Color Visual Evoked Potentials in Children with Type 1 Diabetes: Relationship to Metabolic Control

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PURPOSE. To examine the association between metabolic control (HbA1c) and the chromatic mechanisms of children with type 1 diabetes (T1D), by using the color visual evoked potential (VEP).

METHODS. Fifty children with T1D (age range, 6–12.9 years) and 33 age-matched control subjects were tested. VEPs were recorded by placing five electrodes on the scalp according to the International 10/20 System of Electrode Placement. Active electrodes O1, O2, and Oz were placed over the visual cortex. Short-wavelength (S), and long- and medium-wavelength (LM) color stimuli consisted of vertical, photometric isoluminant (1 cyc/deg) gratings presented in a pattern onset (100 ms)–offset (400 ms) mode. Achromatic vertical gratings were presented at 3 cyc/deg. Primary outcome measure was VEP latency. The relationship between S, LM, and achromatic VEP latency, and HbA1c, was determined by ANCOVA regression.

RESULTS. S, LM, achromatic VEP latencies were not associated significantly with HbA1c. Pubertal status, however, was associated significantly (P = 0.0114) and selectively with S-VEP latency. Pubertal children with T1D had delayed (mean delay, 9.5 ms) S-VEP latencies when compared with the prepubertal children with T1D. However, there was no statistically significant difference (P = 0.1573) in the effect of pubertal status on S-VEP latency between the T1D and control groups.

CONCLUSIONS. Pubertal status rather than HbA1c appears to affect selectively the S-VEP latency of preteen children with T1D. Further study is warranted to determine whether the delay in S-VEP latency in pubertal children with T1D changes over time and whether this change could be a predictive marker for future development of background diabetic retinopathy. (Invest Ophthalmol Vis Sci. 2005;46:4107–4113) DOI:10.1167/iovs.05-0178

Diabetic retinopathy is one of the long-term microvascular complications of diabetes and a major source of morbidity, causing vision impairment and blindness. In the United States, diabetes is responsible for 8% of legal blindness, making it the leading cause of new cases of blindness in adults aged 20 to 74 years. Each year, approximately 12,000 to 24,000 U.S. residents1,2 and 400 Canadians3 become blind as a result of diabetes. As such, one of the primary goals of managing children with type 1 diabetes (T1D) is to avoid the future risk of diabetic retinopathy by maintaining blood glucose levels close to the normal range.2 The Diabetes Control and Complications Trial4 used fundus photography to examine the retinal vasculature of adolescents with T1D (age range, 13–17 years) and demonstrated a close relationship between metabolic (blood glucose or HbA1c levels) control and the onset and progression of retinopathy. Those in the intensive treatment group who maintained good blood glucose control (low HbA1c levels) showed a 53% decreased risk of the development of diabetic retinopathy when compared with the conventionally treated group, whose HbA1c levels were higher.5

Before the onset of the microvascular lesions of diabetic retinopathy, the neural retina of the diabetic eye undergoes subtle functional changes that are undetectable by fundus photography.6,7,8 However, electrophysiologic techniques have served to detect early neuroretinal functional changes that occur in T1D.9,10 For instance, prepubescent children with T1D and no sign of diabetic retinopathy show significantly reduced focal ERG responses compared with control subjects.6,11 These findings suggest that diabetes has an early and selective effect on the neural retina before the appearance of microvascular complications.

One functional change previously shown to precede the appearance of overt retinopathy, one that may reflect early neuroretinal dysfunction in T1D, is a change in color vision.6,7 Color vision is a central or foveal function that may be impaired by any retinal disease that affects the neural retina9,10 or the neural pathway to the visual cortex.6,9,10,11 Specifically, a change or deficit in the short-wavelength chromatic pathway that is responsible for blue-yellow color discrimination has been described in both adults9,10,12,13 and adolescents10,14–20 with T1D who have no evidence of retinopathy. More important, the short-wavelength deficit found in adults with T1D is associated with elevated long-term glucose control or HbA1c levels20–23 and elevated short-term ambient blood glucose levels (blood glucose levels at time of color vision testing).11,24

The color visual evoked potential (VEP), which assesses the integrity of the neural pathways responsible for color vision, is a useful electrophysiologic indicator of early color vision changes in T1D.10,11 Crogna et al.10 demonstrated that the latency of the short-wavelength (S)-VEP response of adults with T1D is delayed compared with that of control subjects, whereas Schneck et al.11 observed in adults with T1D that the delay in S-VEP latency is associated with an increase in ambient blood glucose levels.

The purpose of the present study was to examine the association between long-term metabolic control or HbA1c,
levels and the chromatic mechanisms in preteen children with T1D, by using the color VEP technique. The latency of the color VEP was the primary outcome measure of the study.

METHODS

Subjects

Fifty children with T1D were recruited from the Diabetes Clinic at The Hospital for Sick Children (Sick Kids) in Toronto, Canada and 44 met the inclusion criteria. Inclusion criteria were diagnosis of T1D, age between 6 and 12.9 years (mean, 9.1 ± 1.9 SD), and duration of diabetes between 1 and 7 years (mean, 3.6 ± 2.1). Exclusion criteria were background diabetic retinopathy (BDR), hemoglobinopathy, inherited or acquired color defects or ocular abnormalities, and any medication or medical condition that affects the retina. Thirty-three age-matched typically developed children or control subjects (mean age, 9.1 ± 1.8 years) were also recruited, to permit exploratory data analysis.

Approval for this study was obtained from the Research Ethics Board at Sick Kids. Parents and/or guardian(s) signed informed consent to confirm their child’s participation in the study, whereas children older than 7 years provided oral and written assent. The study was conducted in accordance with the tenets of the Declaration of Helsinki.

Vision Examination

Vision was assessed in the Visual Electrophysiology Unit (VEU), Department of Ophthalmology and Vision Sciences, Sick Kids. All children with and without T1D had visual acuities correctable to 20/20 or better. Children with and without T1D had normal scores on the clinical color vision tests: H-R R Pseudoisochromatic Plates (Hardy, Rand and Rittler, 1991) and Mollon Reffin Minimalist. Ophthalmoscopy revealed no retinal abnormalities in children without T1D, and refractive errors ranged from −2.50 to +2.50 D in subjects with T1D and from −3.25 to +3.50 D in those without.

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Seven-field stereo color fundus photographs were used to determine whether children with T1D had BDR. Photographs were graded according to the modified Airlie House classification system used in the Early Treatment Diabetic Retinopathy Study. Five children with T1D had BDR: microaneurysms, hemorrhages, and cotton wool spots. Of these five children, one had a history of nephrotic syndrome and had BDR: microaneurysms, hemorrhages, and cotton wool spots. Of these five children, one had a history of nephrotic syndrome and another was receiving L-thyroxine (0.1 mg) for hypothyroidism, but BDR was also receiving L-thyroxine (0.1 mg) for hypothyroidism, but was not excluded from the study. No other ocular conditions or systemic conditions that affect vision were found in children with T1D.

Pubertal Status

Pubertal status was assessed both in children with and in those without diabetes by means of a physical development self-rating questionnaire based on the Tanner stages of sexual maturity. The pubertal-assessment questionnaire was scored as follows: Tanner stage 1 was considered prepubertal, whereas Tanner stage 2 and higher represented onset of puberty. Breast development independent of pubic hair status determined pubertal status on the female questionnaire, whereas the most advanced stage of development, either pubic hair or genital development or testicular size, determined pubertal status on the male questionnaire.

Metabolic Control

The most recent HbA1c measurements (i.e., HbA1c measurement taken on day of color VEP testing) were obtained from the Sick Kids diabetes database. HbA1c is an index of blood glucose control over the preceding 3 months. Mean HbA1c levels in the children with T1D was 7.8% ± 0.9%. To control for variations in ambient blood glucose levels during color VEP testing that would influence the study outcome, three blood glucose measurements using the a glucose monitoring system (One Touch Ultra; Lifescan, Burnaby, British Columbia, Canada) were taken.

Color Visual Evoked Potential

Stimuli were created using Vision Research Graphics (VRG) software (Durham, NH) and presented on a rectangular 21-in. RGB color graphics monitor (FlexScan F930; Eizo, Cypress, CA) with 26° × 20° field dimensions. Color stimuli were presented along two axes in CIE color space: the Tritanopic confusion axis stimulated selectively the S-cone pathway, and the axis orthogonal to this stimulated the long-medium-wavelength (LM)-cone pathway. S and LM stimuli passed through white (CIE x, y-coordinates, 0.33, 0.33). CIE coordinates used were: S-axes x = 0.3409, y = 0.3523 (greenish-yellow) and x = 0.2893, y = 0.2496 (purple) and LM-axes x = 0.3594, y = 0.3099 (red) and x = 0.3064, y = 0.3372 (green). The cone contrasts for both chromatic stimuli were calculated using the Cole and Hine formula. The respective cone contrasts for S and LM gratings were: S-axis (L = 0.00, M = 0.00, S = 0.39) and L-M-axis (L = 0.06, M = 0.11, S = 0.00). Achromatic stimulus with CIE coordinates x = 0.3260, y = 0.3358 for white and x = 0.3250, y = 0.3340 for black was also presented.

Stimulus parameters were chosen to optimize the chromatic response and differentiate between the chromatic and achromatic VEP responses. Chromatic and achromatic stimuli were vertical sinusoidal wave gratings of 1 and 3 cyc/deg respectively. Low spatial frequency for chromatic gratings was chosen to minimize chromatic aberration. Chromatic stimuli were presented at photometric isoluminance. Both chromatic and achromatic stimuli were presented in an onset (100 ms)-offset (400 ms) mode at a repeat rate of 2 Hz. The offset mode, composed of a uniform field, was equated in mean luminance and chromaticity (36.50 cd/m²) to the onset mode. S and LM stimuli were presented at 40% contrast. Achromatic stimulus was presented at 90% Michelson contrast. Mean luminance and chromaticity (36.50 cd/m²) were identical for all three stimuli.

To extract cortical responses to color stimuli, we placed 6-mm diameter gold disc electrodes (Genuine F-E56G; Grass Instrument Division, Astro-Med, Inc., West Warwick, RI) equipped with protected terminals (Safelead; Grass) on the scalp according to the international 10-20 system of electrode placement. Three active electrodes were placed over the occipital or visual cortex in positions O2, O1, and O2, whereas two additional electrodes were positioned on nonvisual areas of the cortex at Pz (ground) and Cz (reference). Color VEPs were recorded monocularly with a viewing distance of 75 cm. Results are reported for the right eye only.

Sample Size Calculation and Statistical Analysis

A sample size calculation using a multiple linear regression power analysis (Power & Sample Size, NCS/SPASS; Statistical and Power Analysis Software, Kaysville, UT) with a power of 0.8, an α of 0.05, and an estimated β = 0.4 determined that 44 subjects with T1D were needed for the study. ANCOVA regression was used both for the primary analysis to examine the relationship between S-VEP latency and HbA1c, and for the exploratory analyses: (1) to evaluate the interaction term between the effect of pubertal status on S-VEP latency and group, and (2) to examine the relationship between LM- and achromatic VEP latencies and HbA1c. A computer (SAS software; SAS Institute Inc., Version 8.0) was used to perform the statistical analysis and produce the graphics (S-Plus, 6.2. Academic Site Edition; Insightful Corp. Seattle, WA) software was used for graphics.

Results

In analyzing the VEP data, latency served as the main measure because it is a more reliable parameter than amplitude; and also, among typically developed subjects, VEP latency shows less interindividual variation than does amplitude. Figure 1
shows the characteristic waveforms for the three axes: S, LM, and achromatic.

The latency for chromatic onset–offset VEP data was measured from pattern onset (time of stimulus presentation at 0 ms) to the trough of the first negative component, which is often followed by a positive component. For achromatic onset–offset stimuli, latency was measured from pattern onset to the peak of the first positive component.

The relationship between S-VEP latency and HbA1c across T1D subjects was determined. The results of the ANCOVA regression modeling S-VEP latency as a function of HbA1c after adjustment for sex, pubertal status, duration of diabetes, and average ambient blood glucose levels during VEP testing, are summarized in Table 1.

ANOVA Regression

**S-VEP Latency Versus HbA1c.** The results shown in Table 1 indicate no statistically significant association between S-VEP latency and HbA1c in preteen children with T1D. The Pearson correlation coefficient between S-VEP latency and HbA1c was $r = 0.134$ ($P = 0.3905$). The relationship between SVEP latency and HbA1c in the children with T1D is shown in Figure 2. The covariates of sex, disease duration, and average ambient blood glucose during VEP testing were also not found to be associated significantly with S-VEP latency.

### ANOVA Regression Model

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<th>Parameter</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>$P$</th>
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<td>HbA1c</td>
<td>1.76</td>
<td>1.93</td>
<td>0.3662</td>
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<tr>
<td>Sex: female vs. male</td>
<td>3.21</td>
<td>3.61</td>
<td>0.3788</td>
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<tr>
<td>Pubertal Status: prepubertal vs. pubertal</td>
<td>$-9.52$</td>
<td>3.57</td>
<td>0.0114*</td>
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<tr>
<td>Disease duration</td>
<td>$-0.44$</td>
<td>0.79</td>
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<tr>
<td>Average blood glucose</td>
<td>$-0.02$</td>
<td>0.28</td>
<td>0.9288</td>
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</table>

Data are from children with T1D after adjustment for the covariates sex, pubertal status, disease duration, and average ambient blood glucose levels during VEP testing.

*Statistically significant.

An association between S-VEP latency and pubertal status was found to be statistically significant. The mean S-VEP latency of pubertal T1D children ($n = 22$, $144.27 \pm 12.24$ ms) was delayed significantly when compared with prepubertal children with T1D ($n = 20$, $134.75 \pm 6.65$ ms; Fig. 3).

Sample S-VEP waveforms of pubertal and prepubertal children with T1D are provided in Figures 4A and 4B, respectively.

To explore further the statistically significant association between S-VEP latency and pubertal status, we computed the interaction term examining the difference in pubertal status effect on S-VEP latency after adjusting for sex between the T1D and control groups. ANCOVA regression results for the interaction term (pubertal status × group) are summarized in Table 2.

**Interaction Term Pubertal Status × Group.** The pubertal status effect on S-VEP latency was greater in the T1D group than in the control group (estimate value = 6.67). However, this effect was not significant. After a Bonferroni adjustment...
be associated significantly with HbA1c levels in this cohort of children with T1D. Furthermore, LM- and achromatic VEP latencies also were not associated with HbA1c levels.

One possible explanation for the lack of association between HbA1c levels in preteen children with T1D and their S-VEP latencies, contrary to the findings in adults with T1D,11,21–24 is that preteen children with T1D, unlike older individuals with T1D, generally maintain good blood glucose control and low HbA1c levels (≤8%).3,41,42 As such, children with T1D generally show decreased frequency and/or severity of diabetic retinal complications compared with older individuals with T1D.3 The range of HbA1c levels in the participants in the present study may have been too restrictive to obtain a significant correlation between S-VEP latency and HbA1c levels. Indeed, 52% of the diabetes sample in the present study demonstrated good blood glucose control (≤8% HbA1c), whereas 39% were in the 8% to 9% HbA1c range. Only 9% had an extremely elevated level (>9% HbA1c).

Instead, we found an association between pubertal status and S-VEP latency in the T1D group. The pubertal T1D group had significantly delayed S-VEP latencies when compared with the prepubertal T1D group. There are several possible explanations for this finding.

A luminance (nonchromatic) artifact resulting from a large field stimulus and/or testing at photometric isoluminance35,36,38,43 may have contaminated the data. A luminance artifact would appear as an early (approximately 100 ms) positive peak preceding the first negative component or chromatic response.35 S-responses are more vulnerable to luminance contamination than LM-responses.36,38,43 The most likely sources of luminance artifact under such conditions are chromatic aberration35,36,45 and varying macular pigmentation among subjects.38,43

Testing with a 3° circular stimulus field along with a restricted number of spatial cycles (3–6 spatial cycles) minimizes luminance artifact in S-VEP responses35,36,38,44. We attempted to collect S-VEP responses using a 3° circular blue-yellow stimulus on the most compliant children with and without diabetes (ages, ~6–12years). The data collected in this age group were unrepeatable and unreliable. However, we managed to collect S-VEP data on the children by using a 9° circular field. When comparing the morphology of S-VEP responses from the 26° × 20° rectangular stimulus and the 9° circular field stimulus, we found that responses to either stimulus produced a predominantly chromatic waveform (i.e., no significant positive peak; Fig. 5). Most important, stimulus field size did not affect S-VEP latency significantly. The test-retest variability for our large 26° × 20° rectangular stimulus, which is defined as mean S-VEP latency difference (trial 1 minus trial 2) ± 1 SD, was 5.77 ± 4.40 ms. The mean difference in S-VEP latency (n = 8, 4.26 ± 2.11 ms) between the 26° × 20° and 9°

**TABLE 2. ANCOVA Regression Modeling the Interaction Term Pubertal Status × Group**

<table>
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<th>Parameter</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>( P )</th>
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<tr>
<td>Intercept</td>
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<td>2.65</td>
<td>&lt;0.0001</td>
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<td>Sex: female vs. male</td>
<td>2.89</td>
<td>2.33</td>
<td>0.2203</td>
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<tr>
<td>Pubertal status: prepubertal vs. pubertal</td>
<td>−9.85</td>
<td>2.99</td>
<td>0.0016*</td>
</tr>
<tr>
<td>Group: control vs. diabetes</td>
<td>−5.79</td>
<td>3.08</td>
<td>0.0603</td>
</tr>
<tr>
<td>Pubertal status × group</td>
<td>6.67</td>
<td>4.66</td>
<td>0.1573</td>
</tr>
</tbody>
</table>

The difference in pubertal status effect (difference in mean S-VEP latency between the pubertal and prepubertal subgroups) on S-VEP latency between the T1D and control groups after adjusting for sex. Main effects in the model are pubertal status and group.

*Statistically significant.
appear to arise as a result of luminance contamination. 

responses found in the pubertal children with T1D does not all mature or adultlike until the onset of puberty, which occurs usually between 12 and 14 years. At this time, the morphology of the chromatic VEP waveform changes from a positive-negative to the classic negative-positive, causing a subtle shift in latency (i.e., earlier or shorter). Consequently, insulin sensitivity or the ability of insulin to stimulate glucose uptake into peripheral tissues may be reduced in adolescents with T1D, thereby resulting in poor metabolic control. However, because mean HbA1c in the pubertal group (HbA1c, 7.8%; range: 6%-10.7%) was similar to that of the prepubertal group (HbA1c, 7.8%; range: 6.5%-9.5%), metabolic control in the present study does not seem to explain the S-VEP latency delay in pubertal children with T1D. The lenses of young adults (mean age, 30 years) with T1D, with a long disease duration (median, 21 years), become yellow at an accelerated rate when compared with those of nondiabetic control subjects. Moreover, premature lens yellowing in T1D has been attributed in part to elevated blood glucose levels, which may lead to the accelerated glycosylation of lens proteins. In the present study, however, the pubertal children with T1D were young (mean age, 10 years), had good glucose control (mean HbA1c, 7.8%), and had a short disease duration (mean disease duration, 4.3 years), and therefore this explanation is unlikely.

Last, the retina is an insulin-sensitive tissue. Retinal electrophysiology has demonstrated a dose-dependent reduction in the amplitudes of both the a- and b-wave components of the electroretinogram after administration of insulin in vitro. Abnormal levels of insulin are needed by children with T1D, which are typically increased to control for elevation in HbA1c during puberty. As insulin dosage administered in units per kilogram body weight does not increase significantly during the early stages of puberty, changing insulin levels during puberty is an unlikely explanation of delayed S-VEP latency.

In light of recent findings by Verrotti et al., which showed an association between delayed VEP latencies in response to a luminance pattern-reversal stimulus and high HbA1c levels (mean HbA1c, 9.4%) in adolescents between the ages of 10 to 19, it is important to study the chromatic mechanisms of older adolescents as well as young adults with T1D. Because the short-wavelength deficit in T1D is a functional change that arises before the onset of diabetic retinopathy it is also important to determine whether the S-VEP latency delay in pubertal children with T1D changes over time and whether this change could be a predictive marker for the onset of background diabetic retinopathy.

In summary, pubertal status, rather than metabolic control, disease duration, sex, and average ambient blood glucose during VEP testing, appears to delay S-VEP latency in pubertal children with T1D. Factors such as chromatic VEP maturation, hormonal milieu, hyperopic refractive error, lens yellowing, hypoglycemia, and attention deficits cannot explain the delay in S-VEP latency of pubertal children with T1D. However, further investigation into the pubertal status effect on S-VEP latency in the diabetes group is needed, since this effect seemed to be greatest in the diabetes group, even though the interaction term was not found to be statistically significant.

Acknowledgments

The authors thank Carole Panton, Assistant Director of the VEU, for invaluable comments and criticisms after reviewing the manuscript; Thomas Wright, research technologist, for excellent technical assistance with the color VEP and for help with the illustrations; ophthalm-
mic imaging specialists Leslie MacKeen and Cynthia VandenHoven for obtaining fundus photographs in the children with TID; Joyce Robinson, Administrative Assistant in the Division of Endocrinology, for helping to identify the children with TID who were eligible to take part in the study; and all the participants for their cooperation in the study.

References


44. Robson AG, Kulikowski JJ. Verification of human visual evoked potentials (VEPs) elicited by gratings containing tritanopic pairs of hues (abstract). *J Physiol*. 1996;485:22P.