Effect of Short-Term Hyperglycemia on Multifocal Electroretinogram in Diabetic Patients without Retinopathy

Kristian Klemp,1 Michael Larsen,1 Birgit Sander,1 Allan Vaag,2 Per Bruun Brockhoff,3 and Henrik Lund-Andersen1

PURPOSE. To investigate the effect of variations in blood glucose level on the multifocal electroretinogram (mfERG) in patients with type 1 diabetes without retinopathy.

METHODS. Fourteen patients were clamped sequentially and in random order at 5 and 15 mM blood glucose for 75 minutes on the same day. mfERG was recorded in one eye at each level of blood glucose level.

RESULTS. Compared to euglycemia (5 mM), hyperglycemia was associated with an overall decrease in the implicit times and an increase in the amplitudes of the multifocal ERG. The effect of hyperglycemia on implicit time reached statistical significance for the summed first positive (P1) and second negative component (N2) of the first-order kernel ($P = 0.0020$ and $0.0005$, respectively) and all components of the second-order kernel ($P = 0.014$, $0.0037$, and $0.0034$, respectively). These changes in the mfERG demonstrated no significant variation in relation to retinal location or long-term blood glucose level.

CONCLUSIONS. Steady-state hyperglycemia induced shorter first- and second-order kernel implicit times in patients with type 1 diabetes without retinopathy. The effects of hyperglycemia were not significant with retinal location. These results support the hypothesis that hyperglycemia accelerates retinal metabolism. (Invest Ophthalmol Vis Sci. 2004;45:3812–3819) DOI:10.1167/iovs.03-1260

Long-term blood glucose elevation in the absence of diabetic retinopathy is accompanied by changes in retinal functions: alterations in the production of oscillatory potentials in the full-field ERG,1,2,3 increased implicit times of the multifocal ERG (mfERG),3,4,5 and changes in color discrimination.3,4

Glucose enters retinal cells by an insulin-independent facilitated transport mechanism governed by the blood glucose concentration.7,8 Consequently, acute changes in blood glucose concentration probably cause acute changes in neuroretinal function. This is supported by full-field ERG studies in animals and humans. The effect of acute changes in blood glucose has been studied in the isolated arterially perfused cat eye9 and in the anesthetized cat eye.10 These studies demonstrate large and reliable reductions in the rod-driven b-wave amplitude when blood glucose is decreased. In human volunteers, in whom circulating blood glucose was manipulated by fasting followed by high sugar intake, the latency of the full-field ERG flicker response decreases when blood glucose is elevated.11

Both anatomy and function of the healthy retina vary with eccentricity. Thus, the Müller cells of the peripheral retina in the cat contain more glycerogen than Müller cells in the central retina.12 Additionally, the pathologic changes in diabetic retinas (e.g., microaneurisms, hard exudates, edema) are highly localized. These features raise the possibility that retinal glucose metabolism also differs with eccentricity. As the full-field ERG reflects the activity of the entire retina, this method does not enable assessment of the sensitivity of local retinal function to changes in glucose concentration.

In the multifocal technique, small retinal areas are independently stimulated, and the local ERG contributions from each stimulated area to the mass potential are extracted using a cross-correlation technique.13 A previous study showed that the biphasic response of the multifocal cone ERG is closely related to the a-wave and the positive peak of the full-field cone ERG, which thus appears to be generated by the same retinal cells.14 The spatial resolution of the multifocal ERG offers an opportunity to study the local retinal sensitivity to changes in blood glucose.

Here, the glucose clamp technique was used to study the effect of hyperglycemia on the mfERG. With this technique, a desired blood glucose concentration in a test person can be achieved and held constant for several hours using infusion of glucose in titrated doses adjusted to blood glucose measurements at short intervals. The technique enables investigation of the effect of blood glucose on electrophysiological parameters during steady state conditions with constant insulin levels.

Our aim was to investigate the effect of acute hyperglycemia at precisely defined levels of blood glucose on the mfERG responses during steady state conditions in patients with type 1 diabetes without diabetic retinopathy. mfERGs were recorded for each patient twice on the same day, with one recording during steady-state euglycemia and one recording during steady-state hyperglycemia. The implicit times (time from stimulus onset to peak response) and amplitudes of responses of the three major components (N1, P1, and N2) of the mfERG were compared between euglycemia and hyperglycemia. Additionally, this response was examined in relation to patients' chronic level of glycemia, expressed as their mean HbA1c over the 6 months before the mfERG examination.

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**TABLE 1. Clinical Characteristics of Patients with Type 1 Diabetes without Retinopathy (n = 14)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)</th>
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<tbody>
<tr>
<td>Age (years, mean (SD))</td>
<td>37.9 (7.3)</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>5/9</td>
</tr>
<tr>
<td>Duration of Diabetes (years, mean (SD))</td>
<td>13 (3.1)</td>
</tr>
<tr>
<td>HbA1c (% , mean (SD))</td>
<td>8.5 (1.2)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg, mean (SD))</td>
<td>129.2 (7.3)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg, mean (SD))</td>
<td>81.0 (7.2)</td>
</tr>
<tr>
<td>Bodyweight, kilograms (median (range))</td>
<td>7.35 (6.6-99.2)</td>
</tr>
</tbody>
</table>

**METHODS**

**Subjects**

Fifteen eyes of 15 patients with type 1 diabetes and no diabetic retinopathy were included. One of the 15 patients was excluded after the first study day because of noise-contaminated mfERG recordings. Clinical characteristics are listed in Table 1. All patients were in diabetological care of the Steno Diabetes Center (Gentofte, Denmark), and had best visual acuities of 20/20 or better and no detectable complications of diabetes or chronic diseases other than diabetes. All patients received fast-acting insulin before meals and intermediate-acting insulin at bedtime. Ophthalmic examination included determination of visual acuity, slit-lamp biomicroscopy, and ophthalmoscopy. Color stereo fundus photographs of the macula and the optic disc were evaluated with a stereo viewer by a retinal specialist. No patients had diabetic retinopathy or other retinal disease. Patients with spherical equivalent refraction 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.

The study followed 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.

**Procedure**

Patients were randomly assigned to have either their left or right eye tested. Pupils were dilated to a diameter of ≥7 mm using 10% phenylephrine hydrochloride and 1% tropicamide. After topical anesthesis with 0.4% oxybuprocaine hydrochloride, a Burian Allen bipolar contact lens electrode with two built-in infrared light sources for fundus illumination (Veris IR Illuminating Electrode; EDI Inc., San Mateo, CA) was placed on the test eye and the contralateral eye was occluded with enough pressure on the lids to prevent blinking. For better electrode- cornea contact, 1% carboxymethylcellulose sodium was applied to the inner surface of the contact lens. A ground electrode was attached to the forehead after cleaning of the skin with an abrasive gel (Nuprep; D.O. Weaver & Co., Aurora, CA).

**Stimulus Characteristics**

The stimuli were displayed on a Veris 1.5-in. stimulator/fundus camera (Veris; EDI Inc.), which permitted optimal refraction without changing the size of the visual image and ensured fixation by real-time infrared viewing of the fundus. To facilitate monitoring of fixation, the stimulus array grid was projected on the infrared fundus image on the examiner’s monitoring screen.

An array of 103 hexagons scaled with eccentricity was displayed at a frame rate of 75 Hz. Responses were band-pass filtered outside 10 to 300 Hz, amplified at a gain of 100,000, and sampled every 0.835 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 re-recorded. The luminance of the white stimulus was 200 cd · m⁻² and 2 cd · m⁻² or less for the black stimuli. The surround luminance was set to 50% of the bright test luminance (i.e., 100 cd · m⁻²). Standardized ambient room lighting was used throughout the study day. Stimulus luminance was calibrated with the EDI auto calibrator and the stimulus grid was calibrated with the EDI 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.¹⁵

**Response Analysis**

A single iteration of artifact rejection was applied to the raw data and no spatial smoothing was performed. The first- and second-order (first slice) kernels were derived and implicit times and amplitudes of N1 (first negative component), P1 (first positive component), and N2 (second negative component) were measured. The N1 response amplitude was measured from the starting baseline to the base of the N1 trough; the P1 response amplitude was measured from the N1 trough to the P1 peak; and the N2 response amplitude was measured from the P1 peak to the N2 trough.

Average responses were calculated for nine regions specified by coordinates relative to the fovea, as illustrated in Figure 1.

**Glucose Clamp Protocol**

The study was designed as an open labeled, randomized crossover study including a 75 minute euglycemic (blood glucose: 5 mM; 90 mg/dl) and a 75 minute hyperglycemic (blood glucose: 15 mM; 270 mg/dl) period. For each patient, a multifocal ERG was recorded twice on the same day, one after 75 minutes of hyperglycemia and one after 75 minutes of euglycemia. Examinations were started at 7:30 AM after a 10-hour overnight fast. 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 of the contralateral arm for infusion of insulin and glucose. After baseline blood samples were drawn, a constant infusion of insulin (Insulin Actrapid; Novo Nordisk, Bagsværd, Denmark) was started and continued at a constant rate of 0.5 ml/kg per min so as to maintain a stable plasma insulin concentration within the physiological range. Patients were randomized to start with the euglycemic period followed by the hyperglycemic period, or vice versa. At 8:00 AM, the infusion of 20% glucose at a variable rate was started. The glucose metabolism of Vision (ISCEV) guidelines for basic mfERG.¹⁵

![Figure 1. The geometry of the hexagonal array of 103 stimulus elements with circles drawn to indicate radii 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 midperiphery, and four quadrants dividing the outer periphery.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932925/)
The mean implicit times for the nine regions during euglycemia were shown in Table 2 and the mean implicit time differences for the nine regions between 5 and 15 mM blood glucose are shown in Table 3 and Figure 3. The raw data distributions of P1 and N2 implicit times for the 14 patients during 5 and 15 mM blood glucose are illustrated in Figure 4.

Compared to euglycemia, hyperglycemia was associated with decreased P1 and N2 implicit times (Fig. 3; Table 3). In the majority of patients (94.4%), the P1 implicit time decreased or remained unchanged during hyperglycemia compared to euglycemia (Fig. 4A). As for P1, the majority of patients (98.4%) had decreased or unchanged N2 implicit times during hyperglycemia compared to euglycemia (Fig. 4B). This effect is illustrated by first- and second-order responses from a single patient (Fig. 5). Compared to euglycemia, the first-order responses are left-shifted (i.e., implicit times are decreased, during hyperglycemia). When analyzed between distant regions. Mathematically, the model assumes that the effect of distance (i.e., the nonindependence of the response) follows an exponential decay. In summary, in each case a mixed model for the differences was applied in which region was included as a fixed effect and the exponential model was used to model the correlation structure.

Here, the main parameter of interest is the difference in implicit times and amplitudes between 5 and 15 mM blood glucose. In cases of regional differences in the response, the mixed model results in significant values for the effect of region together with the P value for a hypothetical difference between high and low glucose.

To assess the effect of the patients' chronic glucose level—expressed as their mean HbA1c over the 6 months before the mfERG examination—responses were compared from patients with mean HbA1c over and under 8.0%.

**RESULTS**

During the euglycemic period of 75 minutes + recording time, the mean of all blood glucose values in all patients was 5.0 mM (SD 0.66) and during the hyperglycemic period + recording time, it was 15.6 mM (SD 1.34; Fig. 2).

**First-Order Response**

**Implicit Time.** The mean implicit times for the nine regions during euglycemia are shown in Table 2 and the mean implicit time differences for the nine regions between 5 and 15 mM blood glucose are shown in Table 3 and Figure 3. The raw data distributions of P1 and N2 implicit times for the 14 patients during 5 and 15 mM blood glucose are illustrated in Figure 4.

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**Mean blood glucose during clamps**

![Mean blood glucose during clamps](image-url)
TABLE 3.

Mean First- and Second-Order Implicit Times during Euglycemia Averaged from Nine Regions

<table>
<thead>
<tr>
<th>Region</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>15.53±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>
</tr>
<tr>
<td>P1</td>
<td>28.64±1.52</td>
<td>27.26±1.58</td>
<td>27.52±1.10</td>
<td>27.26±1.19</td>
<td>26.95±0.95</td>
<td>27.56±1.28</td>
<td>27.50±1.15</td>
<td>27.26±1.29</td>
<td>27.37±1.08</td>
</tr>
<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>
</tr>
</tbody>
</table>

Data shown are mean ± SD.

using a mixed model, the mean decrease reached statistical significance for P1 (0.49 ms, P = 0.002) and N2 (0.64 ms, P = 0.0005; Table 4). The mixed-model analysis showed that the effect of hyperglycemia was diffuse: that is, the change in implicit time induced by hyperglycemia was not significantly different between regions.

The patients’ chronic blood glucose level was not significantly correlated to the timing or amplitude of mfERG responses (P > 0.15 for all components in all regions).

Amplitudes. When compared to euglycemia, hyperglycemia was associated with slightly increased amplitudes of all mfERG components. This change in amplitude, however, did not reach statistical significance (Table 4). As demonstrated for implicit times, the increase in amplitude during hyperglycemia showed no significant variation with retinal region.

Second-Order Response

Implicit Time. The same overall pattern was found for the second-order response, although the decrease in implicit times during hyperglycemia was less than for the first-order response (Table 3). The decrease in implicit time reached significance for N1, P1, and N2, decreasing during hyperglycemia by 0.48 ms (P = 0.014), 0.32 ms (P = 0.004), and 0.31 ms (P = 0.034), respectively (Table 4). Again, change in implicit time induced by hyperglycemia was not significantly different between regions.

Amplitudes. The second-order amplitudes of N1, P1, and N2 did not show any significant change in response to hyperglycemia (Table 4).

DISCUSSION

The effect of steady state hyperglycemia on the human mfERG was investigated by applying a glucose clamp protocol to 14 patients with type 1 diabetes without retinopathy. Elevation of mean blood glucose from 5.0 mM to 15.6 mM resulted in significantly decreased first- and second-order implicit times. The results demonstrated that the mfERG implicit time changed with blood glucose concentration in diabetic patients without retinopathy. Our findings were consistent with previous full-field ERG studies in humans, showing decreased implicit time in response to hyperglycemia.11 Elevation of blood glucose increases the b-wave amplitude of the full-field ERG in the perfused cat eye and in

TABLE 2.

Mean First- and Second-Order Implicit Times during Euglycemia Averaged from Nine Regions

<table>
<thead>
<tr>
<th>Region</th>
<th>1</th>
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<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>18.76±1.96</td>
<td>14.71±1.71</td>
<td>14.59±1.84</td>
<td>14.27±1.49</td>
<td>14.36±1.50</td>
<td>14.46±0.83</td>
<td>15.24±1.10</td>
<td>14.59±0.97</td>
<td>14.59±0.95</td>
</tr>
<tr>
<td>P1</td>
<td>24.54±1.62</td>
<td>21.91±1.28</td>
<td>22.68±0.72</td>
<td>22.02±0.78</td>
<td>22.61±0.96</td>
<td>22.50±0.44</td>
<td>23.09±0.61</td>
<td>22.68±0.65</td>
<td>22.79±1.02</td>
</tr>
<tr>
<td>N2</td>
<td>30.46±1.24</td>
<td>27.92±0.96</td>
<td>28.16±1.26</td>
<td>27.79±1.10</td>
<td>28.03±0.94</td>
<td>28.16±0.93</td>
<td>28.75±1.11</td>
<td>27.91±1.36</td>
<td>28.92±1.19</td>
</tr>
</tbody>
</table>

**P < 0.01, *P < 0.05.
vivo in anesthetized cats under glucose clamp conditions. This study found the rod ERG to be much more sensitive to changes in glucose than the cone ERG. This could account for the absence of an effect of hyperglycemia on amplitude measures in our study of the mFERG, which is a cone-driven response.

A feature of the multifocal technique is the ability to detect regional variation. Here the data averaged from nine retinal regions (Fig. 1) were analyzed to evaluate the regional variation in retinal sensitivity to changes in blood glucose level. The effect of changes in blood glucose on the multifocal responses did not vary between the nine retinal regions, indicating that hyperglycemia had the same effect on the entire stimulated area of the retina.

In patients with diabetes and no diabetic retinopathy, hyperglycemia increases retinal oxygen consumption and increases retinal blood flow when blood glucose is elevated, suggesting an increased retinal metabolism when retinal energy supplies are increased. This is consistent with a more rapid impulse transduction in the retina being induced by hyperglycemia. This agrees with studies of full-field ERG responses during hyperglycemia, showing shorter implicit times and larger amplitudes when compared to responses during euglycemia. In long-term hyperglycemia, this may lead to permanently increased metabolic stress to the retinal neurons and/or glial cells and increased vascular stress from increased retinal blood flow. The increased permeability of the blood-retina barrier in diabetic retinopathy may also be interpreted as a response to an increased demand for nutrients and removal of waste products. A compensatory process such as this is likely to begin soon after the onset of diabetes and to precede clinically detectable changes in retinal morphology.

In conclusion, first- and second-order implicit times decreased during hyperglycemia. The effect was relatively small but significant and blood glucose should be taken into consideration in future studies. The mFERG demonstrated no statistically significant effect of long-term glucose level.

**FIGURE 3.** The distributions of P1 (A) and N2 (B) implicit times during euglycemia (black) and hyperglycemia (gray) grouped by nine regional response averages. The box indicates quartiles (25th and 75th percentiles) with a middle line indicating the group median and the whiskers indicating 5th and 95th percentiles. *P < 0.05, mixed model analysis.
FIGURE 4. P1 (A) and N2 (B) implicit times during euglycemia (x axis) plotted against implicit times during hyperglycemia (y axis). Responses are averaged from nine areas as illustrated in Figure 1. Patients with shorter implicit times during hyperglycemia are plotted under the line of identity and patients with longer implicit times during hyperglycemia are plotted above the line of identity.
Figure 5. The first-order (left) and second-order (right) mfERG responses recorded from a patient during euglycemia (bold curve) and hyperglycemia (thin curve). Responses are averaged from nine regions, as specified in Figure 1. First-order traces are shifted to the left during hyperglycemia (i.e., implicit times are decreased compared to euglycemia). Second order traces are not affected by hyperglycemia to the same extent.
No local differences in response to hyperglycemia were found. Thus, the hypothesis of a regional difference in retinal glucose sensitivity was rejected. Our findings, therefore, did not provide evidence that local retinal differences in sensitivity to hyperglycemia contribute to the localized nature of lesions typical in diabetic retinopathy. These results supported and extend previous human reports that indicate photopic retinal sensitivity to variations in circulating glucose.

References