

Association between Local Neuroretinal Function and Control of Adolescent Type 1 Diabetes

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PURPOSE. To evaluate associations between neuroretinal function measured with multifocal electroretinogram (mfERG) and disease variables in adolescents with type 1 diabetes and no retinopathy.

METHODS. Fundus photographs, blood glucose (BG) concentration, HbA1c, and monocular mfERG were performed on 115 adolescent patients (mean age \pm SD; 15.7 ± 1.8 years) and 30 controls (18.0 ± 2.8 years). All subjects had best-corrected visual acuity $\geq 20/20$. The 45° mfERG stimulus included 103 hexagons, reversing between dark and bright according to a pseudorandom m-sequence. Amplitudes (AMPs) and implicit times (ITs) were derived from local mfERG response waveforms, and Z-scores were calculated. Retinal maps of abnormality frequencies were generated. Differences between controls and patients were evaluated using *t*-tests. Associations between mfERG and age, duration, and diabetes control were examined using linear regression analysis.

RESULTS. Mean mfERG IT was significantly longer in the patients compared with that in the controls ($P = 0.019$), but AMP was not different ($P > 0.05$). In all, 26 eyes (23%) of the patients had abnormal IT and 3 eyes (3%) had abnormal AMP. IT abnormalities were essentially distributed randomly across the retina. There were too few AMP abnormalities to examine their retinal distribution. IT was positively correlated with HbA1c ($P < 0.0002$) but not correlated with diabetes duration, BG, or age.

CONCLUSIONS. Higher long-term blood glucose concentration is associated with degraded neuroretinal function in adolescents with type 1 diabetes and no retinopathy. Over 20% of these patients have abnormal neuroretinal function. It will be important to determine longitudinally whether the relationship between mfERG IT and diabetes control exists within individual adolescent patients. (*Invest Ophthalmol Vis Sci*. 2012;53:7071-7076) DOI:10.1167/iops.12-10570

Type 1 diabetes is among the most common chronic diseases in children and is characterized by an autoimmune, T-cell-mediated response against pancreatic beta cells.^{1,2} Globally, there is an increase of approximately 3%

annually in the incidence of this disease.³⁻⁵ The effect of long-term diabetes on the body includes neurologic, macrovascular, and microvascular abnormalities. One of the major complications is diabetic retinopathy, affecting approximately 50% of patients with disease duration over 10 years, and leading in some cases to loss of vision.^{6,7}

New treatment approaches for type 1 diabetes are being developed, including gene therapy, islet cell/pancreas transplantation, residual beta cell regeneration, and artificial pancreases, but these are still at the early stages.⁸⁻¹² In addition, continuous glucose monitoring devices are emerging. Thus far, however, there is limited evidence for the advantage of using such devices for achieving better blood glucose (BG) control.¹³ Although progress is being made toward improved treatment and a cure for type 1 diabetes, insulin administration remains the standard therapy.

Previous studies of adults with diabetes in our laboratory have shown that multifocal electroretinogram implicit time (mfERG IT) delays, a measure of local neuroretinal function, combined with other clinical measures, are highly predictive of development of nonproliferative diabetic retinopathy and diabetic macular edema in specific retinal locations.¹⁴⁻¹⁹ Additionally, we and other laboratories recently have shown that it is not uncommon for adolescents with diabetes to present with mfERG IT delays in the absence of any clinical (vascular) signs of retinopathy.²⁰⁻²³

Maintaining good glycemic control is still the gold standard of diabetes treatment. Accordingly, if a correlation between neuroretinal function measured by mfERG IT and BG control exists, it would suggest that good glycemic control could help in slowing down the progression of the disease and its retinal complications. In a preliminary study that included 32 adolescent patients with type 1 diabetes and no retinopathy, our laboratory found a positive correlation between long-term BG control measured by HbA1c (an index of average BG concentration over the past 1-3 months) and mean mfERG IT.²¹

In the present study, we evaluated local neuroretinal function in a relatively large group of adolescents with type 1 diabetes and no retinopathy, and compared this to a group of age-similar controls. We also determined the prevalence and frequency of local neuroretinal function abnormalities in this patient group, and examined potential relationships between neuroretinal function and BG control, duration of diabetes, and age.

METHODS

Subjects

The right eyes of 137 adolescents with type 1 diabetes recruited from Children's Hospital and Research Center Oakland (Oakland, CA), and 30 healthy control subjects were examined. Three adolescents in the

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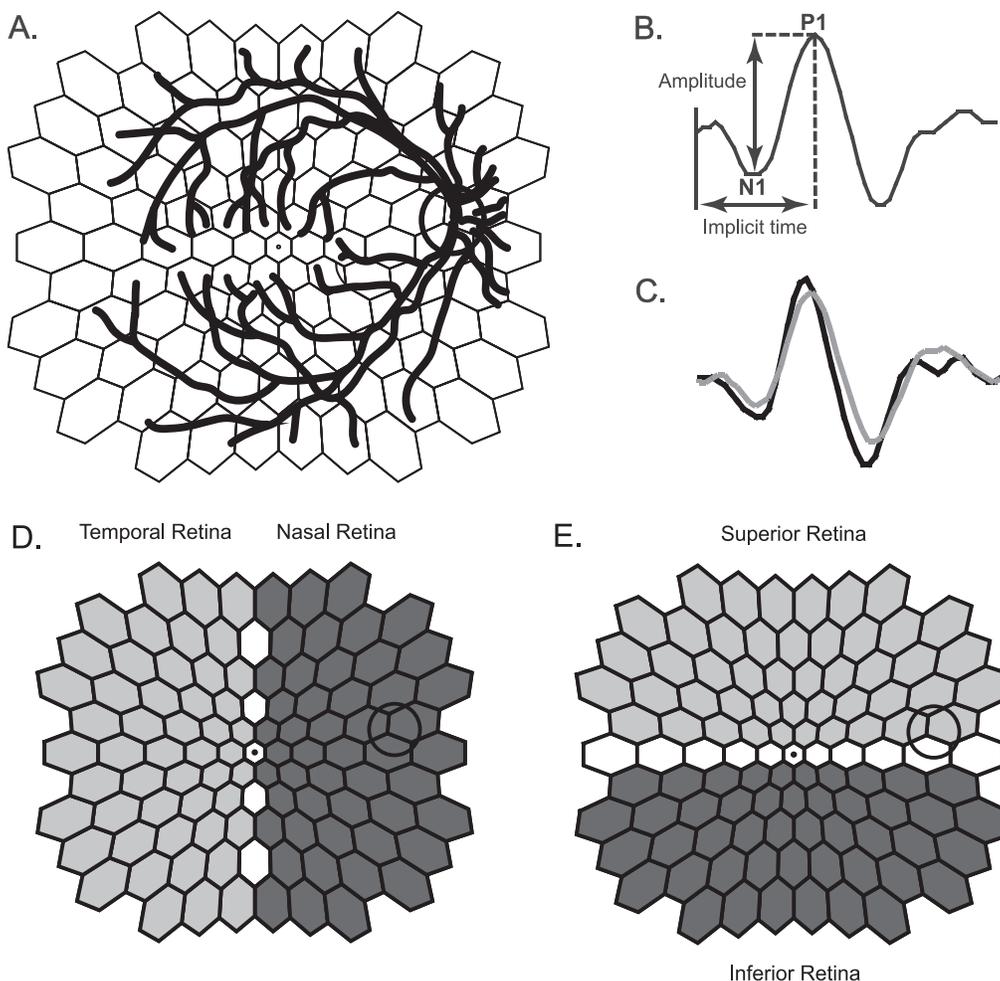


FIGURE 1. The mfERG stimulus array superimposed on the major blood vessels and the optic disc of the right eye (A). mfERG AMP is the voltage difference between the first trough (N1) and the first peak (P1), and mfERG IT is the time between the local flash and P1 (B). An mfERG waveform from a diabetes patient (*gray*) is superimposed on a control subject's waveform (*black*) (C). The temporal and nasal areas (D) and superior and inferior areas (E) examined for asymmetry of mfERG abnormalities.

patient group did not have mfERG recordings per their request, leaving 134. Six of these patients had also participated in our preliminary study,²¹ but their data from that study are not included in this study. Patients (M:F = 69:65) and controls (M:F = 11:19) ranged from 13 to 21 years of age (mean \pm SD was 16.0 ± 2.0 for patients; and 18 ± 1.8 for controls). All patients and control subjects had best-corrected visual acuity (BCVA) of 20/20 or better. Random BG was measured with a glucose meter (OneTouch; Lifescan, Milpitas, CA). The patients' glycated hemoglobin (HbA1c), a measure of average historic BG levels over 1 to 3 months, was taken (DCA2000; Bayer HealthCare, Elkhart, IN), and ranged between 5.3% and >14% (mean \pm SD: $9.3 \pm 1.9\%$; the two values >14% were assigned 14%). Forty-five degree fundus photographs were taken through a dilated pupil from all subjects at their visit. A retina specialist, who was masked to the subjects' identity and medical status, graded the photographs. Patients with retinopathy were excluded. Other exclusion criteria were spherical equivalent refractive error less than $-6.00D$ or more than $+4.00D$, and any disease or injury that could affect the visual system.

All procedures were explained before enrollment in the study and informed consent was obtained from all subjects and the parents/legal guardians of minors. Procedures adhered to the tenets of the Declaration of Helsinki, and the University of California Committee for Protection of Human Subjects approved this research.

Multifocal Electroretinogram

mfERGs were recorded using a commercial system (VERISscience 4.3 system; EDI, San Mateo, CA). Pupils were fully dilated at least 30 minutes before recording with 1.0% tropicamide, 2.5% phenylephrine, and the cornea was anesthetized with 0.5% proparacaine. The stimulus was displayed on a cathode ray tube monitor with a refresh rate of 75 Hz. Subjects fixated a small cross at the center of a 45° 103-hexagon array (Fig. 1A). The hexagons were scaled in size with eccentricity to approximate cone density, and followed a pseudorandom sequence (m-sequence) of luminance polarity reversal. Mean stimulus luminance was 100 cd/m^2 with dark hexagons $< 3 \text{ cd/m}^2$ and bright hexagons 200 cd/m^2 . Subjects viewed the stimulus through a refractor unit and an infrared camera that allowed the examiner to monitor for eye movements during recording. First-order mfERG responses were acquired using a bipolar contact lens electrode (Burian-Allen; Hansen Ophthalmic, Solon City, IA), with a ground electrode attached to the right earlobe. Electrode impedances were $< 5 \text{ k}\Omega$ s.

The template scaling method of Hood and Li²⁴ was used to measure the local first-order kernel responses. Response amplitudes (AMPs) of the local mfERG waveforms were calculated as the difference in voltage between the first trough (N1) and the first positive peak (P1), and implicit times (ITs) were calculated as the time from the local flash to P1 (Fig. 1B).

TABLE. Subject Demographics

	Age, y (Mean ± SD)	Sex M/F	BCVA	DM Duration, y Mean ± SD	BG, mg/dL Mean ± SD	HbA1c, % Mean ± SD
Diabetes (<i>n</i> = 115)	15.7 ± 1.8	57/58	20/20 or better	6.3 ± 3.9	237 ± 98	9.2 ± 1.9
Controls (<i>n</i> = 30)	18.0 ± 2.8	11/19	20/20 or better	N/A	97 ± 11	N/A

DM, diabetes; N/A, not applicable.

Statistical Analysis

Differences in IT and AMP between the diabetes patients and control subjects were examined using two-tailed Student's *t*-tests. *Z*-scores were calculated for each of the 103 local mfERGs using the corresponding mean and SD values obtained from the control subjects. A *Z*-score ≥ 2 for IT, or ≤ -2 for AMP, was considered to be abnormal ($P < 0.023$ for both), and an eye was defined as abnormal if it had 6 or more abnormal locations ($P < 0.03$). Frequencies of abnormalities were compared between the patient and control groups, including nasal versus temporal hemiretinas (in the retinal areas shown in Fig. 1D), and superior versus inferior hemiretinas (as shown in Fig. 1E), using proportions tests. Retinal maps summarizing the frequencies of mfERG IT and AMP abnormalities across the patients were generated. Linear regression analyses were used to test potential associations between the mfERG and (1) BG concentration, (2) HbA1c, (3) diabetes duration, and (4) age.

RESULTS

Of the 134 patient eyes, 19 (14%) were excluded from analysis due to clinical signs of retinopathy, leaving 115 right eyes in the diabetes patient group. The summary characteristics of the patient and control groups are shown in the Table. Figure 1C shows local mfERG waveforms obtained from a patient and a control subject. These waveforms demonstrate the main mfERG response difference, longer IT in the diabetes patients, as compared with the control subjects.

The mean mfERG IT and AMP obtained from each subject are shown in Figures 2A and 2B, respectively. The average mfERG IT of the adolescent patient group was significantly longer compared with that of the control group ($P = 0.019$; Fig. 2C). Mean mfERG AMP did not, however, differ significantly between the patient and control groups ($P > 0.05$; Fig. 2D). Twenty-six eyes (23%) in the patient group were defined as abnormal for IT versus only 3 eyes (10%) in the control group ($P = 0.20$). Only 3 eyes (3%) in the patient group, versus 2 eyes (7%) in the control group, were defined as abnormal for AMP ($P = 0.28$).

Frequency of IT abnormality, defined as the ratio of overall number of abnormal local mfERG responses divided by the total number of responses, was significantly greater in the diabetes group (8.8%) compared with 2.3% in the control group ($P < 0.0002$). As shown in Figure 3A, the mfERG IT abnormalities appear to be fairly randomly distributed across the retina, with the exception that the central region appears to have lower frequencies than more peripheral retinal eccentricities. There were no significant differences in the number of mfERG IT abnormalities between either superior compared with inferior hemiretina ($P = 0.13$), or nasal compared with temporal hemiretina ($P = 0.31$).

The frequencies of mfERG AMP abnormalities did not differ between the subject groups ($P = 0.96$), and the distribution of abnormalities in the diabetes patient group did not differ between nasal and temporal hemiretinas ($P = 0.13$). The superior hemiretina had significantly more AMP abnormalities compared with the inferior hemiretina in the patient group ($P < 0.0001$; Fig. 3B). However, this hemiretinal difference in

AMP abnormalities is not meaningful because the percentage of eyes defined as abnormal at each location was approximately at the chance level ($\leq 3.5\%$ for all locations).

Mean mfERG IT was positively correlated with long-term BG control measured by HbA1c in the adolescent diabetes patient group ($r = 0.35$, $P < 0.0002$), as shown in Figure 4. This indicates that poorer control of diabetes (higher HbA1c level) is associated with worse neuroretinal function (longer mfERG IT). IT, however, was not correlated with the duration of diabetes ($r = 0.016$, $P = 0.86$), random BG concentration at the time of testing ($r = 0.12$, $P = 0.20$), or age ($r = 0.14$, $P = 0.14$) in the patient group. AMP was not correlated with any of the other measures in the patient group (all $P > 0.3$). However, in the control group, mfERG AMP was negatively correlated with age ($r = 0.47$; $P = 0.009$), but mfERG IT was not correlated with age ($r = 0.23$; $P = 0.21$).

DISCUSSION

In this study, we evaluated neuroretinal function in a relatively large group of adolescents with type 1 diabetes and no clinical evidence of retinopathy. First-order mfERG IT in the diabetes patient group was significantly longer compared with that in the control subjects. The frequency of local mfERG responses with abnormal IT was also significantly greater in the patient group, and 23% of the patient eyes were classified as abnormal based on IT. These findings confirm and extend previous studies that have shown the high sensitivity of mfERG in evaluating neuroretinal function in adolescent diabetes patients, and show that neuroretinal function is compromised before the appearance of the clinical signs of retinopathy.²⁰⁻²³

More important, mfERG IT was positively correlated with HbA1c values. These results indicate that worse control of adolescent type 1 diabetes is associated with greater compromise of neuroretinal function in patients with no clinically apparent retinopathy. They also confirm the results of our earlier preliminary study that was performed on a smaller, independent group of adolescent type 1 diabetes patients.²¹ Although HbA1c percentage is considered to be the gold standard measure of long-term BG control, it has limitations. Because it reflects the average BG level over the past 1 to 3 months, HbA1c measurement does not capture shorter-term glucose level fluctuations (e.g., over days). An HbA1c level of 9% could result, for example, from either relatively stable or very variable short-term BG levels, which in turn could affect neuroretinal function in different ways. It is possible, therefore, that different patterns and amounts of historic short-term BG fluctuations contributed to the scatter of the data points around the regression line representing the relationship between mfERG IT and HbA1c in Figure 4.

Klemp and colleagues^{25,26} previously reported, in a small group of adults with type 1 diabetes and no retinopathy, that longer mfERG IT was correlated with higher HbA1c under both normoglycemic and hyperglycemic clamping conditions. More directly relevant to the present study, Lakhani and colleagues²³ developed a negative binomial regression model that demonstrated an association between higher HbA1c and the number of local mfERG responses with abnormal IT in adolescents with

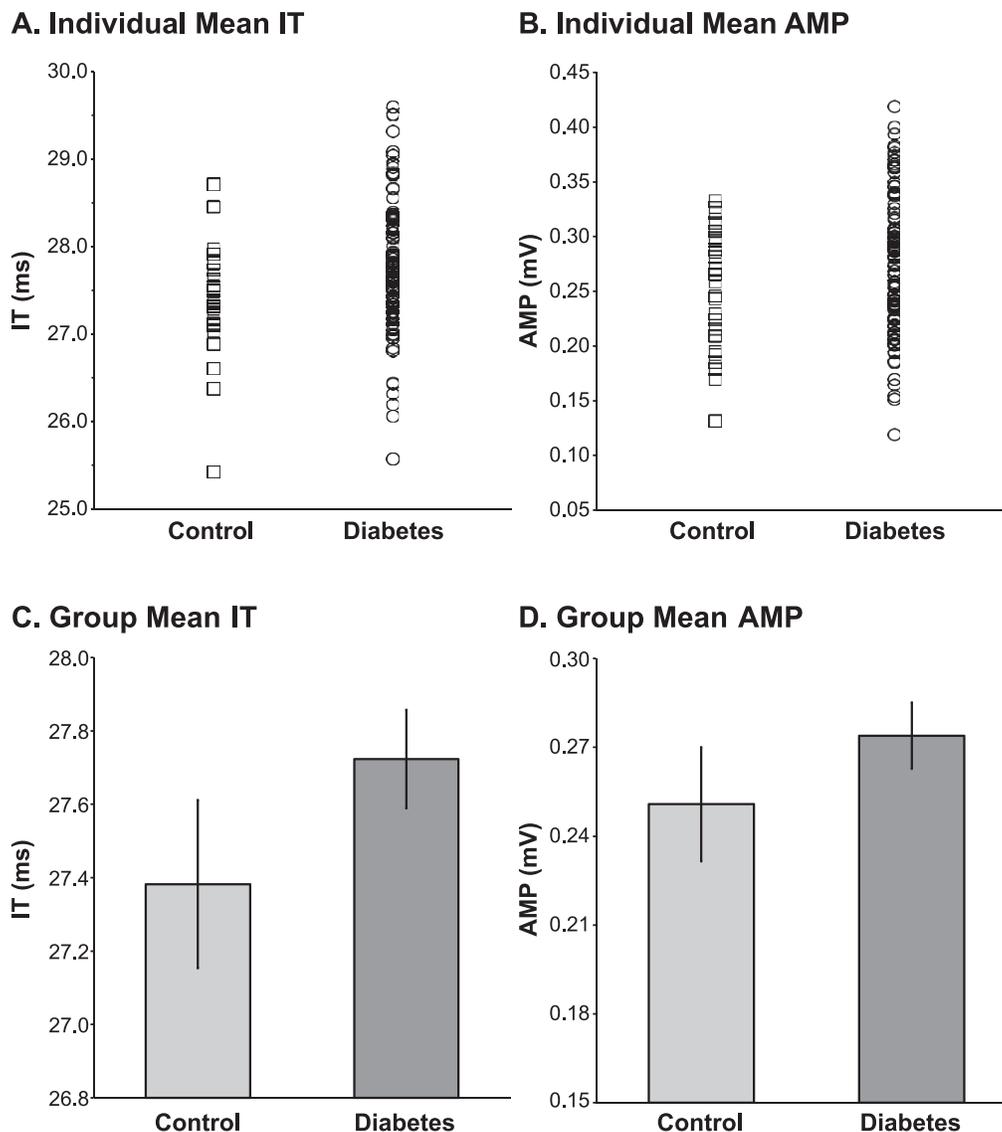


FIGURE 2. Mean mfERG IT (A) and AMP (B) of each study subject. The diabetes patient group had longer mean mfERG IT ($P = 0.019$) than that of the control group (C). Mean mfERG AMP did not differ ($P > 0.05$) between the groups (D). Error bars: (C) and (D) ± 2 SEM.

type 1 diabetes and no retinopathy. This group did not, however, find a significant difference in either the mean mfERG IT or the mean number of abnormal ITs between patients and controls, and did not report examining the correlation between HbA1c and average mfERG IT.²⁵ The methods used in our present study differ from those in the study reported by Lakhani et al.²³ in potentially important ways: we examined a larger sample of adolescent type 1 patients; and we measured HbA1c at the time of mfERG testing.

Across eyes, mfERG IT abnormalities in our adolescent type 1 diabetes patients did not appear to occur with greater frequency in any specific hemiretina or retinal quadrant. They did, however, appear to occur less frequently in the central retina, compared with more peripheral eccentricities. Even though AMP abnormalities were more frequent in the superior retina, there were generally very few AMP abnormalities in our sample. Superior retina susceptibility as seen in our sample is, therefore, probably related to chance alone, given that it is characterized by low frequencies of abnormalities at all retinal locations (<3.5%). The fact that mfERG IT appears to be

affected to a greater degree than AMP in early diabetic eye disease is consistent with a number of previous studies in both adolescents and adult patients, and also in both type 1 and type 2 diabetes.^{14–18,20–23,27}

Although mfERG AMP in our patient group was not significantly different from that in the control group, AMP was negatively correlated with age in the control group but not in the patients. The lack of correlation observed in the patient group could be related to their relative hyperglycemic state at the time of the mfERG recordings. Short-term hyperglycemia induced by clamping was shown to increase mfERG AMP and decrease mfERG IT in adult type 1 diabetes patients.^{25,26} Blood glucose levels in our diabetes group, measured approximately 30 minutes before mfERG recording, were 237 ± 98 mg/dL (mean \pm SD), with a range from 52 to 513. In comparison, the control group's blood glucose level was 97 ± 11 mg/dL. The relative hyperglycemic states of our diabetes patients, and the short-term effect of decreasing mfERG IT, may also have resulted in an underestimation of the IT delays and neural dysfunction in this group.

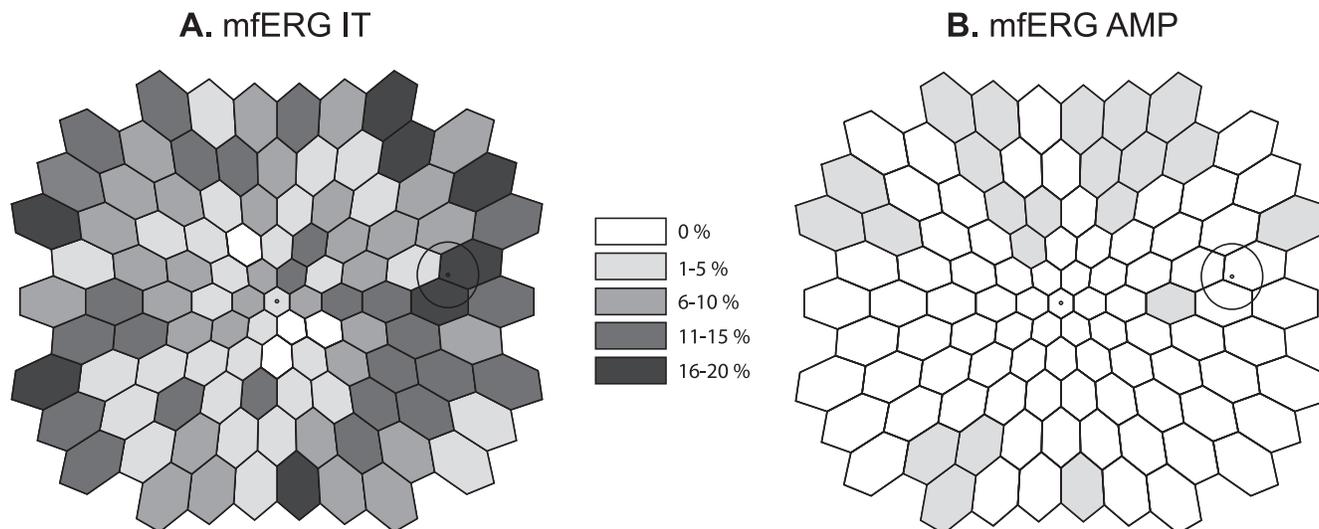


FIGURE 3. The percentage, at each retinal location, of adolescent patient eyes with abnormal mfERG IT (A). Percentages of abnormal mfERG AMP (B). Darker hexagons represent retinal locations with higher percentages of patients defined as abnormal, as indicated by the grayscale key in the center.

We have shown, in a series of adult patient studies, that mfERG IT delays, in combination with other risk factors such as duration of diabetes and blood glucose levels, are highly predictive of the onset of diabetic retinopathy, and the development of diabetic macular edema.¹⁴⁻¹⁹ Therefore, the adolescent patients with longer mfERG IT, who also tend to have the highest HbA1c levels (worse long-term blood glucose control), could be at greater risk for retinopathy development in the future compared with the patients with shorter mfERG IT. Although we do not know for certain whether mfERG IT is predictive of diabetic retinopathy in adolescent patients, it seems reasonable to expect that the neuroretinal dysfunction present in many of their eyes could be associated with the future appearance of the vascular abnormalities defining the clinical signs of retinopathy.

In conclusion, we found a significant difference in mfERG IT between healthy controls and adolescents with type 1 diabetes and no retinopathy. More important, the patients' IT delays were correlated with long-term blood glucose control. In the future, it will be important to learn whether the

neuroretinal dysfunction represented by mfERG IT delays can be improved by decreasing long-term blood glucose levels. The patients who participated in this study are being followed longitudinally, and will be retested 1 year after the initial visit reported herein. We will examine whether improved blood glucose control over the year between examinations can reduce mfERG IT, making it "more normal," or whether the IT delays reflecting compromised neuroretinal function cannot be improved.

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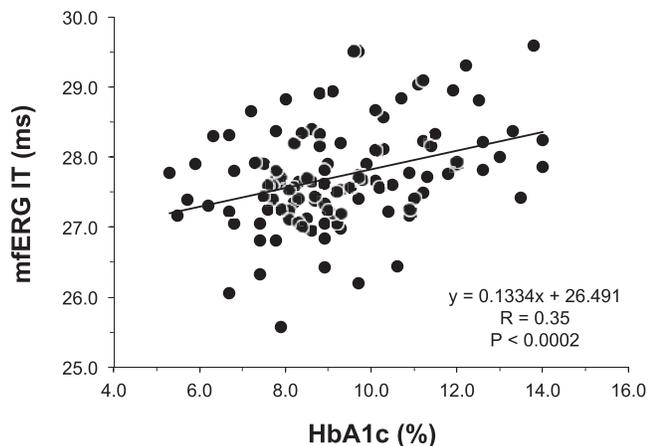


FIGURE 4. mfERG IT is correlated with long-term blood glucose control, as measured by HbA1c percentage, in adolescent patients with type 1 diabetes ($P < 0.0002$).

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