Acute Effects of Blood Glucose on Chromatic Visually Evoked Potentials in Persons With Diabetes and in Normal Persons

Marilyn E. Schneck,* Brad Fortune,* Eugene Switkes,† Michael Crognale,* and Anthony J. Adams *

Purpose. To determine whether specific chromatic pathways are selectively affected by short-term variations in blood glucose levels in observers with and without diabetes.

Methods. Ten subjects with diabetes, all with type 1 diabetes and no retinopathy, and eight age-similar normal subjects were tested. Cortical visually evoked potentials (VEPs) in response to stimuli designed to selectively activate the short-wavelength-sensitive (S) or long- and middle-wavelength-sensitive (LM) chromatic (isoluminant) pathways or the achromatic pathway were recorded over a period of several hours. Capillary blood glucose also was measured repeatedly over the same period. The relation between VEP latency and blood glucose was determined.

Results. The S-pathway VEP latency was correlated significantly with blood glucose in a slight majority (6/10) of persons with diabetes; S-pathway latency was longer at higher blood glucose levels. This association between S-pathway latency and blood glucose was not dependent on the pattern of blood glucose variation over time (i.e., significant correlations between blood glucose and latency were observed in persons for whom blood glucose increased, decreased, or rose and then fell over time). No dependence on blood glucose was observed for LM- or achromatic-pathway VEP latency in subjects with diabetes.

Conclusions. Acute variations in blood glucose of subjects with diabetes over hours selectively affect the function of the short-wavelength-sensitive chromatic pathway. The findings are discussed within the context of known mechanisms by which elevated glucose affects cellular metabolism with a time course consistent with the transient nature of the effects observed.


It is well-recognized that in diabetes, changes in the function of the short-wavelength-sensitive system (S-pathway) precede changes in more standard measures of visual function, such as visual acuity. Changes in S-pathway function may be found before or in the absence of diabetic retinopathy.1,2 Such findings have motivated researchers to seek explanations other than retinopathic changes for the early vision loss in diabetes. In fact, the suggestion that functional changes may precede, and not be dependent on, retinal vascular changes in diabetes is not a new one.3 More recently, others4,5,6 have affirmed the point of view that the direct effects of diabetes on retinal function be considered and that with respect to vision, diabetes should be regarded as a neurosensory rather than a vascular disorder.

Diabetes is fundamentally a metabolic disorder, characterized by elevated blood glucose. Thus, it is reasonable to consider the altered metabolic state of the persons with diabetes as the underlying cause of the early loss of S-pathway sensitivity. It has, however, proved difficult to separate metabolic from structural factors as the basis for the early vision loss, because the development of retinopathy is itself affected by metabolic control7-10 and vascular changes may be present, though undetected, in people with "no retinopathy."
Many other factors related to metabolic control also vary among persons. Any of these factors may, in turn, affect or produce the apparent correlation of color vision with metabolic control observed in cross-sectional studies, which rely on comparisons between persons. Studies showing acute or reversible changes in color vision in association with variations in blood glucose within persons are not confounded in this way.

In previous studies, we explored the relation between acute variations in metabolism and psychophysical measures of vision function in diabetes. Fluctuations in blood glucose occurring over a period of hours or months as well as large induced variations in blood glucose over hours were found to produce rapid, systematic, and selective changes in S-pathway sensitivity in observers with diabetes. Higher blood glucose levels were associated with lower S-pathway sensitivity. No such dependence was seen for the L- and M-cone-mediated pathway. We concluded that in diabetes, metabolism directly and selectively affects S-pathway function.

In those studies, S-pathway sensitivity was assessed by presenting short-wavelength (blue) test stimuli on bright (200 cd/m²) yellow backgrounds to which subjects had preadapted. Threshold measures for yellow spots on the same backgrounds provided our measure of L- and M-cone function. As pointed out by others, there are limitations of interpretation of such data. For example, the mechanism detecting the short-wavelength (S-pathway) stimulus under our test conditions, is mediated by a postreceptoral opponent channel influenced by activity of L and M cones. Thus, changes in threshold for blue spots on yellow backgrounds can reflect changes in L- or M-cone function; conversely, absence of such changes can reflect changes in S-cone function that are offset by changes in L- or M-cone function or both.

In addition, the intense yellow backgrounds used in the earlier studies adapt differentially the postreceptoral opponent pathways detecting the blue and yellow test stimuli. The blue–yellow opponent pathway, through which the blue test stimulus (S cone) signal travels, is polarized strongly, and thus desensitized, by the yellow background. In contrast, the response to the yellow (L–M) test is carried by either the red–green opponent or luminance pathway which is not polarized strongly by the yellow background and thus maintains its sensitivity. In comparing the behavior of thresholds for blue and yellow targets, we are thus making comparisons between systems that are in rather different adaptation states; the comparisons would be more meaningful when all systems are in a similar state of adaptation.

In the current study, we address the issue of the dependence of chromatic mechanisms on short-term variations in blood glucose using stimuli that activate selectively the individual chromatic mechanisms without the use of adapting fields. Further, we use an objective technique, the visually evoked potential (VEP). In this study, stimuli were sinusoidal gratings modulated around a chromatically neutral point along three cardinal axes of a color space described by Krauskopf et al., based on the cone activation space put forth by MacLeod and Boynton. The VEPs were measured repeatedly over several hours, as was blood glucose. The association between VEP latency and blood glucose was determined for each observer.

For a majority of persons with diabetes tested, S-axix VEPs latency varied with blood glucose; longer S-axis VEP latencies were associated with higher blood glucose levels. The consistent finding of both our earlier psychophysical study and the current study is that lower S-pathway sensitivity is associated with higher blood glucose levels, suggesting that the steady-state early loss of S-cone sensitivity in diabetes may be attributable, at least in part, to metabolic factors rather than being secondary to vascular damage.

METHODS

Subjects

The characteristics of the 10 type 1 insulin-dependent subjects with diabetes who participated in the study are given in Table 1. All had visual acuities correctable to 20/20 or better and normal color vision as assessed by the Adams desaturated D-15 test. None had diabetic retinopathy, other complications of diabetes, nor other ocular conditions or systemic conditions that affect vision.

Blood Glucose Measurements

Capillary blood glucose was measured repeatedly throughout each test session. Blood was obtained by finger stick, and glucose levels were determined using a glucometer with digital readout (One Touch II, Lifescan; Johnson & Johnson, Milpitas, CA). The glucometer was calibrated (using the solution provided by the manufacturer) before use.

Test sessions were scheduled to occur at times that normally would include a meal and insulin injection for subjects with diabetes. By varying the relative timing of the meal and medication, three different patterns of blood glucose variation across time were created: a continuous increase in blood glucose during

Downloaded From: http:// iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/ iovs/933200/ on 04/02/2017
TABLE 1. Characteristics of the Subjects with Type 1 Insulin-Dependent Diabetes

<table>
<thead>
<tr>
<th>Identification Number</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Diabetes Type</th>
<th>Duration (years)</th>
<th>High BG (mg/dl)</th>
<th>Low BG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>M</td>
<td>I</td>
<td>10</td>
<td>328</td>
<td>93</td>
</tr>
<tr>
<td>2</td>
<td>47</td>
<td>F</td>
<td>I</td>
<td>10</td>
<td>251</td>
<td>98</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>F</td>
<td>I</td>
<td>3</td>
<td>558</td>
<td>84</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>F</td>
<td>I</td>
<td>10</td>
<td>536</td>
<td>72</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>M</td>
<td>I</td>
<td>20</td>
<td>190</td>
<td>131</td>
</tr>
<tr>
<td>6</td>
<td>27</td>
<td>M</td>
<td>I</td>
<td>1 month</td>
<td>143</td>
<td>60</td>
</tr>
<tr>
<td>7</td>
<td>33</td>
<td>M</td>
<td>I</td>
<td>10</td>
<td>271</td>
<td>143</td>
</tr>
<tr>
<td>8</td>
<td>24</td>
<td>F</td>
<td>I</td>
<td>4</td>
<td>222</td>
<td>86</td>
</tr>
<tr>
<td>9</td>
<td>28</td>
<td>M</td>
<td>I</td>
<td>5</td>
<td>555</td>
<td>177</td>
</tr>
<tr>
<td>10</td>
<td>27</td>
<td>M</td>
<td>I</td>
<td>15</td>
<td>325</td>
<td>82</td>
</tr>
<tr>
<td>Mean</td>
<td>29.4</td>
<td></td>
<td>I</td>
<td>7.7</td>
<td>277.9</td>
<td>102.6</td>
</tr>
</tbody>
</table>

BG = blood glucose.

the test session (subject 1), a continuous decrease in blood glucose over time (subjects 2, 3, 4, 5, and 10), and a nonmonotonic variation in blood glucose over time in which blood glucose levels first decreased and then increased (subject 6) or increased and then decreased during the test session (subjects 7, 8, and 9).

Subjects without diabetes were tested in the morning after an overnight fast. After initial fasting blood glucose and VEP measures were made, a solution containing 100 g of d-glucose was consumed by the subject. VEP and blood glucose were measured as blood glucose rose, and in most cases declined, over approximately 2 hours.

**Stimuli**

The VEPs were recorded using stimuli composed of sinusoidal gratings with luminance and chromatic variations chosen to activate selectively independent mechanisms of early visual processing. VEPs are reported for stimuli varying along three chromatic directions: an achromatic white–gray luminance-varying grating, an isoluminant LM-grating that corresponds to complementary L-cone and M-cone activation–deactivation (L+ M− activation along a reddish direction and L− M+ along a blue–green direction), and an isoluminant S-grating that gave only S-cone activation–deactivation along a violet and yellow–green direction. The stimuli were presented on a Sony (San Jose, CA) GDM-1604 color monitor under computer control (SUN 3/160 [Sun Microsystems, Mountain View, CA] with TAAC graphics facility). The mean chromaticity and luminance of each of the three stimuli and the surround were identical, a white corresponding to illuminant C with a luminance of 18 cd/m². Stimuli were calibrated using a Minolta (Ramsey, NJ) Chroma Meter, Model CS-100. Cone activation and luminance were calculated using the methods of Smith and Pokorny and Judd. Contrasts of 40%, 32%, and 40% (relative to the maximum available on our monitor) were used for the achromatic, LM-varying, and S-varying gratings, respectively, for all but one subject. The contrast levels were chosen to fall on the steep portion of the functions relating VEP latency and amplitude to contrast in normal subjects. One subject with diabetes required higher contrasts to obtain reliable responses. He was tested using 63% contrast for all axes.

To optimize responses, the achromatic (luminance) VEPs were recorded using 3 cyc./deg sinusoids reversing temporally at 2 Hz. Isoluminant LM- and S-varying stimuli were 1 cyc./deg sinusoids in a 2-Hz temporal onset-offset presentation (on for 106 msec followed by a 394 msec off to mean luminance period at a repeat rate of 2 Hz). In all cases, the stimulus was

![FIGURE 1. Visually evoked potential responses to short-wave-length-sensitive-axis (top), long- and middle-wavelength-sensitive-axis (middle), and achromatic-axis stimuli (bottom). The latency measure is indicated by the horizontal lines.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933200/ on 04/02/2017)
FIGURE 2. Regression lines relating visually evoked potential (VEP) latency to blood glucose for the 10 subjects with diabetes. Solid lines indicate a statistically significant dependence of VEP latency on blood glucose (P < 0.05). Dashed lines indicate nonsignificant associations between VEP latency and blood glucose. Data are plotted separately for short-wavelength-sensitive-axis (left), long-and middle-wavelength-sensitive-axis (center), and achromatic-axis stimulation. In each case, the line spans the range of blood glucose levels exhibited by the subject.

The VEPs were recorded by conventional techniques with the active electrode 1.5 cm above the inion and ground and reference electrodes on the earlobes. Signals were sampled at 2000 Hz, amplified (×10⁴), and band-pass filtered (1 to 30 Hz) on an LKC System (LKC Technologies, Gaithersburg, MD). Responses were averaged over 30 cycles for each recording. The three stimulus axes were tested sequentially. The three-axis sequence usually was repeated three times at each measurement timepoint followed by a prolonged (10- to 20-minute) rest period. A blood glucose measure was taken in close temporal proximity (±5 minutes) to each set of VEPs. Blood glucose was interpolated linearly between successive mea-

<table>
<thead>
<tr>
<th>Identification Number</th>
<th>S Axis</th>
<th></th>
<th></th>
<th></th>
<th>LM Axis</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Achromatic Axis</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P &lt;</td>
<td>r</td>
<td>P &lt;</td>
<td>r</td>
<td>P &lt;</td>
<td>r</td>
<td>P &lt;</td>
<td>r</td>
<td>P &lt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.55</td>
<td>0.002*</td>
<td>0.16</td>
<td>0.40</td>
<td>0.04</td>
<td>0.84</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.45</td>
<td>0.01*</td>
<td>0.13</td>
<td>0.46</td>
<td>0.22</td>
<td>0.23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.004</td>
<td>0.98</td>
<td>0.008</td>
<td>0.95</td>
<td>0.03</td>
<td>0.84</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.19</td>
<td>0.08</td>
<td>0.19</td>
<td>0.10</td>
<td>-0.37</td>
<td>0.001*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.48</td>
<td>0.05*</td>
<td>0.049</td>
<td>0.84</td>
<td>0.08</td>
<td>0.76</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.74</td>
<td>0.01*</td>
<td>0.403</td>
<td>0.25</td>
<td>0.48</td>
<td>0.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.46</td>
<td>0.02*</td>
<td>0.25</td>
<td>0.22</td>
<td>0.03</td>
<td>0.87</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.08</td>
<td>0.77</td>
<td>0.32</td>
<td>0.20</td>
<td>-0.52</td>
<td>0.03*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.35</td>
<td>0.03*</td>
<td>0.56</td>
<td>0.01*</td>
<td>0.18</td>
<td>0.26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.02</td>
<td>0.92</td>
<td>0.26</td>
<td>0.14</td>
<td>-0.39</td>
<td>0.02*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Statistically significant.
FIGURE 3. Blood glucose (left panels) and visually evoked potential (VEP) latency for the short-wavelength-sensitive (S)-, long- and middle-wavelength-sensitive (LM)-, and achromatic-axes are plotted separately against time for three observers with diabetes (rows). Each subject illustrates a different pattern of blood glucose variation across time. VEP latency (data points) for the S-axis stimuli follows blood glucose variation. LM- and achromatic-axis VEPs do not. The solid lines in the VEP data panels are the averages of the three repeat measures for each axis at each measurement time. Blood glucose and latency scales differ among persons. The range of latencies differs among the axes for a given person (but the extent of the range is the same for all axes for a given person).

Latency is a more reliable measure than amplitude in terms of repeatability within subjects and shows less interindividual variation among normal subjects and is used in all analyses reported here.

Figure 1 shows characteristic responses for each axis. The responses to our chromatic onset-offset stimuli are characterized by a negative deflection occurring 120 to 220 msec after pattern onset. This negativity often is followed by a positive-going component that does not correspond to pattern offset. Latency is measured to the trough of the negative deflection. The latency of the response to S-axis stimuli typically is considerably longer than that to LM-axis stimuli. In contrast, the achromatic pattern-reversing stimulus produces VEPs that are characterized by a large positive deflection (sometimes preceded by a smaller negativity). Latency is measured to the peak of the positivity.

For each subject, for each of the three stimulus axes, the degree to which VEP latency was correlated linearly with blood glucose was determined. The resulting regression lines relating VEP latency to blood glucose for subjects with diabetes are plotted for the S, LM, and achromatic axes in Figure 2 from left to right. Refer to Table 2 for correlation coefficients and significance levels of the association between latency and blood glucose for each subject with diabetes for each axis. For most subjects with diabetes, regression lines for the S-axis have positive slope, indicating that longer VEP latencies are associated with higher blood glucose levels. In fact, 6 of 10 persons with diabetes showed statistically significant positive correlations (solid lines) between S-axis VEP latency and blood glucose. In contrast, there is no systematic association between LM-axis VEP latency and blood glucose; only 1 of 10 subjects showed a significant correlation (latency increasing with blood glucose). VEP la-
Blood glucose effects on chromatic VEPs

**FIGURE 4.** Visually evoked potential (VEP) latency as a function of blood glucose level for the three subjects with diabetes shown in Figure 2. VEP latencies for short-wavelength-sensitive-axis (left), long- and middle-wavelength-sensitive-axis (middle), and achromatic-axis (right) stimulation are plotted separately. Lines show best-fitting linear regression of VEP latency to blood glucose. Solid lines indicate significant associations, and dashed lines indicate nonsignificant associations between VEP latency and blood glucose. Latency scales differ among persons. The range of latencies differs among the axes for a given person (but the extent of the range is the same for all axes for a given person).

The association of S-axis VEP latency with blood glucose does not depend on the pattern of variation of blood glucose over time. Figure 3 shows results for three persons with diabetes, each of whom illustrates a different pattern of variation in blood glucose during the course of the VEP measurements. For subject 1 (top), blood glucose increased monotonically over the 2.5-hour test session. The blood glucose of subject 5 decreased over a similar time scale. For subject 7, blood glucose first rose and then fell over the approximately 5-hour measurement period. In all three cases, it is apparent that S-axis latency followed blood glucose variations, whereas the LM-axis and achromatic-axis VEP latency did not.

In Figure 4, VEP latency is plotted against
blood glucose (rather than time) for each stimulus axis for these same three subjects with diabetes. For all three subjects, S-cone VEP latency increases with blood glucose, whereas the LM and achromatic VEPs show no dependence on blood glucose. For subjects 1 and 7, the relation between S-axis VEP latency and blood glucose is roughly linear. For subject 5, VEP latency essentially is independent of blood glucose at levels above approximately 150 mg/dl; VEP latency is much shorter at lower blood glucose levels.

To summarize results in subjects with diabetes, a majority show an association between S-axis VEP latency and blood glucose; latency increases as blood glucose increases and decreases as blood glucose decreases over time. For a minority of subjects with diabetes, VEPs for achromatic stimuli show slightly shorter latency at higher blood glucose levels. No association with blood glucose is seen for LM-axis VEPs.

Figure 5 compares the results of subjects with diabetes and subjects without diabetes at high and low blood glucose. Each point represents the latency associated with the highest and lowest blood glucose level exhibited for each subject. The latencies at the blood glucose extremes were determined from the equations that describe the regression lines in Figure 1 for subjects with diabetes and for subjects without diabetes (not shown). Although, strictly speaking, it is inappropriate to use nonsignificant regressions to determine latency values, it is superior to using single measurements.* At low blood glucose, the mean S-axis VEP latency for this group of young healthy subjects with diabetes is close to that of normal subjects. Both groups show longer mean S-axis latencies at high rather than low blood glucose, although only the subjects with diabetes show a significant change with blood glucose (Mann–Whitney U test, $P = 0.04$). VEPs for stimulation along the LM-axis have similar latencies for subjects with diabetes and normal subjects at high and low blood glucose; LM-axis latency is not affected by blood glucose in most (9/10) subjects with diabetes nor in any normal persons. At low blood glucose, the VEP latencies for achromatic stimuli are similar for subjects with diabetes and normal subjects. As a group, normal subjects showed a nonstatistically significant increase in achromatic VEP latency with blood glucose. However, four of eight normal persons showed highly statistically significant increases in achromatic latency with blood glucose ($r = 0.65$, $P < 0.0001$; $r = 0.64$, $P < 0.001$; $r = 0.55$, $P < 0.0003$; $r = 0.53$, $P < 0.01$). Only two of eight showed a significant increase in S-axis latency with blood glucose ($r = 0.49$, $P < 0.0004$; $r = 0.63$, $P < 0.002$).

**DISCUSSION**

In a small sample of subjects with type 1 diabetes with normal vision and no retinopathy, we show a selective association between blood glucose and latency for VEPs in response to S-cone stimulation. S-axis VEP latency follows acute increases and decreases in blood glucose level. The increase in VEP latency in subjects
with diabetes for S-cone axis stimulation is the most consistent and largest blood glucose-associated VEP latency change found. The mean change in latency was 21 msec for the subjects with diabetes who showed significant correlations between S-axis VEP and blood glucose and 15.4 msec for all subjects with diabetes tested. Significant associations between S-axis VEP latency and blood glucose were observed in 5 of 10 subjects with diabetes. These data, obtained under neutral adaptation with an objective technique, are consistent with our earlier psychophysical findings of a correlation between thresholds for blue increments on bright yellow backgrounds and blood glucose; lower S-pathway sensitivity was associated with higher blood glucose levels. In those studies, thresholds for yellow spots on yellow backgrounds, detected by L- or M-cone-mediated pathways, showed no association with blood glucose in observers with diabetes. The current study also shows no decrement in LM- or achromatic pathway function with elevated blood glucose in subjects with diabetes. These results indicate that the S-pathway is more sensitive to acute variations in blood glucose and presumably is more vulnerable to insult from more long-term elevations in blood glucose.† The observed changes in S-pathway function that occur early in diabetes and precede changes in other more standard measures of visual function may be a consequence of this heightened vulnerability of the S-cone pathway to elevated blood glucose levels. As we and others have suggested,⁵⁻⁶ early vision changes in diabetes may be linked directly to metabolic changes of diabetes and may precede and be separate from visual disturbances that are caused by those vascular changes of diabetes that alter retinal structure.

The chromatic VEP and increment threshold

† It has been argued that the apparent heightened vulnerability of the S-cone system is attributable to differences between the S-cone system and other systems in terms of response properties (most notably, steeper threshold versus intensity functions), which make it easier to detect changes in S-cone system function. In fact, over the range of contrasts used here, the slope of the short-wavelength-sensitive-axis (S-axis) latency versus contrast (as percent of maximum) is slightly steeper (X1.27) than that of the long- and middle-wavelength-sensitive-axes (LM-axes),⁷ which, in turn, is steeper than that of the achromatic axis. An equal change in blood glucose-associated effective contrast for the three axes would, in that case, result in a larger change in S-axis latency. One might therefore expect that this would manifest the apparent association of latency and blood glucose for the S-axis stimuli. We do not believe that this underlies the observed selective S-axis latency increase with blood glucose in subjects with diabetes. The relative steepness of the achromatic- S-, and LM-axis latency versus contrast functions depends on the contrast metric used. In fact, when contrast is scaled in terms of cone activation, the LM-axis latency versus contrast function is 2.83 times steeper than that of the S-axis. Nonetheless, neither the subjects with diabetes nor the normal subjects show a dependence of LM-axis latency on blood glucose. In fact, although the achromatic-axis latency versus contrast function is the most shallow, it shows the most common and strongest association with blood glucose in normal subjects. Thus, slope is not the critical factor. Techniques are both sufficiently sensitive to detect glucose-associated S-pathway functional changes. Such associations have not been shown with the FM 100-hue test, a suprathreshold color discrimination test.²⁹

The selectivity of the effect of blood glucose for the S-pathway seen in subjects with diabetes is not present in normal subjects. In subjects without diabetes, VEP latencies for both achromatic and S-cone stimuli tend to increase with increasing blood glucose. Only VEPs for stimuli that modulate selectively the LM-chromatic pathway were invariant with blood glucose. In our earlier psychophysical studies, normal subjects’ thresholds for both yellow (L- or M-cone detected) and blue (S-cone detected) increments on yellow backgrounds increased during acute induced elevations in blood glucose. The findings of the two studies are consistent, if the reasonable assumption is made that the visual signals from the L or M cones detecting the yellow test increment are carried in a luminance, rather than chromatic, pathway.

Sannita et al³⁰ reported a small but significant change in achromatic VEP latency with normal variations in blood glucose in a group of subjects without diabetes; latency increased with increasing blood glucose. The current result for achromatic axis latencies of subjects without diabetes is of similar magnitude (6.3 msec in those showing a significant association, 3.7 msec for all subjects without diabetes).

Although there are no previous reports on the influence of blood glucose variation on chromatic VEPs in diabetes, others have explored the acute dependence of VEP latency on glucose levels in subjects with diabetes using luminance-varying pattern-reversing stimuli. Ziegler et al²⁸ were able to show a decrease in latency after a minimum of 3 days of improved glycemic control (i.e., continuously lowered glucose) in 7 (58%) of 12 subjects with poorly controlled diabetes. The mean latency change was 4.3 msec for the group. In a study designed to look at more acute effects, Martinelli et al³¹ fixed blood glucose at a euglycemic (120 mg/dl) level for 1 hour, after which blood glucose was elevated and fixed at a moderate level of hyperglycemia (250 mg/dl) for 1.5 hours. No change in VEP latency was observed after as much of 1.5 hours of elevated blood glucose in the 10-patient group. Our current study also shows little or no variation in achromatic latency but does show large (up to 51 msec) variations in S-axis VEP latency that follow variations in blood glucose over hours in subjects with diabetes.

The question of the mechanism by which blood glucose or related factors affect the change in S-cone pathway function remains unanswered. However, our results, showing a rapid shift in S-pathway function
with blood glucose changes, place constraints on possi-
ble mechanisms.

Explanations based on changing refractive error, which have been reported to accompany variations in blood glucose in subjects with diabetes,\textsuperscript{5–9} must be rejected. In our ancillary studies of the effect of diop-
tric blur on VEP latency using plus lenses, we found that unusually large refractive error changes would be required to produce changes in S-axis VEP latency of the magnitude exhibited by our subjects with diabetes. Furthermore, we found that LM-axis VEPs are as affected by blur as are S-axis VEPs, yet no blood glucose-associated change in LM-axis VEP latency was found in our studies. Both the rapidity of the change in S-axis function and its reversibility make yellowing of the crystalline lens an untenable explana-
tion for our results.

Transient hypoxia has been shown to produce a selective deficit in S-system-mediated color vision function.\textsuperscript{37} Elevated blood glucose produces a pseudohypoxia, evidence of which is the increase in retinal blood flow in response to elevations in blood glucose.\textsuperscript{38–40} It may be the case that the glu-
cose-induced pseudohypoxia that stimulates in-
creased blood flow also affects the S-pathway in a manner similar to that of true hypoxia and contri-
butes to the S-cone VEP latency increases observed here.

Three interrelated mechanisms by which tissue function is altered in diabetes as a direct result of persistent hyperglycemia have been described: the action of the polyl pathway, myoinositol depletion, and nonenzymatic protein glycosylation. High glucose levels leads to glycosylation of proteins, al-
tering their function. Long-term hyperglycemia re-
results in the formation of advanced glycated end-products, which cross-link with other proteins to produce permanent structural changes in dia-
betic tissue, such as cataract and vascular damage (retinopathy). A cross-sectional study reported an association between glycosylated hemoglobin and FM 100-hue score in diabetes.\textsuperscript{17} Thus, nonenzy-
matic protein glycosylation may play a role in the more stable, early S-pathway changes reported in diabetes, but the relatively long time course of these changes indicates that they cannot account for the rapid transient and reversible effects seen in our study.

Mechanisms by which elevated glucose can alter cell osmolarity and ion transport are more rele-
vant to the time scale of the effects observed in the current study. In tissues that do not require insulin for glucose transport across the cell membrane (e.g., cornea, lens, and retina of the eye), glucose enters the cells at a rate directly proportional to that of ambient glucose levels. Elevated glucose has been shown to impair the operation of sodium–potassium–adenosine triphosphatase,\textsuperscript{41} pro-
duce alterations in the Na\textsuperscript{+}–H\textsuperscript{+} exchange process and in pH,\textsuperscript{42} and increase intracellular sorbitol through the action of the polyl pathway.\textsuperscript{43} These three processes are highly interconnected and have been shown to alter myoinositol uptake and metab-
olism, leading to further decreases in sodium–po-
tassium–adenosine triphosphatase and thus changes in essential sodium, calcium, and potas-
sium-dependent cellular processes. Such processes are essential for the normal function of all cells, and their importance for the normal function of the photoreceptors, retinal pigment epithelium, and neural tissues of the visual system is readily apparent. These processes are sufficiently rapid to account for the changes in S-cone pathway function observed in the current study.

The S cones differ from L and M cones in a number of ways that may make them more sensitive to blood glucose variations. For example, the mor-
phology of S cones differs from that of M or L cones in ways that reduce their ability to exchange ions and make them more vulnerable to changes in their environment.\textsuperscript{44} In addition, S cones, like rods, lack carbonic anhydrase, found in M and L cones.\textsuperscript{44} Carbonic anhydrase is involved in regulation of intracellular fluid and pH and transport of carbon dioxide and ions, bicarbonate generation, and cal-
cium metabolism.\textsuperscript{44–46} The lack of carbonic anhy-
drase would result in a reduced ability of S cones to cope with metabolic insult.

The absence of carbonic anhydrase also may make S cones more prone to osmotic-induced changes in the configuration of the outer seg-
ments. The decreased light absorption of S-cone outer segments\textsuperscript{47} certainly is consistent with the loss of waveguide properties of cones that would accompany osmotic changes.

There are, thus, candidate mechanisms to ex-
plain rapid and selective S-pathway sensitivity loss due to increases in blood glucose in the cellular environment. These mechanisms also may contrib-
ute to the early S-pathway changes in diabetes that have been shown by a variety of techniques.26,18,46–50

In summary, we find that selective shifts in the function of the short-wavelength-sensitive pathway accompany transient fluctuations in blood glucose in observers with diabetes. These changes in S-pathway sensitivity, found using an objective technique, the VEP, under conditions of neutral adaptation, indicate that the acute effects of blood glucose truly are selective for the S-pathway in diabetes. The heightened sensitivity to ambient blood glucose levels may predispose the S-pathway to diabetes-induced damage, accounting for the early changes in S-pathway function found commonly in diabetes.

Key Words
blood glucose, diabetes, diabetic retinopathy, metabolic control, S cones

References


