Multifocal Electroretinogram Delays Predict Sites of Subsequent Diabetic Retinopathy

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PURPOSE. To examine the potential of abnormal mfERGs to predict the development of diabetic retinopathy at corresponding retinal locations 1 year later.

METHODS. One eye of 11 diabetic patients with nonproliferative diabetic retinopathy (NPDR) and 11 diabetic patients without retinopathy were retested 12 months after initial testing. At each time, mfERGs were recorded from 103 retinal locations, and fundus photographs were taken within 1 month of each recording. Local mfERG implicit times were measured and their z-scores were calculated based on results obtained from 20 age-matched control subjects. mfERG abnormalities were defined as z-scores of 2 or more for implicit time and z-scores of 2 or less for amplitude (P ≤ 0.025). mfERG z-scores were mapped onto fundus photographs, and the relationship between baseline abnormal z-scores and new retinopathy at follow-up was examined.

RESULTS. New retinopathy developed in 7 of the eyes with NPDR after 1 year. In these eyes, 70% of the mfERGs in areas of new retinopathy had abnormal implicit times at baseline. In contrast, only 24% of the responses in regions that remained retinopathy free were abnormal at baseline. Relative risk of development of new retinopathy over 1 year in the areas with abnormal baseline mfERG implicit times was approximately 21 times greater than that in the areas with normal baseline mfERGs (odds ratio = 31.4; P < 0.001). Eyes without initial retinopathy did not develop new retinopathy within the study period, although 4 of these 11 eyes had abnormal implicit times at baseline. mfERG implicit times tended to be more delayed at follow-up than at baseline in NPDR eyes, but not in eyes without retinopathy and control eyes. mfERG amplitudes had no predictive power.

CONCLUSIONS. Localized functional abnormalities of the retina reflected by mfERGs delays often precede the onset of new structural signs of diabetic retinopathy. Those functional abnormalities predict the local sites of new retinopathy observed 1 year later. (Invest Ophthalmol Vis Sci. 2004;45:948–954) DOI:10.1167/iovs.03-1101

Diabetic retinopathy is the leading cause of irreversible blindness in people 20 to 74 years of age in the United States.1 Even with good blood glucose control, more than 76% of diabetic patients will develop diabetic retinopathy within 20 years.1 Laser therapy, the only available clinical treatment for this disease, can slow the progression of retinopathy but cannot reverse vision loss.

The multifocal electroretinogram (mfERG) technique, which can measure and map retinal function at more than 100 locations within 8 minutes, has been used to examine a large number of eye diseases2–4 including diabetes.5–12 For diabetic patients with retinopathy, the magnitude of mfERG implicit time delays correlates with the severity of retinopathy, and the locations of abnormal mfERG implicit times correlate spatially with anatomic abnormalities.5–7 By contrast, response amplitude, although often reduced in eyes with retinopathy, has no such correspondence with the presence of retinal lesions.5–8,12 Moreover, implicit times are often significantly delayed in retinal locations without retinopathy and in diabetic eyes without retinopathy.5,9 This raises an interesting question of whether the abnormal implicit times identify retinal locations where retinopathy will develop in the future. The ability to identify eyes and retinal sites at greatest risk for retinopathy would strengthen future clinical trials of new preventative pharmacologic therapies now being developed.13–15

The primary purpose of this study was to examine whether local mfERG implicit time abnormalities in diabetic eyes are predictive of the development of diabetic retinopathy identified in fundus photographs at corresponding retinal locations. Diabetic eyes with little or no diabetic retinopathy at baseline were retested 1 year after initial study. We examined the incidence of newly developed diabetic retinopathy, the change in the mfERG over 1 year, and the spatial relationship between new retinopathy and baseline mfERG results.

METHODS

Subjects

At the first visit, 11 diabetics with nonproliferative diabetic retinopathy (NPDR; 6 men and 5 women) and 11 diabetic patients without retinopathy (5 men and 6 women) were tested with the mfERG. Examinations were performed in dilated eyes, and central stereoscopic 50° color fundus photographs of these eyes were taken within 1 month of mfERG testing. The 50° fundus photographs were chosen for this study to cover the testing field of the mfERG, the central 45°. The 50° fundus photography is the standard protocol defined in the Field Guide Book and applied in the EURODIAB study.16 It is reported that assessment of diabetic retinopathy using this protocol compares well with the standard 30° stereo photograph.17,18 After 1 year (1 year ± 1.8 months), the eyes were retested according to the same procedures as at baseline. In diabetic subjects, the eye with less retinopathy at baseline was the study eye.

The diagnosis of diabetic retinopathy was masked to the mfERG results and was made on the basis of the eye examination and fundus photograph grading performed by a retinal specialist. The severity of diabetic retinopathy was classified according to the Early Treatment Diabetic Retinopathy Study (ETDRS) criteria.12 In the NPDR group, two subjects had moderate NPDR (each one had a small patch of edema in the midperipheral retina) and the other nine had only mild retinopathy (95% of the lesions are microaneurysms or dot hemor-
All eyes in the diabetic groups had 20/25 or better corrected visual acuity. Patients with visible media opacity or history of other ocular disease or surgery were excluded from the study. Subjects with NPDR were aged 32 to 59 years (mean ± SD, 47.2 ± 6.4) with duration of diabetes of 2 to 20 years (9.7 ± 6.8) and the patients without retinopathy were aged 26 to 62 years (49.2 ± 9.8) with duration of diabetes of 3 to 20 years (7.8 ± 4.5).

Twenty eyes of 20 normal subjects, 9 men and 11 women, aged 28 to 60 years (47.2 ± 9.5) were tested (14 right and 6 left eyes, based on the subject’s preference). All normal subjects were free of ocular and systemic disease and had 20/20 or better corrected visual acuity. No subjects with refractive errors outside the range of −6.00 and +4.00 D were included in this study. Half of the control subjects had their mfERGs retested at a 1-year follow-up to examine repeatability.

The purposes and potential risks of the study were explained, and informed consent was obtained from all subjects before testing. Procedures followed the tenets of the Declaration of Helsinki, and the protocol was approved by the University of California Committee for the Protection of Human Subjects.

### mfERG Recording

mfERGs were recorded using a visual evoked response imaging system (VERIS 4.3; EDI, San Mateo, CA; Fig. 1A). Pupils were dilated to 7 to 8 mm with 1.0% tropicamide and 2.5% phenylephrine. After the cornea was anesthetized with 0.5% proparacaine, a bipolar contact lens electrode (Hansen Ophthalmic, Solon City, OH) was placed on the eye, and a ground electrode was clipped to the right earlobe. The fellow eye was occluded. An array of 103 hexagonal elements was delivered by an eye camera-display-refractor unit (EDI) driven at a 75Hz frame rate. The hexagons were modulated between white (200 cd/m²) and black (<2 cd/m²) according to an m-sequence during the 7.5-minute recordings. Observers adjusted the stimulus unit for best focus of the central fixation target before the recording. Recordings were made in sixteen 30-second segments. Recording quality and eye movements were monitored by real-time display and the eye camera, respectively. Contaminated segments were discarded and repeated. Retinal signals were filtered 10 to 100 Hz.²¹ mfERGs were processed in the usual way with one iteration of artifact removal and spatial averaging with one sixth of the surrounding responses.

### Data Analysis

The implicit time and amplitude of local first-order mfERG responses were analyzed using a template-stretching method described in detail by Hood and Li.²⁰ A normal waveform template was obtained at each stimulated retinal location by averaging the mfERGs recorded from the control subjects, and local responses from each subject were compared with the templates. Each template was independently scaled in amplitude and time dimensions by multiplying the amplitude and the time vectors by a scale factor for each parameter until the best least-square fit to each local response was obtained. The amplitude and implicit time of each local response was then derived from the scale factors. Amplitude was calculated as the voltage difference between the first trough and the first peak of the scaled template. Implicit time was measured to the first prominent response peak (P1) of the scaled template. The quality of fitting was assessed by a goodness-of-fit measure referred to as a statistic. All the local responses had a statistic of less than 0.75, indicating a false alarm rate below 3%.²⁰ Based on the mean and SD of each local mfERG measure obtained from the normal subjects, mfERG z-scores were calculated for all diabetic subjects’ responses. Abnormal implicit time was defined as a z-score ≥ 2.0 (P ≤ 0.025) and abnormal amplitude as a z-score ≤ −2.0 (P ≤ 0.025). The cutoff values for abnormality defined by these criterion z-scores and those defined by the 0.025 percentile are similar at each location, with a maximum difference of 3% (mean difference in implicit time measure is 1%). This similarity suggests that our results are not dependent on, or a consequence of, using z-scores to define abnormalities. Previous studies showed that the local mfERG amplitude measure detects fewer functional abnormalities than implicit time measures in diabetics,⁵⁻⁶ and is not correlated with the visible retinal lesion sites.⁷ Therefore, in this study we focus on implicit time measures and briefly report amplitude results.

The spatial correspondence between the mfERG stimulus array and fundus photograph grading is shown in Figure 1B. The comparison between mfERG and fundus photograph grading was performed in “zones.” A zone is a retinal area comprising three to seven adjacent mfERG stimulus elements (described in detail in the Results section). Zones, instead of individual elements, were chosen for study for three reasons. First, the actual size of the anatomic retinal lesion could extend beyond the lesion identified in a fundus photograph. Second, the location of visible retinopathy might not lie directly over the site of actual anatomic lesion. Third, for each individual the use of zones helps to offset the possible spatial mismatch between the retinal locations of the mfERG stimulus array and fundus photographs.

### Results

#### Development of New Retinopathy and Change in mfERG over a Year

In this analysis, a “retinopathy zone” is defined as the stimulus hexagon overlaying a visible retinal lesion plus its nearest stimulus neighbors. In practice, retinopathy zones were constructed from the mfERG stimulus array beginning in the top left corner of the array and continuing horizontally by rows to the lower right corner while avoiding overlap among adjacent zones. The first stimulus element covering a lesion and its immediate neighbors were identified and assigned to a zone, and the process continued until all lesions were assigned to zones. The remaining elements were assigned to a “no retinopathy area.” If a retinopathic lesion was already included in a

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**Figure 1.** (A) mfERG stimulus display. The luminances for white, black, and surround were 200 cd/m², less than 2 cd/m², and 100 cd/m², respectively. The frame rate of the monitor was 75 Hz. (B) Approximate spatial correspondence of the multifocal stimulus array and the fundus photograph.
retinopathy zone, it did not generate a new zone. In fact, because most of the subjects with NPDR had only mild retinopathy with scattered microaneurysms and dot hemorrhages, most of the retinopathy zones were not adjoining. In the hypothetical example illustrated in Figure 2, there are two retinopathy zones at baseline, each composed of 7 hexagons, together covering 13.6% (14/103) of the stimulated retinal area. The remaining 86.4% of the locations are categorized as a no retinopathy area. After 1 year, new retinopathy is observed in the no retinopathy area, and a new retinopathy zone comprising seven hexagons (6.8% of the retinal area) is constructed at this location.

Seven of the 11 NPDR eyes showed development of new retinopathy at follow-up, whereas none of the eyes of diabetic patients without retinopathy did. The two NPDR eyes that had a small patch of edema showed development of additional retinopathy at follow-up. All the newly developed retinopathies were microaneurysms or dot hemorrhages, and, like the lesions at baseline, they were much smaller than an mfERG stimulus element. At baseline, retinopathy zones covered 16% of the total testing area across all NPDR eyes. After 1 year, newly developed retinopathy zones accounted for another 10% of the total tested retinal area free of retinopathy.

The changes in mfERG implicit time z-scores over the study year were analyzed for each subject group. For the subjects with NPDR, mfERG z-scores were further separated into three retinal categories based on the retinopathy stages at baseline (Fig. 2): (1) Old Retinopathy Zone (retinopathy was present at baseline and remained at follow-up); (2) New Retinopathy Zone (newly developed retinopathy was present at follow-up); and (3) Still-No-Retinopathy Area (retinal regions remained lesion-free after 1 year). Table 1 shows the number of Old Retinopathy Zones and the number of New Retinopathy Zones in the NPDR eyes in which new retinopathy developed.

To compare the mfERG implicit times among subject groups, distributions of the implicit time z-scores were constructed by combining all the z-scores from all the eyes within each group and category. The results are shown as box plots in Figure 3. For the normal subjects, mfERG implicit time z-scores at both testing times were normally distributed, with the median of the distributions close to the expected value of zero. Only 2.2% of the responses had significant delays, as expected. The z-score distributions for the 10 normal control subjects tested at both times were not significantly different (P = 0.50, sign test), with 64% of the results being identical.

At both testing times, the z-score distributions for the three NPDR categories were shifted in the abnormal direction, with most of the mfERG z-scores being greater than 0 (i.e., delayed compared with the normal mean; Fig. 3). Even in the retinal areas that remained retinopathy free, 16% of the initial responses were abnormal. The percentage of abnormal z-scores in New Retinopathy Zones was higher than that in Old Retinopathy Zones (67.8% vs. 37.0%, P < 0.008). In general, implicit times of mfERGs within the Old Retinopathy Zones and the still-no-retinopathy areas were significantly longer at follow-up than at baseline (P < 0.01). However, response implicit times within the New Retinopathy Zones did not change over the study year (P = 0.89). In the diabetic subjects without retinopathy, mfERG implicit times did not show significant change over the year (P = 0.34; Fig. 3), although the distributions were skewed in the abnormal direction and some of the responses were abnormal (15.0% at baseline and 12.0% at follow-up).

Local mfERG amplitudes did not change over the year in the normal eyes and also did not change in New and Old Retinopathy Zones of NPDR eyes. However, the eyes of diabetic subjects without retinopathy and the zones in NPDR eyes that remained retinopathy-free had significantly more reduced amplitudes at follow-up than at baseline (4.8% abnormal amplitude at baseline vs. 12.6% at follow-up; P < 0.01).

### Association between New Retinopathy and Baseline mfERG

In the seven NPDR eyes in which new retinopathy developed, the baseline implicit time z-scores in two types of retinal areas were compared: New Retinopathy Zones (n = 118) and still-no-retinopathy areas (n = 798). An example (subject 3) of the zone analysis is shown in Figure 4A. First, retinal areas with preexisting retinopathy (Old Retinopathy Zones), represented by the black hexagons, were excluded from subsequent analysis. On follow-up, New Retinopathy Zones, represented by the gray hexagons, were constructed around the instances of new retinopathy and the remaining (white) area was categorized as a still-no-retinopathy area. The numbers at some of the retinal locations indicate the instances and the z-scores of abnormal initial implicit times. In this example, 6 (46%) of 13 initial implicit times within the two New Retinopathy Zones were abnormal, whereas only 13 (17%) of 76 initial implicit times in the still-no-retinopathy area were abnormal. Figure 4B

### Table 1. Retinopathy Zones and Initial Abnormal mfERG Implicit Times

<table>
<thead>
<tr>
<th>Subject</th>
<th>Old Retinopathy Zones</th>
<th>New Retinopathy Zones</th>
<th>Initial Abnormal mfERG Implicit Times Associated with New Retinopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>1</td>
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<td>7</td>
<td>2</td>
<td>8</td>
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</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>21</td>
<td>19</td>
</tr>
</tbody>
</table>

Data are the number of retinopathy zones at baseline (Old) and follow-up (New) for individual subjects and the number of initial abnormal mfERG implicit times associated with the New Retinopathy Zones.
In this analysis, mfERGs and graded fundus photographs were divided into “mfERG zones” comprising three to seven adjacent stimulated locations without regard to where new retinopathy later developed (Fig. 6). These nonoverlapping mfERG zones were constructed concentrically with a center element. This process started in the upper left corner of the stimulus array and proceeded horizontally across consecutive rows to the lower right corner. The center element was chosen in such a way as to include the maximum number of elements per zone. Because of the irregular shape of the border of the entire stimulus field, coupled with the variations in the locations of old retinopathy zones, the number of elements per zone varies from three to seven. Each mfERG zone was then classified as either normal or abnormal, based on whether at least one mfERG z-score in that zone exceeded 2.0. In the hypothetical example shown in Figure 6, the dark region is an Old Retinopathy Zone that is excluded from further analysis, and the remaining stimulated retinal area is divided into 17 mfERG zones (3 abnormal and 14 normal) whose borders are represented by thick dark lines. On follow-up, new retinopathy is observed in 3 of the zones, indicated by the gray shading, 2 (66.7%) of which occur in abnormal mfERG zones and in 1 (7.1%) in the 14 normal mfERG zones.

In the 11 NPDR eyes, 63 (34.6%) of the 182 mfERG zones were defined as normal on the basis of baseline implicit time z-scores (Table 2). After 1 year, 22 (35%) of the 63 abnormal mfERG zones showed development of new retinopathy, whereas only 2 (1.7%) in the 119 normal mfERG zones did. Consequently, development of retinopathy within 1 year was 21 times more likely in abnormal than in normal mfERG zones. The odds ratio for development of new retinopathy in the locations with abnormal baseline mfERG implicit times is 31.4 \( (P < 0.001) \). Even if the criterion of implicit time abnormality is defined more conservatively as a z-score of 3 or more \( (P \leq 0.0014) \), the abnormal mfERG zones are approximately 9 times more likely to develop new retinopathy than normal mfERG zones (odds ratio = 16.6; \( P < 0.001 \)).

Response amplitude does not predict development of diabetic retinopathy. When initial response amplitude is used to identify abnormal mfERG zones, there is no difference in development of future retinopathy between abnormal and normal mfERG zones (17% vs. 13%; odds ratio = 1.5; \( P = 0.71 \)).

**DISCUSSION**

In this study, diabetic subjects with mild to moderate NPDR and diabetic subjects without retinopathy were examined by...
mfERG and had stereoscopic color fundus photographs taken at baseline and after 1 year. Seven of the 11 subjects with NPDR showed newly developed retinopathy during the study period, whereas none of the diabetic subjects without retinopathy did. Over the 1-year study, mfERG implicit times increased in most retinal areas of the NPDR eyes, but they did not change in normal subjects and in diabetic subjects without retinopathy. More important is the fact that abnormal mfERG implicit times at baseline were associated with and predicted the locations of newly developed diabetic retinopathy observed at the 1-year follow-up. In contrast, the initial mfERG amplitude measures were not associated with and did not predict retinopathy.

These results can be expected to have an impact on diabetes care and clinical trials. The mfERG implicit time measure, a sensitive measure of retinal function, can be used to monitor the progression of diabetic retinopathy at a very early stage and evaluate the effectiveness of preventative drug therapies currently being developed.

The mfERG is well suited to the study of the diabetic retina for several reasons. First, diabetic retinopathy is a retinal disease with local lesions typically confined to the posterior pole, where the standard mfERG techniques test local retinal function (across the central 45°). Second, diabetic retinopathy is largely caused by defects of retinal capillaries in the inner nuclear layer, where the cell bodies of the bipolar cells, the primary generators of the mfERG, are located. Thus, there is an anatomic basis for the detection of mfERG abnormality in diabetes.

We used the template-stretching method instead of the cross-correlation method implemented in the visual evoked...
response imaging software (VERIS; EDI) to measure mfERG implicit times. This method has an advantage over peak implicit time measurement, because it considers the entire response waveform instead of a single waveform feature, and it is therefore less affected by noise. In addition, we have recently compared cross-correlation measures and the template-stretching method and found that the template-stretching method is more sensitive to diabetic retinal dysfunction than the cross-correlation measures (Schneck ME, et al. IOVS 2002; 43:ARVO E-Abstract 3474).12

What causes the mfERG delays associated with, and even occurring before, the diabetic lesions? Pathophysiological studies on this retinal disease provide some insights. Diabetic retinopathy is a disease of small retinal vessels, even before the appearance of visible fundus lesions, and early characteristic changes in the retinal vasculature of diabetic eyes are pericyte apoptosis and basement membrane thickening, resulting in acellular capillaries.24-26 Three major theories have been proposed to explain how chronic hyperglycemia and subsequent retinal hypoxia might lead to those anatomic changes: increased formation of advanced glycosylation end products,27-31 abnormal by-pass of glucose metabolism through the sorbitol pathway,32-35 and the activation of growth factors (such as vascular endothelial growth factor) and the protein kinase C pathway.13,14,25,31,36-38 Oxidative stress and free radical generation also promotes the development of diabetic lesions.39-42 Compromised local metabolism may affect the function of mfERG generators, leading to delayed neural conduction and prolonged mfERG implicit times. Moreover, in diabetic eyes early or undetected perfusion defects associated with choriocapillaris degeneration13,15,24,43 may also result in the implicit time delays that occur before the anatomic signs of abnormal vasculature within the inner retina.

In contrast to the findings with implicit times, mfERG amplitudes were not correlated with, nor did they predict, the sites of new retinopathy. This is consistent with earlier studies comparing the relative usefulness of implicit time and amplitude measures with mfERG recordings in diabetes.5-8,12 One possible reason for the insensitivity of amplitude to diabetic dysfunction is that this measure has larger intersubject variability than implicit time in normal subjects. Another consider-

<table>
<thead>
<tr>
<th>Initial mfERG Zone</th>
<th>Retinopathy Development at Follow-up</th>
</tr>
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<tbody>
<tr>
<td>Abnormal</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>22</td>
</tr>
<tr>
<td>Normal</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
</tr>
</tbody>
</table>

Odds ratio = 31.4; \( P < 0.001 \).

The three gray areas indicate mfERG zones within which new retinopathy was observed at follow-up. Two of these areas occurred within abnormal baseline mfERG zones and one in a normal mfERG zone.

FIGURE 5. The z-score distributions of the initial mfERGs implicit times in the New Retinopathy Zones (n = 118) and still-no-retinopathy areas (n = 798) of seven subjects with NPDR. All z-scores in the New Retinopathy Zones are greater than zero, and the distribution of z-scores within New Retinopathy Zones is significantly more abnormal (\( P < 0.001 \)) than the responses from still-no-retinopathy zones.

FIGURE 6. Example of data analysis based on mfERG zones. Responses within initial retinopathy zones were excluded. The remaining initial responses were then divided into nonoverlapping mfERG zones comprising three to seven adjacent stimulated locations. Each mfERG zone was then classified as either normal or abnormal, based on whether at least one mfERG z-score in that zone exceeded 2.0. The number in each hexagon is the implicit time z-score measured at baseline. In this example, the remaining regions were divided into 17 mfERG zones (borders represented by thick dark lines; 3 abnormal and 14 normal). The three gray areas indicate mfERG zones within which new retinopathy was observed at follow-up. Two of these areas occurred within abnormal baseline mfERG zones and one in a normal mfERG zone.
ation is that amplitude measures reflect the strength of the summed responses generated by retinal cells and may be significantly affected only at a later stage when the generators are severely damaged or cell loss occurs.

For the normal subjects the mfERG responses did not change over the study year, indicating that the mfERG measures were stable and reliable for follow-up studies. We found that in the areas where new retinopathy developed, mfERG implicit times were more delayed at baseline than in the areas that had retinopathy at the onset of the study. A possible explanation is that the metabolic alterations affecting neural retinal function might be at a more severe stage before signs of retinopathy develop. The results from the second-year follow-up of our subjects will help to investigate this possibility.

In summary, we believe that this study is the first to demonstrate that abnormal mfERG implicit times are predictive of the sites of new retinopathy observed 1 year later. The results suggest that mfERG implicit time is a sensitive candidate metric for the assessment of new treatments of retinal disease. It will be interesting as we follow these subjects to examine whether the mfERG results also predict the development of diabetic retinopathy in eyes without retinopathy at baseline. If so, such predictive power will have additional implications for the prevention of diabetic retinopathy.

References