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The fast oscillation of the electrooculogram reveals sensitivity of the human outer retina/retinal pigment epithelium to glucose level

Marilyn E. Schneck *, Brad Fortune, Anthony J. Adams

Visual Sciences Program, School of Optometry, University of California, Berkeley, CA 94720-2020, USA

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Abstract

The effect of acute blood glucose elevations on human outer retinal function was examined. Electrooculograms were recorded as the background light cycled on/off with a 2-min period, eliciting rapid changes in the corneo-retinal standing potential known as the fast-oscillation of the electrooculogram. Recordings were made while subjects fasted and after they consumed 100 g of D-glucose. In all subjects, blood glucose levels strongly affected fast oscillation amplitude, which reflects photoreceptor-driven changes in RPE cell chloride concentration. The sensitivity of RPE metabolism to glucose fluctuations may relate to changes in the blood-retinal barrier that are known to occur in diabetes (e.g. macular edema). © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Diabetes mellitus; Electrooculogram; Retinal pigment epithelium; Electroretinogram; Blood retinal barrier

1. Introduction

The retinal pigment epithelium (RPE), site of the outer blood-retinal-barrier (BRB), is critical for normal function of the photoreceptors and for control of fluid volume and ionic concentrations in the subretinal space. In diabetes, loss of integrity of the inner and outer BRBs, with subsequent development of macular edema, is the major cause of visual impairment. Failure of the RPE to effectively move the fluid out of the retina may contribute to the persistence and progression of macular edema.

Although the full manifestations of BRB breakdown (e.g. macular edema) are usually associated with advanced retinopathy, deterioration of the BRB actually begins at a very early stage. In fact, studies employing vitreous fluorophotometry (VFP) have shown it to be one of the earliest ocular functional lesions that can be detected in human and experimental diabetes, occurring prior to the development of ophthalmoscopic or angiographic signs of retinopathy. (Cunha-Vaz, Faria de Abreu, Campos, & Figo, 1975; Waltman et al., 1978; Schalnus & Ohrolff, 1995; Benitez del Castillo, Castillo, Fernandez, & Garcia-Sanchez, 1993). Abnormally elevated VFP values have also been shown to correlate with the degree of diabetic retinopathy, predict further breakdown of the BRB, predict the development and progression of retinopathy, as well as herald the need for future laser-photocoagulation therapy (Castillo et al., 1996; Krupin & Waltman, 1985; Benitez del Castillo et al., 1993; Cunha-Vaz, Leite, Sousa, & Faria de Abreu, 1993; Cunha-Vaz et al., 1998; Engler, Krogsaa, & Lund-Andersen, 1991). Increased VFP values are associated with increased duration of diabetes and elevated glycated hemoglobin values (a measure of poor long-term control of blood glucose), suggesting that this functional abnormality is directly related to chronic hyperglycemia (Castillo et al., 1996). Reversal of abnormal VFP results can be achieved with improvement of blood glucose control (White, Waltman, Krupin, & Santiago, 1982; Bleicher et al., 1980), providing additional evidence that early breakdown of the BRB is directly related to hyperglycemia.

Several studies of experimental diabetes have demonstrated that structural and functional alterations of the RPE precede changes in the retinal vasculature (inner-BRB) (Kirber, Nichols, Grimes, Winegrad, & Laties,

* Corresponding author. Tel.: +1-510-6425904; fax: +1-510-6435109.

E-mail address: mes@spectacle.berkeley.edu (M.E. Schneck).

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1980; Krupin et al., 1982; Tso, Cunha-Vaz, Shih, & Jones, 1980; Vinores, Gadegbeku, Campochiaro, & Green, 1989; Klein, Engerman, & Ernest, 1980). Structural signs of RPE damage include: thickening of the basal laminae of the RPE and Bruch's membrane, marked dilation and redundancy of RPE basal membrane infoldings, formation of large vacuoles within RPE cells, RPE cell swelling, and focal necrosis (Vinores et al., 1989; Grimes, McGlinn, Laties, & Naji, 1984; Grimes & Laties, 1980; Tso et al., 1980). Early stage abnormalities of RPE barrier function have been demonstrated using tracer elements, such as FL and horseradish peroxidase (HRP), which appear within and pass through RPE cells (i.e. into the sub-retinal space) of diabetic animals, but not in non-diabetic animals (Kirber et al., 1980, Tso et al., 1980).

In summary, animal studies show altered structure and function of RPE membranes early in experimental diabetes. Human clinical studies show the manifestations of this early BRB breakdown as FL accumulation. Additional evidence indicates that accumulation of FL within the human diabetic vitreous reflects dysfunction of active outward transport mechanisms for fluid and organic anions, such as chloride and sodium fluorescein, (e.g. Engler, Sander, Larsen, Dalgaard, & Lund-Andersen, 1994a; Engler et al., 1994b; Tsuboi, 1990). Active transport of fluid across the RPE in the retina to choroid direction is primarily driven by the RPE chloride ion transport pathway, which consists of an apical (facing the photoreceptors) membrane Na–K–2Cl cotransporter and a basolateral (facing the choroidal blood supply) membrane Cl^- channel (reviewed by Gallemore, Hughes, & Miller, 1997).

Changes in retinal illumination alter the activity of the RPE chloride ion transport pathway. The molecular events and RPE membrane voltage responses that occur in response to light onset (and offset) can be measured non-invasively in the human eye using the electro-oculogram (EOG, Arden & Kelsey, 1962; Kolder & Brecher, 1966) which reflects changes in the standing potential (or corneo-retinal potential) of the eye. During the first minute of light onset, the standing potential rapidly decreases and then begins a slow steady increase lasting 8–12 min. These light-induced changes in the standing potential are known as the 'fast oscillation' and 'slow oscillation', respectively. Available evidence indicates that the light 'trough' of the EOG fast oscillation (FEOG) is generated by a light-evoked decrease in chloride ion (Cl^-) concentration within RPE cells with subsequent hyperpolarization of the basolateral membrane (Bialek, Joseph, & Miller, 1995; Gallemore & Steinberg, 1993).

Given the mounting evidence that abnormalities of RPE metabolism may be an early ocular manifestation of diabetes, as well as the important role that the RPE chloride ion transport pathway has in moving fluid out of the retina, we chose to use the FEOG technique to evaluate changes in RPE function during acute metabolic challenges common to the diabetic state. Here we examine the sensitivity of the normal human outer retina and retinal pigment epithelium to alterations in blood glucose levels, using the fast-oscillation of the clinical electro-oculogram. Our results show that in normal humans, the FEOG is very sensitive to changes in blood glucose, showing large amplitude increases with increasing blood glucose.

2. Methods

2.1. Subjects

Nine healthy individuals (age 22–42) with no history of eye disease or systemic disease known to affect vision were tested.

All subjects gave written informed consent after the experimental procedures were described to them. All subjects were tested in accordance with the tenets of the Declaration of Helsinki and with approval of the institutional human experimentation review board.

2.2. Electro-oculogram recording

Pupils were dilated using Phenylephrine 2.5% and/or Tropicamide 1.0% following corneal anesthesia (Proparacaine 0.5%). The skin was scrubbed in preparation for electrode attachment. Silver/silver chloride electrodes filled with conductive gel were attached to the skin with tape rings at the lateral and medial canthi of each eye, with forehead as ground.

EOG's were recorded using an LKC electrodiagnostic system (Gaithersgurg, MD). The subject was seated with his/her head positioned on a chin rest at the opening of a white Ganzfeld dome. Room lights were off during testing. For EOG recordings, two red LED's at the rear of the dome, separated horizontally by 30°, were alternately lit for 2 s (4 s per left/right-right/left saccade pair) to elicit regular fixed amplitude saccadic eye movements (see Fig. 1A). Three hundred milliseconds after each saccade prompt (to allow time for completion of the saccade), the voltage signal was averaged from ten 30-ms epochs (total sample time/saccade 300 ms). The voltage difference between left-rotated and right-rotated eye positions (e.g. points labeled b and a in Fig. 1A) were taken as the amplitude of the standing potential at that time. The Ganzfeld dome light (30 cd/m^2) was cycled off/on at 1 min intervals for 10 min (five light/dark cycles per EOG recording) to permit recording of the FEOG.

EOG data were analyzed using standard LKC system software. Following smoothing and spike removal, a sine wave (peaks occurring in dark, troughs in light

periods) was fit to the five-cycles of raw data (Fig. 1B). Three parameters of the sine wave are computed by the software: amplitude, peak/trough ratio, and phase. Amplitude was considered in analyses reported here, because it is conceptually straightforward. The general results and conclusions would have been the same if peak to trough ratio were used instead. The amplitude data from the two eyes of each subject were averaged, as both eyes produced very similar results.

2.3. Protocol

Upon arrival, subjects were prepared for recordings, given instructions, and practiced making eye movements of appropriate timing/amplitude in response to the LED's.

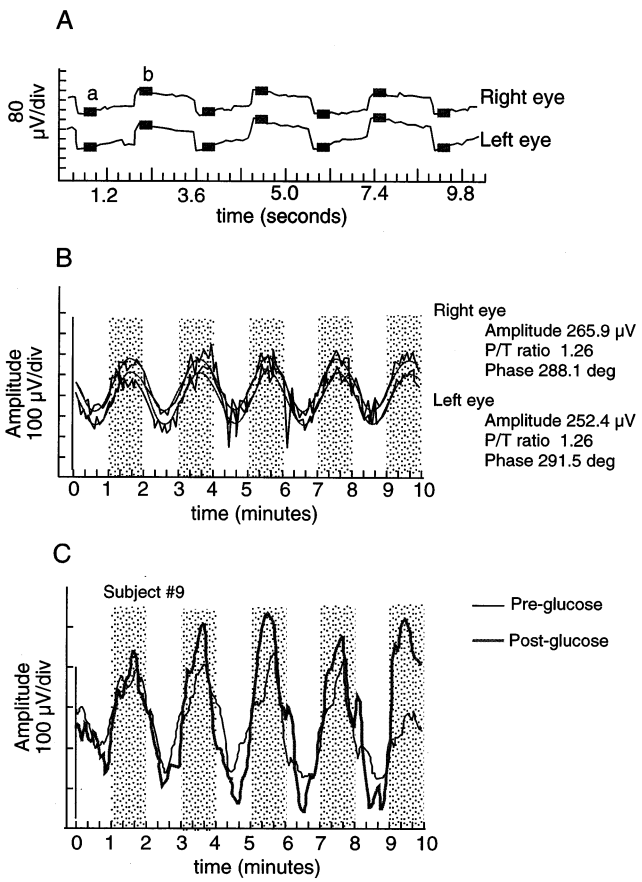

Fig. 1. Recording the FEOG. (A) Eye movement traces (voltages across time) for the right and left eyes of a subject who is tracking the alternately illuminating LED's. The gray rectangles indicate the 300 ms during which voltages are averaged following each saccade. The voltages after each saccade are then differenced (e.g. b–a) to continuously derive the amplitude of the standing potential as shown in panel B. (B) Result from each eye of a single FEOG measurement. Dark traces are the derived FEOG data, showing oscillations of the standing potential in response to cyclical light/dark stimulation (white and stippled vertical bars). Values of the FEOG amplitude are computed from the best fitting sine wave for each data set (smooth gray traces). (C) Averaged pre-glucose (thin, black) and post-glucose (thick, gray) FEOG's for subject #9. Note vertical scale difference in panels B versus C. Best fitting sine waves not shown in panel C for clarity.

Blood glucose was measured at the start and end of the test session as well as between 10-min recordings. For each measure, a blood drop was obtained by finger stick, and glucose level was determined using a calibrated One-Touch glucose meter (LifeScan, Johnson&Johnson).

Subjects were tested in the morning following overnight fast. Thus, subjects initially had fairly low blood glucose levels, and after two or three 'baseline' fasting BG and FEOG's were recorded, the subjects consumed a solution containing 100 g of D-glucose to elevate BG. Subjects were required to drink the entire glucose tolerance test solution within 5 min. BG and FEOG recordings were then recorded alternately as BG rose, and then, in most cases, fell (after ~ 30 min) as a result of the normal insulin response. Experimental sessions lasted 1.5–2 h, including frequent breaks.

3. Results

Fig. 2, which shows results for three subjects, illustrates the general pattern of results for all nine subjects. For each subject, blood glucose (open symbols, dotted lines, right ordinates) and FEOG amplitude (solid symbols and line, left ordinate) are plotted against time into the test session. To the extent that the two curves in each panel follow a similar pattern with similar time course, an association between BG level and FEOG amplitude is indicated. As expected, after subjects drank the glucose solution (arrows), blood glucose began to rise; the rise in blood glucose was accompanied by an increase in FEOG amplitude for all subjects. In all but one case (Panel C), testing was continued for a sufficiently long period so that blood glucose leveled off or declined during the test session as a result of the individual's normal insulin response. FEOG amplitude either leveled off (e.g. Panel A), or, more often, transiently fell markedly (e.g. Panel B) when the increase in blood glucose was curtailed (by endogenous insulin).

Note for the subjects whose data are shown in panels A and B that while the excursion of FEOG amplitude is similar (each spanning 100 μV) the absolute values are different. Also note that the FEOG amplitudes of the subject in panel C span a range three times that of the others, illustrating the inter-subject variation in the magnitude of the glucose-associated change in FEOG amplitude. The range of blood glucose values is similar among these three subjects, though there is somewhat less blood glucose variation across time for the subject in panel B.

The time course of BG change in normal subjects is also quite consistent; BG begins to rise within just a few minutes after glucose ingestion and continues to do so for about 30 min. This pattern makes possible a simplification of the quantitative analysis of the relation

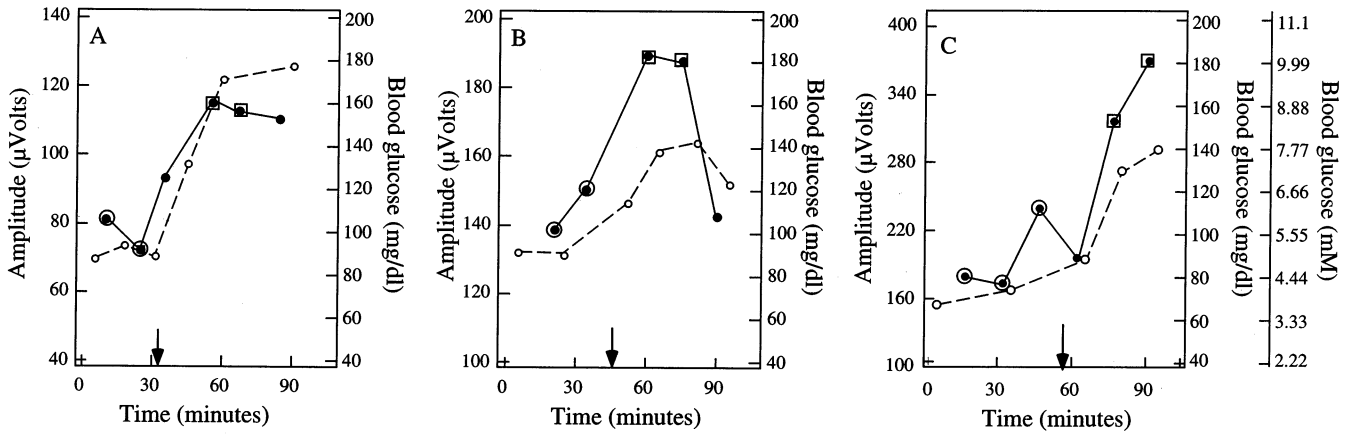

Fig. 2. Variation in FEOG amplitude (solid symbols, line; left ordinates) and blood glucose (open symbol, broken lines; right ordinates) across time in three non-diabetic subjects (Panel A; Subject #4; Panel B, Subject # 5; Panel C, Subject #9). Note: Glucose scales are the same for all subjects (range 40–200 mg/dl). The blood glucose values are also in mM on the panel at the right; (1 mM = 18.018 mg/dl). FEOG amplitude scales vary in range among subjects; scales in panels A and B span 100 μV; panel C spans 300 μV. The relative vertical position of the two sets of data within each panel is arbitrary. Arrows on the ordinate indicate the time at which glucose solution was administered (over a 5 min period).

between BG and FEOG amplitude. For each subject, the mean of the amplitudes of the two to three FEOG's recorded prior to glucose ingestion is used to estimate 'pre-glucose' FEOG amplitude. The estimate of the 'post glucose' FEOG was obtained by averaging all FEOG's obtained during the period starting 10 min after the initiation of the ingestion of the glucose solution and up to 30 min after glucose ingestion. FEOG's obtained within 5 min of a measured leveling off or decline in blood glucose were excluded. The circled and boxed points in Fig. 2 indicate the FEOG amplitudes included in the pre-glucose and post-glucose measures, respectively, for each of these three subjects. Fig. 1C directly compares the averaged pre-glucose FEOG's and post-glucose FEOG's for subject #9, whose data are presented on the right in Fig. 2. The larger amplitude of the averaged post-glucose (thick gray trace) response is readily apparent.

The ratio of post- and pre-glucose FEOG amplitude was calculated for each individual. The ratio simplifies comparisons by eliminating large inter-individual differences in absolute FEOG amplitude (attributable to differences in electrode placement relative to the posterior pole, etc.). Fig. 3 shows the post/pre FEOG amplitude ratios for the nine subjects. All fall above the line of equal amplitude, consistent with all FEOG's being of larger amplitude during the period during which blood glucose was rising. The ratios range from 1.10 to 1.65, representing 10–65% increases in amplitude (median 25.4%, mean 28.7%).

Two subjects (# 8 and 9, who showed average and largest glucose-associated FEOG excursions, respectively) were tested in second sessions to measure the variation of the FEOG amplitude over time in the absence of glucose variation. For these measures water was consumed rather than the glucose solution. Average pre-(water) and post-(water) amplitudes were computed, and from them the percent change for each person. The average percentage amplitude change over the length of a test session in the absence of glucose was 12.3%. A second estimate of non-glucose related variability was obtained by calculating the 95th percentile of all amplitude changes observed between baseline measurements. The percentage amplitude difference between the first and second baseline FEOG (i.e. absolute value of the difference divided by the first measure) was calculated for each subject. One subject (#9) had three baseline measures; for her the differencing was done in

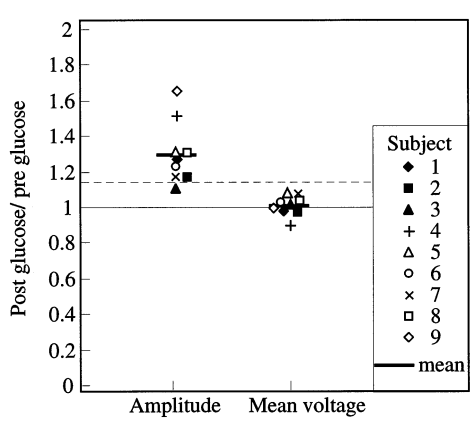

Fig. 3. Ratio of FEOG amplitude (left) and mean voltage (right) post-glucose to pre-glucose for each of the nine subjects. Each symbol represents a subject. Solid line represents no difference post- versus pre-glucose. Dashed line represents the estimated amount of non-glucose-associated FEOG amplitude variation expected to occur during a test session. Solid horizontal line at unity represents no difference between pre and post glucose.

a pairwise fashion. The 95th percentile of the changes for all observers was 12%. Based on our two estimates, a conservative value of 15% was chosen (dashed line Fig. 3) as our estimate of non-glucose associated variation in FEOG amplitude over time. The amplitude increases of all but one subject were larger than this.[1]

What is the basis of the FEOG amplitude increase? Three possibilities are apparent: an increase in peak voltage during the dark phase, a decrease in the 'trough' voltage during the light phase, or a change in both the light trough and the dark peak. To examine this, the mean voltage measured during each 10-min FEOG recording was calculated for each subject. The means of the included pre-glucose and post-glucose recordings were then computed for each subject. The data set on the right in Fig. 2 plots the ratio of post- to pre-glucose mean voltage for each individual. In contrast to FEOG amplitude (left), which increased substantially and systematically following glucose ingestion, mean FEOG voltage did not show systematic variation across blood glucose levels. A few observers had slightly higher mean voltages post glucose, most showed no change, and a few showed lower voltages than at pre-glucose. Further comparisons show no systematic association between mean voltage and FEOG amplitude or in terms of relative magnitude of the glucose-associated effect. Thus, the association between FEOG amplitude and glucose level is independent of mean voltage. Further evidence for this independence is that the findings are unchanged if FEOG amplitude is 'corrected for' (divided by) mean voltage for each individual both pre and post-glucose. For this derived ratio measure the mean difference between pre- and post glucose is 27.6%, very close to the 'raw' FEOG values. These results suggest that the amplitude increase observed most likely reflects a nearly symmetrical increase in the dark peak height and the light trough depth. That this is the case can be seen in the example in Panel 1C, which shows the averaged pre-glucose and averaged post-glucose FEOG's of subject #9. The curves have not been vertically shifted with respect to one another. The large and nearly-symmetrical increase in dark peak and decrease in light trough voltages, with no underlying change in mean voltage, is apparent.

[1] Similar systematic changes with blood glucose were found for the peak to trough voltage ratio, another ERG parameter used by investigators (e.g. Nakao et al., 1995; Weleber, 1989). All subjects showed larger peak to trough ratios post-glucose, with changes ranging from 0.01 to 0.16. The mean peak to trough ratio pre-glucose for all subjects was 1.20 (S.D. 0.03, range 1.15–1.32), while the post glucose mean was 1.25 (S.D. .04, range 1.19–1.36). The apparently small size of these changes is misleading, as they reflect large absolute voltage changes. The peak to trough ratios are very similar to those reported in normal subjects by Weleber (1989).

4. Discussion

Existing evidence indicates that the fast-oscillation of the electro-oculogram (FEOG) is generated by light-evoked changes in RPE chloride concentration, which, in turn, produce a decrease in basal membrane Cl^- conductance and concomitant basolateral membrane hyperpolarization (Bialek et al., 1995; Gallemore & Steinberg, 1993). Here we have shown that large increases in the amplitude of the FEOG accompany increases in blood glucose in normals. FEOG changes induced by acutely elevated glucose may thus reflect alterations in RPE function and, more specifically, ion transport processes related to fluid transport in the retina-to-choroid direction.

The glucose associated change in the FEOG appears to be symmetrical, with the light trough and dark peak both increasing to produce the observed amplitude increase. The data further suggest that the mean recorded voltage, and thus the standing potential of the eye, did not change following glucose ingestion. Rather, only the relatively fast processes that are modulated by light and dark are altered. This suggests that the FEOG amplitude changes do not reflect the growth of an underlying 'EOG light-peak'. It also indicates that the variation in FEOG amplitude is not attributable to glucose-associated variation in extraneous factors, such as skin resistance, volume conductance through extraocular tissues, or other factors that may affect electrode impedance and simply amplify the voltage signal. It is also unlikely that these changes are secondary to osmotic effects related to increased glucose, as they are of opposite polarity (Macaluso, Onoe, & Niemeyer, 1992; Kawasaki, Yonemura, & Madachi-Yamamoto, 1984).

The events which lead to generation of the FEOG begin with photoreceptor light and dark responses with subsequent changes of potassium concentration in the sub-retinal space. Rod responses are likely to make the major contribution to this initial event (Weleber 1989; Rabin, 1991). To our knowledge, a cone response contribution to initiation of the fast oscillation has not been established. However, we find no change in FEOG amplitude with large increases (1.3 log units) in background light level within the photopic range, consistent with absence of cone involvement (data not shown). As part of a separate study, we have used the ERG to measure the sensitivity of retina to increased blood glucose concentration (Schneck, Fortune, & Adams, 1999). We found that dark adapted rod responses (a-waves) are affected little, if at all, by glucose changes like those induced here. Thus, the glucose effect is likely to be further downstream, within the RPE, consistent with the results of Macaluso et al. (1992) in vitro. They showed, in recordings in isolated perfused cat eyes showed that the fast P-III (and hence the rod photore-

ceptors) are insensitive to changes in glucose concentration and showed further evidence that the measured glucose-associated changes in light-evoked potentials originating in outer retina are attributable to alterations in the RPE. Assessing the glucose effects on the c-wave (as Macaluso et al. were able to do in cat eyes) would further localize the glucose-dependence; however, c-wave recordings in alert humans were unreliable.

The effects of BG on the human FEOG observed here, although rapid and reversible, may occur by the same mechanisms as the more permanent retinal and vision changes that are well known to result from chronic (and/or chronic acute) hyperglycemia, such as those related to development of BRB dysfunction, diabetic retinopathy and macular edema. Numerous experimental diabetes studies have shown that changes in RPE metabolism, such as reduction of Na/K ATP-ase activity and protein synthesis, are an early consequence of prolonged hyperglycemia (e.g. MacGregor & Matschinsky, 1986a,b; Dircks, Williams, & Campochiaro, 1987).The association between glucose and retinal/RPE function demonstrated here, using simple, non-invasive techniques, highlights the value of studies of acute effects of blood glucose on retinal function for understanding the more devastating retinal consequences of diabetes.

In summary, in normal human subjects, abrupt elevation of BG affects the amplitude of the FEOG, a non-invasive (electrophysiologic) measure of outer-retinal/RPE function. Alterations in the FEOG may reflect alterations in RPE transport mechanisms and/or metabolism that could lead to a chronic reduction in net fluid transport across the RPE. Such alterations may be contribute to the development and/or persistence of macular edema, a major cause of sight loss in diabetes. The question remains whether diabetics and non-diabetics differ in terms of their responses to this simple metabolic challenge.

Acknowledgements

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