Ocular Glucose Extraction Using Vitreoperfusion in the Cat

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Quantitative methods to measure ocular glucose consumption in vivo, which is dominated by the retina, could provide considerable information about retinal metabolism in healthy and disease states. Eyes that are totally ischemic for at least 4 hr retain good retinal histopathologic features if treated with vitreoperfusion. In such cases, the vitreoperfusion fluid essentially is the only extraretinal source of glucose. We developed a mathematical model to estimate the rate at which glucose is extracted from the vitreoperfusion solution by metabolism ($E_{\text{glu}}$), compensating for losses via the outflow pathways. With a glass model eye ($E_{\text{glu}} = 0$) with a known, simulated outflow facility, the measured decline in glucose closely approximated the decline predicted by the equation ($r = 0.97$). In six formaldehyde-glutaraldehyde fixed cat eyes ($E_{\text{glu}} = 0$), the measured vitreous glucose concentrations at various times correlated with the calculated values ($r = 0.96$). With the use of general anesthesia, lensectomy and vitrectomy were performed in 10 cat eyes. Vitreoperfusion was initiated, and the cats were killed to establish total ocular ischemia. The mean ± SD $E_{\text{glu}}$ values for intervals between 15 and 75, 105 and 165, and 195 and 255 min after ischemia were 8.0 ± 6.9, 14.4 ± 10.4, and 19.9 ± 11.0 µg/min, respectively ($P < .05$). We conclude that the eyes retained their ability to extract glucose for at least 4 hr but that $E_{\text{glu}}$ values increased during this period for undetermined reasons. In the future, measurements of $E_{\text{glu}}$ may become useful in the evaluation of physiologic and pathologic states of the eye. Invest Ophthalmol Vis Sci 33:2791-2797, 1992

A variety of pathologic states of the retina, such as ischemia, diabetes, and dystrophies, can lead to visual loss. Energy metabolism undoubtedly becomes disturbed in many of these conditions. Therefore, knowledge of retinal energy metabolism is needed to better understand the mechanism of retinal injury in these pathologic states.

Because the primary fuel of energy metabolism in the retina is glucose,1-3 information about glucose use by the retina represents an important component of this knowledge. In vitro studies of glucose metabolism in the retina have involved a variety of mammalian species.1,2,4-8 Glucose metabolism in pigs has been investigated in vivo by determining arteriovenous glucose concentration differences and retinal and choroidal blood flow rates.9 However, these methods require cannulation of ocular blood vessels and are restricted to species that have an unusual vascular anatomy. In vivo10-12 and in vitro12,13 studies of labeled 2-deoxyglucose uptake in the retina also have been performed. This method necessitates removal of the eye to analyze the accumulated 2-deoxyglucose-6-phosphate. The 2-deoxyglucose method was originally developed to evaluate brain metabolism. Consequently, only semiquantitative results for the retina have been obtained, because assumptions and constants were used that apply to the brain.

We propose a new method for evaluating the ocular energy metabolism quantitatively by estimating the ability to extract glucose for metabolism. Although the method is invasive, it has several advantages: it can be repeated, it does not depend on an unusual vascular anatomy, and, in principle, it could be applied in certain clinical situations.

Materials and Methods

Theoretical Considerations

Vitreoperfusion refers to perfusion of the vitrectomized vitreous cavity to supply, study, measure, or manipulate ocular metabolism.14,15 We showed previ-
ously that vitreoperfusion can substantially prevent histopathologic damage when performed during total ocular ischemia for up to 4 hr. Furthermore, vitreoperfusion can limit ischemic retinal injury when initiated at certain intervals after the onset of ischemia. Without ocular blood flow, the vitreoperfusion fluid essentially is the only source of glucose for ocular metabolism, as endogenous glucose and glycogen stores are limited. Accordingly, the extraction rate of glucose from the vitreoperfusion fluid ($E_{glu}$ in micrograms per minute), should be closely related to the rate of ocular glucose use. Because ocular energy metabolism is dominated by the retina, $E_{glu}$ would correlate closely with glucose use by the retina.

The experiments described in the present report were conducted with a recirculating vitreoperfusion system that oxygenated Ames’ solution but did not replace any glucose that was lost (Fig. 1). Under these conditions, we used the following mathematical model of glucose concentration in the vitreoperfusion fluid as a function of time to estimate $E_{glu}$ (see derivation in the Appendix):

$$C(t) = -\frac{E_{glu}}{F} + \left[C(0) + \frac{E_{glu}}{F}\right]e^{-t/V}$$

(1)

where $C(t)$ indicates glucose concentration (in milligrams per milliliter or micrograms per microliter, which are identical) in the vitreoperfusion fluid within the system (we refer to the combination of the apparatus and the intraocular cavity after vitrectomy-lensectomy as the “system”) at time t (in minutes); $C(0)$ indicates $C(t)$ when t was equal to 0; V indicates volume (in microliters) of the vitreoperfusion fluid in the system; and F indicates rate of fluid loss (in microliters per minute) from the system.

Theoretically, F should be entirely related to the normal processes of intraocular fluid loss, because we withdrew no fluid from the system during the measurements. Rather, we sampled the fluid only at the beginning and end of a time interval. In this model, glucose is lost by metabolism or along with fluid as it escapes from the system as F. To keep V constant, we monitored F and replaced this fluid loss with glucose-free Ames’ solution. During vitreoperfusion, flow of fluid occurred within the system that must not be confused with F. Whereas F was the rate at which fluid entered and exited the system, the fluid in the system was recirculated at a rate substantially higher than F to permit reoxygenation of the fluid and to stir the vitreous. Stirring was necessary to ensure that oxygen entering the pars plana was delivered adequately to the posterior retina. Even if F were equal to 0, the recirculation flow still would have been present as long as the vitreoperfusion system was in operation. The recirculation flow rate was approximately 4 ml/min. Glucose concentrations were measured with a glucose analyzer (Glucose Analyzer 2, Model 6517; Beckman Instruments, Fullerton, CA). Multiple measurements of glucose in the same sample gave a standard deviation that was less than 1.5% of the mean. After F, V, $C(0)$, $C(t)$, and t were measured, $E_{glu}$ was calculated with a computer by numerical methods with a nonlinear curve-fitting program from a commercially available statistical package (Systat Inc., Evanston, IL).

**Validation of the Model**

**Glass model eye:** The mathematical model was tested by using a glass model eye in which $E_{glu}$ was equal to 0. We set F, V, and t to equal 80 μl/min, 13,500 μl, and 60 min, respectively. These values were similar to those in preliminary in vivo studies involving cats at normal intraocular pressure. We used linear regression analyses to compare the measured glucose values to those calculated from the theory at specific time points during vitreoperfusion.

**Fixed cat eyes:** The mathematical model was retested in six cat eyes that had undergone lensectomy and vitrectomy and were removed and fixed with formaldehyde-glutaraldehyde. In this case, $E_{glu}$ was equal to 0, but F depended on the eye. F, V, and t were measured. We used linear regression to compare the measured values of glucose to those predicted by the theory at multiple time points (10, 10, 40, 40, 45, and 60 min) after initiating the experiment.
Reproducibility of $E_{glu}$ in the Glass Model Eye

$E_{glu}$ was determined 10 times in the model eye ($E_{glu} = 0$) with data acquired during a 60 min period. We set $F$ at 40 $\mu$L/min and $V$ at 13,900 $\mu$L, based on additional results obtained from experiments involving cat eyes. Samples were obtained at 0 and 60 min, and the glucose concentrations were measured.

Determination of $E_{glu}$ in Living Cat Eyes

Animal procedures: Animal procedures were conducted according to the guidelines of the ARVO Resolution on the Use of Animals in Research. The experiments were performed in normal laboratory illumination, with general anesthesia administered intravenously as a mixture of guaifenesin, ketamine hydrochloride, and xylazine hydrochloride. Unilateral lensectomy and vitrectomy were performed in 10 cats after pupilary dilation with 1% atropine sulfate and 10% phenylephrine. Two cannulas were placed in the pars plana of each eye for inflow to and outflow from the vitreous cavity by the vitreoperfusion system. Vitreoperfusion was performed for 270 min with Ames’ solution bubbled with 95% oxygen and 5% carbon dioxide. The intraocular pressure was monitored and maintained at a constant level (approximately 20 mmHg). Eyes with obvious leaks, usually at the cannula sites, were excluded. Accordingly, it appeared that $F$ was primarily related to fluid loss via the conventional and unconventional aqueous outflow pathways. Fifteen minutes after the onset of vitreoperfusion, the animals were killed by air embolism to induce profound ischemia.

Measurement of parameters: The volume of fluid in the system was evaluated by inspecting the height of the fluid level in a small reservoir in the vitreoperfusion apparatus. $F$ was determined by the rate at which glucose-free Ames’ solution had to be infused into the system to maintain a constant height. A syringe pump with a calibrated syringe was used to add frequent, small amounts of solution based on continuous monitoring.

$V$ equaled the volume of the vitreoperfusion apparatus plus the volume of the intraocular cavity after lensectomy and vitrectomy. The volume of the vitreoperfusion apparatus was determined before the experiment by measuring the volume of fluid necessary to fill it. The measurement of ocular volume was performed in the following manner. First, fluid was evacuated from the eye and replaced with air. Second, measured amounts of fluid were infused until all the air was replaced with fluid and the intraocular pressure equaled that during the glucose measurement. The ocular volume measurements were performed after the completion of vitreoperfusion.

Measurement Protocols: Protocol 1. In five animals, $E_{glu}$ was determined by measuring the initial and final vitreoperfusion fluid glucose concentrations at the beginning and end of a 60 min period. Ten glucose measurements were obtained for each sample of fluid to provide a precise determination of its value. $E_{glu}$ was measured three times in each eye. The system was irrigated again with new Ames’ solution between successive trials for 30 min periods to restore the original glucose concentration. The glucose concentration of the irrigation solution was approximately 1.11 mg/ml (111 mg/dl). The three trials were conducted at 15–75, 105–165, and 195–255 min after the onset of ischemia. These periods are referred to as intervals A, B, and C, respectively. The glucose concentration at the beginning of each measurement interval will be referred to as C(0) and that at the end as C(60). Thus, the start of the measurement period, rather than the start of retinal ischemia, will be used as the reference point.

Protocol 2. In protocol 1, we found that $E_{glu}$ increased steadily over the three time intervals. We wanted to determine whether any segment of the hour interval accounted disproportionately for this increase. Therefore, in another five animals, $E_{glu}$ was determined by sampling the vitreoperfusion fluid for its glucose concentration every 15 min, starting 15 min after the onset of ischemia. Immediately after sampling, the volume of the sample was replaced with an equal volume of new Ames’ solution, the glucose concentration of which was equal to that of the original solution for that day. We accounted for the new mass of glucose introduced into the system after each sample in our calculation of $E_{glu}$. By sampling at 15 min intervals in protocol 2, we were able to select sequences of four intervals whose beginning and end temporally coincided with the 60 min sampling intervals in protocol 1. Intervals 1–4, 7–10, and 13–16 of protocol 2 corresponded with intervals A, B, and C, respectively, in protocol 1. Because the values of $E_{glu}$ over corresponding 60 min intervals were comparable between the two protocols, we combined the results for intervals A, B, and C. Student’s t-test and linear regression were used to analyze the results.

Metabolic contribution to glucose loss: During the measurement, some glucose was lost from the system because of metabolism, and additional glucose was lost because of $F$. We used a computer model of our operational equation to calculate the percentage of total glucose lost due to metabolism. We used the average values for intervals A and C.

A certain amount of glucose was in the system at the beginning of a measurement interval. Similar methods were used to calculate the percentage of the total glucose at the beginning that was metabolized.
Again, we used the average values for intervals A and C.

Retinal Weight

$E_{\text{glu}}$ provides information about glucose extraction per eye. To compare the metabolic rates of eyes of various sizes (for example, between species), $E_{\text{glu}}$ values can be divided by the weight of tissue. This gives units of mass consumed per unit of time per mass of tissue. Assuming the retina is the major metabolizing structure of the eye, we determined the weight of the cat retina to permit rough comparisons to values previously reported in the literature. The retinas of eight cats used in unrelated experiments were isolated by careful dissection to separate them from the vitreous and pigment epithelium. They were touched gently with filter paper to remove excess fluid, weighed, dried in an oven at 57°C for 24 hr, and weighed again. In four cats, one retina was considered unsuitable for inclusion because of damage during the experiment. When both retinas were suitable, we used the average of the two as the result for that cat.

Results

Validation of the Model

Glass model eye: The empirical glucose values at specific times closely approximated those predicted by the mathematical model ($r = 0.97$).

Fixed cat eyes: Figure 2 presents the predicted and measured glucose values in fixed cat eyes at specific times. The correlation between these values was highly significant ($r = 0.96$). The deviation of the experimental values from the theoretical values did not depend on time. This supports the validity of the model.

Reproducibility of $E_{\text{glu}}$ in the Model Eye

The mean ± SD $E_{\text{glu}}$ in the glass model eye, when determined 10 times, was 2.8 ± 3.7 μg/min.

Glucose Extraction in Living Cat Eyes

$F$ was 32.5 ± 20.0 μl/min and was essentially constant over time. $V$ was 13,000 ± 500 μl, and $C(0)$ was constant in protocol 1 (Table 1). In contrast, $C(0)$ varied in protocol 2 because samples were obtained every 15 min throughout the experiment. Immediately after withdrawal of a sample, we injected fluid equal in volume to the sample. The fluid contained the concentration of glucose present at the beginning of interval A. As expected, this did not fully compensate for the glucose lost during the 15 min interval. Accordingly, $C(0)$ declined from interval A to interval C. $C(60)$ decreased from interval A to interval C in both protocols (Table 1).

In protocol 2, all of the $E_{\text{glu}}$ values from the individual 15 min intervals appeared to conform to the overall trend of the $E_{\text{glu}}$ values. The values of $E_{\text{glu}}$ over...
The empirical results from the glass model eye and the glucose concentration in the vitreoperfusion fluid. Cat eyes retain their ability to extract glucose for at least 4 hr during vitreoperfusion, a measure presumed to be dominated by retinal glucose use. Second, totally ischemic eyes had intraocular pressure than was maintained in some of the studies. Nonetheless, we cannot exclude slight leaks in some of our eyes.

Our results varied substantially. In addition to the inevitable contribution of biologic variability, several technical obstacles also cause variability. These are imposed by the magnitude of \( E_{\text{glu}} \) and the relative contribution of \( E_{\text{glu}} \) and \( F \) to glucose loss from the system. First, we calculated that only 3.4–8.3% of the glucose originally in the system was used in 60 min. To derive \( E_{\text{glu}} \) from such small changes demands great accuracy of all measurements. According to our operational equation, this problem could be reduced by decreasing \( V \) or increasing \( t \). The vitreoperfusion system originally was designed to minimize \( V \), but additional efforts to reduce it further appear to be important, based on our study results. Alternatively, we could increase \( t \). However, the time needed to make the measurement would become greater than that needed for significant retinal damage to occur in some of the disease processes we wish to study, such as ischemia.

Second, glucose loss due to \( F \) ranged between 58 and 79% of the total glucose lost. Thus, not only is the amount of glucose lost to metabolism small but it is small compared to the amount lost with \( F \), from which it must be differentiated. Accordingly, accurate determination of \( E_{\text{glu}} \) requires highly accurate determination of the quantities needed to measure \( F \) in addition to those that otherwise would be needed to measure.

### Table 1. Glucose concentrations (mean ± SD) in vitreoperfusion fluid in cat eyes

<table>
<thead>
<tr>
<th>Glucose concentration*</th>
<th>Protocol</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>Protocol</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C(0) ) (mg/ml)</td>
<td></td>
<td>1.11 ± 0.02</td>
<td>1.11 ± 0.02</td>
<td>1.11 ± 0.02</td>
<td>1.06 ± 0.01</td>
<td>0.84 ± 0.06</td>
<td>0.65 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>( C(60) ) (mg/ml)</td>
<td></td>
<td>0.94 ± 0.08</td>
<td>0.92 ± 0.08</td>
<td>0.87 ± 0.09</td>
<td>0.91 ± 0.04</td>
<td>0.67 ± 0.05</td>
<td>0.57 ± 0.07</td>
<td></td>
</tr>
</tbody>
</table>

* Key: \( C(0) \) indicates glucose concentration at the beginning of the 60 min interval (A–C); \( C(60) \), glucose concentration at the end of the 60 min interval (A–C).
sure glucose consumption alone. To overcome this problem involves reducing the loss of glucose via F. The most direct approach to reduce F is to decrease the intraocular pressure, which was approximately 20 mmHg during our experiments. Accordingly, the range available for manipulation of this factor is limited.

We would like to compare our results to those of experiments involving retinal glucose use reported in the literature. Several factors limit comparability, including: (1) species differences; (2) most previous experiments were conducted in vitro; (3) our results were influenced by ocular structures other than the retina; (4) the retinal temperature was lower than the physiologic temperature in our experiments because the vitreoperfusion fluid was not heated to 37°C; and (5) our measurements of retinal weights were performed on cat eyes that were different than those used for glucose measurements. Still, a rough comparison is possible. Measurements of the rate of retinal glucose consumption in vitro have ranged from 0.22–0.65 μmol/hr/mg dry retinal weight in rabbits, rats, oxen, and pigs.17 Conversion of our results to these units yielded values of 0.17, 0.30, and 0.41 μmol/hr/mg dry retinal weight during intervals A, B, and C, respectively. The blood flows in the choroid and retina, and the arteriovenous glucose differences in the choroid and retina of anesthetized pigs also have been measured.9 Based on these measurements, retinal glucose extraction could be calculated to be 26.8 μg/min or 0.198 μg/min/mg wet retinal weight, assuming the wet weight of the pig retina to be 135 mg. During intervals A, B, and C, we obtained values of 0.066, 0.118, and 0.163 μg/min/mg wet retinal weight, respectively. Again, our results are remarkably similar to those previously reported by other investigators.

Further development of our method should include: (1) estimation of E glu of the retina alone by subtraction of that measured in eyes with excised retinas from that obtained in eyes with retained retinas; and (2) determination of E glu at normal body temperature by heating of the vitreoperfusion fluid to 37°C.

Our method assumes that E glu closely correlates with the glucose utilization rate and that this rate is constant. Although E glu was not constant, our method should yield a reasonable estimate of the average E glu during the period of the measurement. The increase in E glu with time was an unexpected finding. In fact, we were concerned there might be a progressive reduction in the ability to extract glucose because of degenerative changes after the onset of ischemia, despite vitreoperfusion. The basis for this increase is not yet apparent, but there are several possibilities. First, the eyes may not have reached a metabolic steady state after conversion from extraction of nutrients from the blood to extraction from the vitreoperfusion fluid. In other words, E glu may have changed with time because the glucose utilization rate was changing. If this were the case, the time needed for equilibration would appear to have been at least 4 hr.

Second, the utilization rate may have been constant, but E glu might have underestimated it initially because of endogenous stores (e.g., glycogen) that were progressively consumed preferentially. Third, if delivery of oxygen were incomplete, there may have been progressive conversion from aerobic to anaerobic metabolism. This would have been accompanied by an increase in glucose use (Pasteur effect)17 in an attempt to maintain adenosine triphosphate levels. Incomplete oxygenation is a possibility because the high concentration of mitochondria in the outer retina had to be supplied with oxygen from the vitreous cavity during the experiment. Nevertheless, the dramatic histologic preservation conferred by vitreoperfusion in ischemic eyes and the similarity of our glucose extraction results to those published previously are consistent with good oxygenation under the conditions of our experiment. The increase in E glu with time does not appear to be attributable to F, because the latter showed no consistent variation with time.

Further development of the technique should clarify the basis for the increase in E glu with time. We plan measurements of retinal glycogen to see whether it becomes depleted during vitreoperfusion. We are developing a method to measure oxygen extraction using vitreoperfusion with a procedure analogous to that described in the present report for glucose. Comparison of glucose extraction to oxygen extraction, particularly over time, will reveal the relative contributions of anaerobic and aerobic metabolism to E glu. Determination of lactate production and its time course (also possible by vitreoperfusion) will provide an assessment of anaerobic glycolysis.

The method we have presented has the potential to determine the status of an important aspect of ocular energy metabolism quantitatively in a variety of physiologic or pathologic states. Virtually any model of disease that occurs spontaneously or that can be induced in animals with eyes large enough to permit vitrectomy can be studied. Further development of the technique may allow it to be applied in certain clinical situations. For example, measurements of glucose extraction could be performed at the time of vitrectomy for a variety of pathologic conditions. In the future, vitreoperfusion may be applied in patients as a treatment for central retinal artery occlusion.14,15 In that case, measurements of E glu could help estimate the amount of glucose-consuming, presumably viable tissue that remains. It also could be useful in deciding when to stop vitreoperfusion. E glu would be expected to decline if blood flow were restored and the tissue preferentially extracted glucose from the blood. Alter-
natively, $E_{glu}$ also would decline if the tissue progressed to infarction and lost its ability to use glucose. Accordingly, low, stable values of $E_{glu}$ could become a criterion for terminating vitreoperfusion.

In conclusion, we have described a new method for assessing ocular energy metabolism. Development and application of the method promises to provide considerable new information about ocular function in healthy and disease states.

**Appendix**

Let $M_v = \text{mass of glucose in vitreous and vitreoperfusion system.}$ $M_m = \text{mass of glucose metabolized by ocular tissue.}$ $M_f = \text{mass of glucose lost by flow out of system, predominantly via the outflow channels.}$

Assumptions:

1. $\frac{dM_m}{dt} = Q$ \hspace{1cm} \text{(2)}

   where $Q$ is the rate of glucose utilization by the tissue. $Q$ is assumed to be constant throughout the duration of and range of $C(t)$ occurring during the experiment.

2. $\frac{dM_f}{dt} = C(t)F$ \hspace{1cm} \text{(3)}

3. Glucose is lost from the system only via $Q$ and $C(t)F$.

4. There are no sources of glucose for the retina, except from the vitreoperfusion fluid, eg, there is no blood flow to the eye. If this is so, $Q = E_{glu}$ the rate of extraction of glucose from the vitreoperfusion fluid.

5. The glucose concentration of the vitreoperfusion fluid is independent of location, ie, the system is well stirred.

6. As fluid flows out of the system, it is replaced with fluid containing no glucose at rate $F$, so $V$ is constant.

7. $F$ is constant during the experiment.

By conservation of mass:

$$\frac{dM_v}{dt} = \frac{dM_m}{dt} - \frac{dM_f}{dt}$$ \hspace{1cm} \text{(4)}

One then can substitute:

$$\frac{dM_v}{dt} = -Q - C(t)F = -E_{glu} - C(t)F$$ \hspace{1cm} \text{(5)}

which can be solved to yield:

$$C(t) = \frac{-E_{glu}/F + [C(0) + E_{glu}/F]e^{-Ft/V}}{F}$$ \hspace{1cm} \text{(6)}

**Key words:** ocular energy metabolism, glucose utilization, vitreoperfusion, glucose, retinal ischemia

**References**


