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 = 0.1575) 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
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.

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 the remaining children therefore were excluded. Another child without T1D was found to have 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.

**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-axes (L = 0.00, M = 0.00, S = 0.59) and LM-axes (L = 0.06, M = 0.11, S = 0.00). Chromatic 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 difference between the chromatic and achromatic VEP responses. Chromatic and achromatic stimuli were vertical sinusoidal wave gratings of 1 and 3 cycles/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 6mm diameter gold disc electrodes (Genuine F-E56GH; 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 Oz, 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, NCSS/PASS; Statistical and Power Analysis Software, Kaysville, UT) with a power of 0.8, an α of 0.05, and an estimated ρ = 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.

**ANCOVA 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 \( \rho = 0.134 \) (\( P = 0.3662 \)). The relationship between S-VEP latency and HbA1c in the children with T1D is shown in Figure 2. The covariates of sex, disease duration, and average ambient blood glucose levels during VEP testing were also not found to be associated significantly with S-VEP latency.

**Table 1. ANCOVA Regression Modeling S-VEP Latency as a Function of HbA1c**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>131.10</td>
<td>14.98</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HbA1c</td>
<td>1.76</td>
<td>1.93</td>
<td>0.3662</td>
</tr>
<tr>
<td>Sex: female vs. male</td>
<td>3.21</td>
<td>3.61</td>
<td>0.3788</td>
</tr>
<tr>
<td>Pubertal Status: prepubertal vs. pubertal</td>
<td>-9.52</td>
<td>3.57</td>
<td>0.0114*</td>
</tr>
<tr>
<td>Disease duration</td>
<td>-0.44</td>
<td>0.79</td>
<td>0.5827</td>
</tr>
<tr>
<td>Average blood glucose</td>
<td>-0.02</td>
<td>0.28</td>
<td>0.9288</td>
</tr>
</tbody>
</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.

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 ± 12.24 ms) was delayed significantly when compared with prepubertal children with T1D (\( n = 20 \), 134.75 ± 8.65 ms; Fig. 3). Sample S-VEP waveforms of prepubertal and pubertal 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...
Moreover, covariates including pubertal status were not significantly associated with either LM- or achromatic-VEP latencies, and HbA1c levels after adjustment for the covariates sex, pubertal status, and average ambient blood glucose levels for multiple testing, left eye results were similar to right eye results.

After adjustment for sex, disease duration, pubertal status, and average ambient blood glucose levels during VEP testing, neither LM- (n = 39, P = 0.6057) nor achromatic (n = 45, P = 0.7250) VEP latencies were significantly associated with HbA1c. Moreover, covariates including pubertal status were not significantly associated with either LM- or achromatic-VEP latency.

**DISCUSSION**

The primary objective of this study was to examine the association between HbA1c and color vision in preteen children with T1D—specifically, the S chromatic pathway. We hypothesized that the S-VEP latency would be associated with HbA1c levels after adjustment for the covariates sex, pubertal status, duration of diabetes, and average ambient blood glucose levels during VEP testing. However, S-VEP latency was not found to 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, is that preteen children with T1D, unlike older individuals with T1D, generally maintain good blood glucose control and low HbA1c levels (<8%). As such, children with T1D generally show decreased frequency and/or severity of diabetic retinal complications compared with older individuals with T1D. 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 isoluminance 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. S-processes are more vulnerable to luminance contamination than LM-processes. The most likely sources of luminance artifact under such conditions are chromatic aberration and varying macular pigmentation among subjects.

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 responses. 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° x 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° x 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° x 20° and 9°

**TABLE 2. ANCOVA Regression Modeling the Interaction Term**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>145.17</td>
<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>
</tr>
<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>3.03</td>
<td>0.0603</td>
</tr>
<tr>
<td>Pubertal status × group</td>
<td>6.67</td>
<td>4.66</td>
<td>0.1575</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. At puberty, which occurs usually between 12 and 14 years, information are not mature or adultlike until the onset of latency was greater in the diabetes group than in the control group. However, this study was not designed to compute the interaction term. With sufficient power and sample size, the difference in pubertal status effect on S-VEP latency in the diabetes group. Major changes in the hor- size, the difference in pubertal status effect on S-VEP latency of pubertal children with T1D does not appear to arise as a result of luminance contamination.

Second, the effect of the pubertal status, per se, must also be examined. Because the interaction term was not statistically significant, the delay in S-VEP latency in the pubertal children with T1D may not be a phenomenon that is confined to the diabetes group. However, this study was not designed to compute the interaction term. With sufficient power and sample size, the difference in pubertal status effect on S-VEP latency between the two groups may have been significant and confined to the diabetes groups, as pubertal status effect on S-VEP latency was greater in the diabetes group than in the control group.

Furthermore, the neural pathways that process chromatic information are not 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). However, because earlier latencies were not seen in pubertal children with T1D, the effect of pubertal status on S-VEP latency in the diabetes group is unlikely to be due to matura- tion of the visual system.

Alternatively, pubertal status may be a marker of blood glucose elevations, which in turn may contribute to the observed significant association between pubertal status and S-VEP latency in the diabetes group. Major changes in the hor- monal environment of adolescents with T1D during puberty are thought to be responsible for reduced metabolic control and increased Hba1c levels during this stage of develop- ment.5,41–46 Poor metabolic control is often attributed to abnormalities in the growth hormone/insulin-like growth factor (GH/IGF)-1 axis, which cause spontaneous hypersecretion of GH and reduction of circulating IGF-1. 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.47–48 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.

S-cone sensitivity reduction or S-deficiency in adults with T1D is associated with duration-dependent lens yellowing.49 The lenses of young adults (median, 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.50 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.50,51 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 explana- tion is unlikely.

Last, the retina is an insulin-sensitive tissue.52 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.53 Abnormal levels of insulin are needed by children with T1D, which are typically increased to control for elevation in Hba1c during puberty.54–55 As insulin dosage administered in units per kilogram body weight does not increase significantly during the early stages of puberty,51–55 changing insulin levels during puberty is an unlikely explanation of delayed S-VEP latency.

In light of recent findings by Verrotti et al.,55 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 impor- tant 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.

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mic imaging specialists Leslie MacKeen and Cynthia Vandenhoven for obtaining fundus photographs in the children with T1D; Joyce Robinson, Administrative Assistant in the Division of Endocrinology, for helping to identify the children with T1D who were eligible to take part in the study; and all the participants for their cooperation in the study.

References

42. Mortensen HB, Robertson KJ, Aanstoot HJ, et al. Insulin management and metabolic control of type 1 diabetes mellitus in child-


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.


