The Multifocal ERG in Diabetic Patients without Retinopathy during Euglycemic Clamping

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PURPOSE. Prolonged multifocal electroretinogram (mERG) implicit times have been observed in diabetes, although the acute response to hyperglycemia is an acceleration of the ERG. The hypothesis for the current investigation was that this discrepancy is caused by a protracted adaptational response of the retina to hyperglycemia.

METHODS. Fourteen patients with type 1 diabetes without retinopathy and 14 age-matched healthy subjects were included. During acute normoglycemia, patients with type 1 diabetes without retinopathy demonstrated an overall 1.36-ms delay of the P1 first-order implicit times (P = 0.0013) and a 0.72-ms delay of the second-order P1 (P = 0.0049) compared with healthy subjects at 4.9 ± 0.28 mM blood glucose. During acute hyperglycemia, the P1 first-order delay was only 0.81 ms (P = 0.02), and the P1 second-order implicit time was comparable to that of healthy subjects (P > 0.05). The magnitude of the diabetes-associated implicit time delay, at both levels of glycemia, was proportional to the level of chronic hyperglycemia at study entry, as expressed by the patients’ HbA1c.

CONCLUSIONS. During acute normoglycemia, patients with type 1 diabetes without retinopathy demonstrated a delayed mERG response compared with the healthy subjects. The delay was more pronounced during euglycemia than during hyperglycemia, and at both levels of glycemia, the delay was proportional to the patients’ habitual hyperglycemia. The results show that chronic hyperglycemia induces an adaptational response that tends to normalize retinal implicit times at a higher level of habitual glycemia.

Diabetic retinopathy is defined clinically by microvascular changes, and the primary pathogenesis is generally assumed to involve the retinal vessels. Nevertheless, abnormalities in the retinal neurons and glial cells have been demonstrated early after the onset of disease, before the onset of ophthalmoscopically visible microangiopathy. Previous studies have found defects in color vision and contrast sensitivity in diabetic patients without visible retinopathy, indicating that pathologic changes may occur in the retina or visual pathways before vascular changes are detectable. Previous studies have indicated that neuropathy may be an early and important component of the pathogenesis of diabetic retinopathy. Electrophysiological tests enable temporal resolution of the response components and therefore more precise localization of the defect. Thus, oscillatory potentials have been shown to decrease with increasing severity of diabetic retinopathy, and loss of oscillatory potential (OP) amplitudes appears to predict the development of proliferative diabetic retinopathy. Results obtained by full-field ERG before and after the onset of retinopathy have been less uniform. Early and moderately advanced stages of ophthalmoscopically visible diabetic retinopathy are often characterized by an uneven distribution over the retina, suggesting that underlying neuroretinal abnormalities may be difficult to detect by full-field ERG. Multifocal (mf)ERG, which can produce 100 or more focal responses from the cone-driven retina in less than 8 minutes, offers a theoretical advantage in this respect; and, indeed, localized mERG implicit time delays have been demonstrated in both diabetic patients with and without retinopathy.

In eyes with diabetic retinopathy, the mfERG delay in implicit time was found to increase with increasing severity of retinopathy when compared between eyes and within single eyes, the largest delay being located where retinopathy was ophthalmoscopically most advanced. Localization of mERG delay also predicts where microangiopathy will appear in eyes with nonproliferative diabetic retinopathy (NPDR). In addition, localized timing abnormalities of multifocal (m)OPs have been demonstrated in some eyes of patients without diabetic retinopathy and in more than 60% of eyes with NPDR.

We have previously shown that accurately controlled hyperglycemia accelerates all mERG responses in patients with type 1 diabetes without retinopathy. The same effect was found in healthy subjects examined with 30-Hz full-field flicker ERGs during fasting and after bolus sugar intake. We hypothesize that the discrepancy between the acute acceleration of implicit times and the delay found in chronic hyperglycemia is caused by a protracted adaptational response of the retina to hyperglycemia.

To adjust for the confounding effect of blood glucose changes on the mfERG, we compared the mfERG of patients with type 1 diabetes without retinopathy during acute normoglycemia (5 mM) with that of healthy subjects.

METHODS. The study adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from the participants after explanation of the nature and possible consequences of the study. The study was approved by the local medical ethics committee. Included were 14 eyes of 14 patients with type 1 diabetes and no retinopathy (Table 1) and 14 eyes of 14 age-matched control subjects with normal fasting blood glucose (<5.5 mM). All patients had best visual acuity of 20/20.

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or better and no complications of diabetes or chronic diseases other than diabetes.

Ophthalmic examination included determination of visual acuity, slit lamp biomicroscopy and ophthalmoscopy. Stereoscopic two-field fundus photographs were evaluated with a stereo viewer by a retinal specialist. Patients with spherical equivalent refractive errors greater than ±3 or −3 were excluded. Blood pressure was measured on the first study day and patients with systolic blood pressure >140 and/or diastolic blood pressure >90 were excluded.

Patients were randomly assigned to have either the left or the right eye tested. Pupils were dilated to a diameter of ≥7 mm with 10% phenylephrine hydrochloride and 1% tropicamide. After topical anesthesia with 0.4% oxybuprocaine hydrochloride, a Burian-Allen bipolar contact lens electrode (Veris IR Illuminating Electrode; EDI Inc., San Mateo, CA) with two built-in infrared light sources for fundus illumination was placed on the test eye by using 1% carboxymethylcellulose contact fluid and occlusion of the contralateral eye. A ground electrode was attached to the forehead after the skin was cleansed with an abrasive gel (NuPrep; D.O. Weaver & Co., Aurora, CO).

Visual stimuli were displayed on a 1.5-in. stimulator/fundus camera (Veris; EDI Inc., San Mateo, CA), which permits optimal correction of refraction without changing the size of the visual image and ensures fixation by real-time infrared viewing of the fundus.

An array of 103 eccentricity-scaled hexagons was displayed at a frame rate of 75 Hz. Responses were band-pass filtered outside 10 to 300 Hz, amplified at a gain of 105, and sampled every 0.833 ms. A standard m-sequence length was used with m = 15, resulting in a total recording time of 7.17 minutes divided into 16 short segments for patient comfort. If loss of fixation or an artifact was observed, the affected segment was discarded and rerecorded. The luminance of white stimuli was 200 and 2 cd/m² or less for black stimuli. The surround luminance was set to 50% of the bright test luminance (i.e., 100 cd/m²). Ambient room lighting was used throughout the study day. Stimulus luminance was calibrated with the autocalibrator, and the stimulus grid was calibrated with a grid calibrator (Veris; EDI, Inc.).

The recording protocol was chosen according to the International Society for Clinical Electrophysiology of Vision (ISCEV) guidelines for basic mfERG.31

A single iteration of artifact rejection was applied to the raw data, whereas no spatial smoothing was applied before derivation of first- and second-order (first slice) kernels (Fig. 1). Implicit times and amplitudes of N1 (first negative component), P1 (first positive component), and N2 (second negative component). Figure 1 illustrates typical first- and second-order waveforms and how the amplitudes and implicit times of the major component were measured. All traces are presented in retinal view orientation.

Average responses were calculated for nine regions specified by coordinates relative to the fovea, as illustrated in Figure 2. This enabled analysis of hypothetical differences in responses between the two groups, both for different eccentricities and the superior-inferior and the temporal-nasal direction.

Examinations were started at 7:30 AM after a 10-hour overnight fast. Blood glucose clamping was instituted at 5 mM and held for 75 minutes before recording the mERG. A polyethylene catheter was inserted into an antecubital vein for blood sampling, and the hand was placed in a heated Plexiglas box to induce hyperperfusion, enabling sampling of venous blood with a glucose concentration approximating that of arterial blood. A second polyethylene catheter was inserted into an antecubital vein in the contralateral arm for infusion of insulin and glucose. After baseline blood samples were drawn, a constant infusion of insulin (Insulin Actrapid; Novo Nordisk A/S, Bagsværd, Denmark) was started and then continued at a constant rate of 0.5 mU/kg per minute, so as to maintain a stable plasma insulin concentration within the physiological range. At 8 AM, the infusion of 20% glucose at a variable rate was started. The glucose infusion rate was constantly adjusted in accordance with the results of blood glucose measurements made at 5-minute intervals (One Touch Profile; Lifescan Inc., New Brunswick, NJ), to maintain the desired blood glucose level. Immediately before and after the mERG recording, blood was sampled for accurate analysis of the plasma glucose concentration, the recording glucose level being defined as the mean of values immediately before and after each recording.

![First-order kernel](image1)

**Figure 1.** The first- and second-order mERG waveforms with arrows indicating how amplitudes (vertical) and implicit times (horizontal) of the major components (N1, P1, and N2) are measured.

| TABLE 1. Clinical Characteristics of Patients with Type 1 Diabetes and Healthy Subjects |
|---------------------------------|-----------------|-----------------|
| Age (y, mean [SD]) | 37.9 (7.3) | 36.1 (3.8) |
| Sex (male/female) | 5/9 | 4/10 |
| Duration of diabetes (y, mean [SD]) | 13 (5.1) | | |
| HbA1c (% mean [SD]) | 8.3 (1.2) | | |
| Systolic blood pressure (mmHg, mean [SD]) | 129.2 (7.3) | | |
| Diastolic blood pressure (mmHg, mean [SD]) | 81.0 (7.2) | | |
| Bodyweight (kg, median [range]) | 73.5 (63.6–99.2) | | |
RESULTS

Blood glucose control in patients with type 1 diabetes was achieved throughout the 75-minute prererecording period and during the subsequent 15-minute ERG recording to the stipulated mean of 5.0 ± 0.66 mM. Before normoglycemic clamping, mean fasting plasma glucose was 9.73 ± 4.78 mM, and mean HbA1c was 8.3% ± 1.2%. In healthy subjects, the mean glucose level during the mfERG recording was 4.9 ± 0.28 mM.

First-order implicit times were significantly delayed in patients with diabetes compared to control subjects for all study components (Table 2; Fig. 3). Mean N1 delay ($\Delta t_{N1}$) was +1.13 ms (+6.8%; $P = 0.0016$; Table 2), mean $\Delta t_{N2}$ was +1.36 ms (+5.2%, $P = 0.0013$), and mean $\Delta t_{P1}$ was +1.39 ms (+3.4%, $P = 0.0009$). Compared with healthy subjects, the P1 delay was 1.60 ms in the superior regions (2, 3, 6, and 7) and 1.23 ms in the inferior regions (4, 5, 8, 9; $P = 0.0002$, mixed model analysis; Table 2; Fig. 3). N1 and N2 demonstrated no significant variation with eccentricity or direction. First-order mfERG amplitudes in patients were not different from those in healthy subjects.

Second-order implicit times of all mfERG components were significantly delayed in patients with diabetes during normoglycemia when compared with healthy subjects (i.e., $\Delta t_{N1}$ was +1.13 ms; +8.4%, $P = 0.0053$; Table 3), $\Delta t_{N2}$ was +0.72 ms (+3.3%, $P = 0.0049$), and $\Delta t_{P1}$ was +1.02 ms (+5.8%, $P = 0.0027$). No subfield variation in implicit time delay was found. Second-order mfERG amplitudes in patients with diabetes were not significantly different from those in healthy subjects during acute normoglycemia.

Controlled hyperglycemia at 15 mM in patients with diabetes resulted in a shift toward the first-and second-order mfERG implicit times in healthy subjects (Table 3; Fig. 4). Compared with euglycemia the first-order delay decreased for all three components of the mfERG—that is, the mean $\Delta t_{N1}$ decreased

![Figure 2. Hexagonal array geometry (retinal view) of 103 stimulus elements. Circles: eccentricity of 5° (dashed) and 25° (solid). For analysis, responses were averaged from nine regions (marked by individual shading and numbers): a central (foveal) area of 5°, four quadrants dividing the mid periphery, and four quadrants dividing the outer periphery.](https://www.iovs.org/content/46/7/2622.large)

### Table 2. First-Order Mean Implicit Times in Diabetic Patients during Euglycemia and Healthy Subjects Averaged from Nine Regions

<table>
<thead>
<tr>
<th>Region</th>
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<tr>
<td>Diabetic patients (5 mM; mean ± SD)</td>
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<tr>
<td>N1</td>
<td>15.59 ± 0.96</td>
<td>14.10 ± 0.89</td>
<td>14.05 ± 0.80</td>
<td>13.87 ± 0.85</td>
<td>13.99 ± 0.88</td>
<td>14.35 ± 0.74</td>
<td>14.52 ± 0.90</td>
<td>14.34 ± 0.81</td>
<td>14.29 ± 0.72</td>
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<tr>
<td>P1</td>
<td>28.64 ± 3.12</td>
<td>27.26 ± 1.58</td>
<td>27.32 ± 1.10</td>
<td>27.26 ± 1.19</td>
<td>26.95 ± 0.95</td>
<td>27.56 ± 1.28</td>
<td>27.50 ± 1.13</td>
<td>27.26 ± 1.29</td>
<td>27.37 ± 1.08</td>
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<tr>
<td>N2</td>
<td>44.31 ± 1.13</td>
<td>41.58 ± 1.35</td>
<td>41.46 ± 1.15</td>
<td>41.59 ± 1.00</td>
<td>41.59 ± 0.95</td>
<td>41.94 ± 1.06</td>
<td>42.05 ± 1.16</td>
<td>42.17 ± 1.15</td>
<td>41.99 ± 0.91</td>
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<td>Healthy subjects (mean ± SD)</td>
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<tr>
<td>N1</td>
<td>14.90 ± 1.12</td>
<td>13.05 ± 0.81</td>
<td>13.05 ± 0.69</td>
<td>12.67 ± 0.85</td>
<td>12.93 ± 0.61</td>
<td>13.49 ± 0.91</td>
<td>13.55 ± 0.81</td>
<td>13.56 ± 0.81</td>
<td>13.79 ± 0.71</td>
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<tr>
<td>P1</td>
<td>27.61 ± 1.37</td>
<td>26.16 ± 0.99</td>
<td>25.59 ± 1.19</td>
<td>25.88 ± 1.01</td>
<td>25.55 ± 1.33</td>
<td>25.83 ± 1.14</td>
<td>25.65 ± 1.14</td>
<td>26.33 ± 1.15</td>
<td>26.17 ± 1.17</td>
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<tr>
<td>N2</td>
<td>45.29 ± 1.42</td>
<td>40.56 ± 0.87</td>
<td>39.99 ± 1.05</td>
<td>40.25 ± 0.92</td>
<td>39.93 ± 1.05</td>
<td>40.69 ± 0.94</td>
<td>40.41 ± 1.25</td>
<td>40.69 ± 1.13</td>
<td>40.53 ± 1.35</td>
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<td>Difference, mean (95% CI)</td>
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<tr>
<td>N1</td>
<td>0.75</td>
<td>(0.07–1.57)</td>
<td>(0.40–1.71)</td>
<td>(0.42–1.57)</td>
<td>(0.56–1.86)</td>
<td>(0.47–1.64)</td>
<td>(0.23–1.49)</td>
<td>(0.32–1.63)</td>
<td>(0.16–1.41)</td>
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<tr>
<td>P1</td>
<td>1.05</td>
<td>1.10</td>
<td>1.73</td>
<td>1.38</td>
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<td>1.73</td>
<td>1.85</td>
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<tr>
<td>N2</td>
<td>1.25</td>
<td>(0.42–2.08)</td>
<td>(0.33–2.10)</td>
<td>(0.64–2.32)</td>
<td>(0.60–2.07)</td>
<td>(0.89–2.43)</td>
<td>(0.48–2.02)</td>
<td>(0.72–2.56)</td>
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### 5 mM Diabetes vs. Healthy Subjects

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<tr>
<td>N1</td>
<td>0.90 (0.38–1.43)</td>
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<tr>
<td>P1</td>
<td>1.36 (0.21–1.92)</td>
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<tr>
<td>N2</td>
<td>1.39 (0.63–2.15)</td>
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<tr>
<td>N1</td>
<td>0.61 (0.11–1.12)</td>
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<tr>
<td>P1</td>
<td>0.81 (0.16–1.61)</td>
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<tr>
<td>N2</td>
<td>0.82 (0.07–1.55)</td>
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* $P = 0.002$ for variation delay between superior and inferior regions.
First-order implicit times in diabetic patients and controls

FIGURE 3. Electroretinographic responses averaged from nine regions as illustrated in Figure 1. First-order N1 (A), P1 (B), and N2 (C) implicit times in patients with diabetes compared with healthy subjects. The box-and-whisker plots include the percentiles 5, 25, 50, 75, and 95. Grouping of the responses into nine retinal regions demonstrated that the first-order P1 delay in diabetic patients was higher in the superior fields than in the inferior fields (P < 0.002).

from +0.9 to +0.61 ms (P = 0.0211), Δt_{N1} from +1.36 to +0.81 ms (P = 0.0200), and Δt_{N2} from +1.39 to +0.82 ms (P = 0.0319). Thus, during hyperglycemia, all first-order components remained significantly delayed compared with the healthy subjects, yet the delays attenuated compared with the euglycemic recordings. The P1 implicit times during euglycemia and hyperglycemia correlated significantly (r = 0.80, P < 0.0001; Fig. 5), and r² was 0.64, indicating that the variability...
in implicit times was determined primarily by the blood glucose level. For the second-order responses, \( \Delta t_{N1} \) decreased from +1.13 to +0.65 ms (NS), P1 from +0.72 to +0.38 ms (NS), and N2 from +1.02 to +0.71 ms \((P = 0.0223)\). The implicit time prolongation found in patients with diabetes was proportional to their level of habitual glycemia, as assessed by their HbA1c, both during hyperglycemic clamping at 15 mM \((r_s = 0.43, P < 0.0001)\) and during normoglycemic clamping at 5 mM \((r_s = 0.37, P < 0.0001)\).

**DISCUSSION**

We have previously shown that acute changes in blood glucose levels in patients with type 1 diabetes without diabetic reti-

![Figure 4](image-url)

**Figure 4.** First-order P1 implicit times averaged from all 103 responses in patients with diabetes during euglycemia and hyperglycemia compared with healthy subjects. The box-and-whiskers plot includes the percentiles 5, 25, 50, 75, and 95. During acute normoglycemia, patients with diabetes demonstrated delayed overall implicit times compared to healthy subjects. \(1.36 \text{ ms; 95% CI, 0.21-1.92; } P = 0.0013\). Acute hyperglycemia in the same patients, at a level near the mean habitual glycemia in the patient population, was associated with partial normalization of implicit times. Thus, the mean delay in comparison with healthy subjects decreased from 1.36 to 0.81 ms \(95\% \text{ CI, 0.16-1.61; } P = 0.02\).

![Figure 5](image-url)

**Figure 5.** Correlation between P1 implicit times summed from all regions during euglycemia (x-axis) and hyperglycemia (y-axis). Pearson’s \( r = 0.80 \) \((P < 0.0001)\), indicating a significant association between implicit times between euglycemia and hyperglycemia. \( r^2 \) was 0.64, indicating that the variability was determined predominantly by the blood glucose level.
nopathy is associated with changes in the multifocal electroretinogram. Thus, an increase in blood glucose leads to acceleration of the ERG. In the present study, we examined the same patients with diabetes to assess the relation between the mfERG response in patients with diabetes at various levels of controlled glycemia and that seen in healthy subjects.

Our diabetic study population was in moderately good habitual metabolic control, a mean HbA1c of 8.3%, corresponding to an average blood glucose level of 12.1 mM. When acutely normalized to a blood glucose level of 5 mM, patients consistently demonstrated delayed ERG responses compared with healthy subjects at the same blood glucose level. The delay was evident in all three components of the first-order response and all three components of the second-order mfERG response. The ERG delay in diabetic patients without retinopathy remains detectable at a glucose level of 15 mM, although it is shortened by acute hyperglycemia. This shift toward a normal ERG response was seen in both the first- and second-order responses; and, indeed, during hyperglycemia the second-order N1 and P1 responses in diabetic patients were not significantly different from those in healthy subjects.

Although there was some variation between regions, the only obvious pattern of regional deviation was that the superior retinal regions demonstrated significantly longer first-order P1 delays than did the inferior regions. Retinal disorders that display altitudinal asymmetry include rare variants of retinitis pigmentosa where the inferior retina is most severely involved, and branch retinal vein occlusion, where the upper retina is more often involved. No obvious relation is apparent between these conditions and diabetic retinopathy.

Normalization of plasma glucose in our diabetic patients was obtained by peripheral insulin infusion. This probably resulted in higher plasma insulin concentrations during euglycemia in the diabetic patients than in the healthy subjects. Although plasma insulin concentrations were not measured, we cannot exclude the possibility that fluctuations in insulin levels influenced the mfERG in our experiments. A previous study, however, failed to show an effect of insulin on ERG signals during euglycemia in perfused cat eyes, suggesting that insulin is of negligible significance.

A diurnal variation in implicit time has been demonstrated for the human photopic full-field ERG. Hence, time of day may have affected the mfERG implicit times in this study. All recordings in the present study in patients with diabetes and in healthy subjects were made at the same time of day, between 8 and 12 AM, and all recordings were made within a period of 1 month. In addition, a previous study investigated whether a circadian rhythm could be observed for the mfERG but found no significant influence of the time of day. Consequently, we expect time of day to have a very limited effect on the findings reported herein.

Neuroretinal cell biology demonstrates abnormalities early after the onset of diabetes, before the development of ophthalmoscopically defined retinopathy, involving ganglion cells and cells of the inner nuclear and inner plexiform layers, where apoptosis has been demonstrated in rats and humans. The functional abnormalities demonstrated in the present study were partially reversible and do not, by necessity, implicate cellular loss or major structural damage. We propose that the implicit time delay is part of a protracted adaptation to chronic hyperglycemia, wherein the tissue partially normalizes retinal function at an abnormally elevated energy substrate level. This hypothesis implies that improved metabolic control of diabetes should be followed by readjustment to normal implicit times. In the present study, we did indeed demonstrate that the higher the habitual level of blood glucose, the longer the delay during normoglycemia.

The implication of our findings is that the retina must be regarded as an organ with substrate-limited performance with an avidity for glucose that in the long run is overridden by homeostatic mechanisms that balance retinal function at a higher level of glycemia than in the nondiabetic subject. The existence of a long-term, glycemia-related adaptational phenomenon in the retina is also suggested by the transient acceleration of retinopathy progression in diabetic patients who undergo permanent improvement of metabolic control.

References


