control was associated with increased localized neuroretinal dysfunction in mfERG.

Mohammed et al.[65] and Adhikari et al.[15] found reduced N1–P1 amplitude with increased P1 IT in subjects with T2DM without DR compared to controls. Some studies have reported normal mfERG responses in diabetic patients[66,67].

The location of mfERG findings can be influenced by both vascular and neuronal factors. Several studies have identified patterns of changes related to vascular autoregulation[68]. Oxygen saturation has been shown to decrease from the upper nasal to the lower nasal, lower temporal, and the upper temporal quadrant, although no differences were found between healthy individuals and T2DM patients[69]. Concerning the neurodegeneration process, differences in caspase 1 activity and increased expression of nitric oxide synthase (iNOS) in the temporal retina have been reported in T2DM patients[70].

Regarding the distribution of vascular signs, Tang et al. noted a higher prevalence of microaneurysms in the temporal retina[70]. Munuera-Gifre et al. observed more vascular signs in the upper temporal quadrant in T2DM subjects[71]. Our study also identified differences in the spatial distribution of mfERG abnormalities, finding a reduction in the P1 RAD in the inferior quadrants compared to the control group, but without a corresponding increase in IT. The variability in results across studies may be attributed to differences in glycemic control, disease duration, and diabetes type. For instance, Laron et al.[37] found IT delays randomly distributed across the retina in a study of 137 adolescent T1DM patients with a mean disease duration of 6.3 years, with a positive correlation with HbA1c levels, while only 3% of their patients showed abnormal amplitudes. Holm and Adrian identified lower amplitude and longer IT specifically in the nasal retina in a study of 27 DM patients with a mean age of 58 years[45], suggesting it is particularly vulnerable to diabetic damage. However, Huang et al.[41] reported prolonged ITs and reduced amplitudes, primarily in the temporal retina of T2DM patients, emphasizing the potential impact of diabetes type and retinal location on mfERG outcomes.

Several studies present conflicting reports on the correlation between age, disease duration, glycemic control and mfERG responses[15,30,37]. The main finding of our study is a significant reduction in RAD across all retinal rings except for R2 and in the inferior quadrants. RAD was negatively correlated with HbA1c levels in R3, Q2, Q3 and Q4. Additionally, we observed an increase in IT with age. These results underscore the importance of good metabolic control. To minimize the influence of confounding factors, we included only patients with moderate-to-good glycemic control and well-managed comorbidities, including controlled blood pressure and lipid levels. Vascular factors unrelated to diabetes could have altered our results, which is one of the reasons for the limited sample size in this study.

Aroca et al.[51] reported that in T1DM, HbA1c levels ≥ 7% were associated with a significant reduction in amplitude in the first ring and an increased in IT across all rings; an effect not observed in T2DM. Furthermore, while diabetes duration did not significantly affect mfERG parameters in T1DM, a duration of ≥ 15 years in T2DM was linked to reduced amplitudes and prolonged IT. These findings suggest that the retina in T1DM is more sensitive to metabolic changes, such as hyperglycemia, whereas in T2DM, retinal function is more strongly influenced by the duration of diabetes.

Ziccardi et al.[42] found that retinal dysfunction positively correlated with aging and disease duration, while HbA1c levels did not correlate with early retinal functional impairment. They noted a correlation between age and the RAD, but not with IT. Mohammed et al.[65] reported that N1–P1 amplitude was negatively correlated with disease duration, while P1-IT was positively correlated with diabetes duration in T2DM subjects without DR. Similarly, Adhikari et al.[15] reported a positive correlation between IT and the duration of the disease in T2DM without DR, though they did not find significant correlations between amplitude and diabetes duration or blood sugar level. However, no significant correlation was found between the duration of the disease and either ITs or RADs in our study.

Our results are consistent with other studies regarding the influence of age and HbA1c on retinal function. However, our study identified a lack of correlation between age and mfERG RAD and a minimal impact of disease duration on IT and RAD. These observations highlight the need for further research to fully understand these relationships. The complexity and multifactorial nature of DR may contribute to these varying results.

Our findings, along with prior studies, enhance the important value of mfERG to evaluate retinal dysfunction. However, despite its potential, the widespread application of mfERG in diabetic eye care is limited by the need for evaluator expertise and is largely confined to research settings and clinical trials. Standardizing mfERG recording

and interpretation protocols is critical for its integration into broader clinical practice and the development of future neuroprotective strategies for DR.

We emphasize the importance of identifying neurodegenerative changes that precede vascular disease to better understand retinal neuronal impairment. The emergence of new therapeutic strategies aimed at preserving retinal function will likely increase the need for comprehensive functional assessments, including neurophysiological tests such as mfERG, as well as complementary tools like microperimetry and contrast sensitivity evaluations.

While ISCEV provides standardized testing protocols, incorporating additional approaches such as mfERG OPs or global flash mfERG (MOFO mfERG) would be beneficial[8]. These additional metrics could offer a more comprehensive assessment of retinal dysfunction in diabetic patients. MOFO mfERG has been shown to detect early functional retinal changes, even in patients without visible DR. Lung et al.[72,73] and Shimada et al.[74] demonstrated that combining MOFO mfERG provides more detailed insights into retinal function. mfOPs have also been identified as sensitive biomarkers for early retinal dysfunction, particularly in diabetic patients with early nonproliferative DR (NPDR)[39,75,76]. However, their full diagnostic potential for retinal diseases like DR still requires further investigation.

Data obtained from mfERG, OCT and OCT angiography can enhance the evaluation of patients at higher risk of developing DR and provide valuable biomarkers for detecting retinal dysfunction. Further prospective studies are required to confirm these findings and to establish optimal strategies for evaluating DM patients. Variations in results may arise from factors such as glycemic control, diabetes type, disease duration, vascular regulation dysfunction, neurovascular coupling dysregulation, undetectable vascular lesions, or the neurodegeneration processes.

To increase the sample size, we included both eyes of each diabetic patient in the study. We acknowledge this as a significant limitation, as the two eyes are not independent and tend to exhibit similar behavior, which may introduce bias. However, due to the difficulty in identifying long-term type 1 diabetes mellitus patients without diabetic retinopathy, we opted to include both eyes to increase the number of studied eyes. Future studies should aim to include a larger sample size with independent eyes to enhance statistical power and minimize potential biases. Additionally, only Caucasian subjects were included to avoid confounding effects related to ethnic variability. Expanding future studies to include longitudinal data and a more diverse participant pool will provide a deeper understanding of the retinal changes associated with DR progression and improve the generalizability of these findings. Longitudinal studies would also be particularly valuable in elucidating the dynamics of retinal dysfunction leading to the onset of DR.

In conclusion, mfERG serves as a valuable tool for assessing retinal function in long-term T1DM patients. Our study found diminished RAD in patients with over 15 years of disease progression and no significant changes in IT. RAD reduction was associated with HbA1c control. Further research is necessary to understand the impact of DR on retinal function and the effects of diabetes type, glycemic control, and other factors.

Data availability

The data that support the findings of this study are available from the corresponding author, Isabel Pinilla (ipinilla@unizar.es), upon reasonable request. Access to the data is restricted to protect patient privacy and confidentiality in accordance with ethical guidelines.

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Author contributions

I.P conceived the original idea, designed, and directed the project; M.A wrote the manuscript with support from M.S, G.F, E.O and D.R. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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