Telemedicine

Comparing the Rise in Glucose Concentration in Blood, Aqueous and Interstitial Fluid During a Glucose Tolerance Test

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Purpose: The purpose of the study was to determine if aqueous glucose levels rise in a comparable time frame to interstitial fluid and could therefore be suitable for a continuous glucose monitoring (CGM) site.

Methods: An intravenous glucose tolerance test was performed on five New Zealand white rabbits. Aqueous humor from the posterior and anterior chamber of the eye and venous blood were sampled for glucose concentration measurement. Glucose concentrations in the interstitial fluids were monitored using a CGM system. A compartment model was created to map the glucose response curves in each compartment. The delay in rising glucose concentrations between blood and interstitial fluid and aqueous humor in the posterior chamber and anterior chamber of the eye were analyzed.

Results: The results showed a statistically similar time lag and rate of change in glucose concentrations between blood and interstitial fluids or aqueous humor in either the posterior or anterior chamber.

Conclusions: The results of this study add further support to the aqueous humor being used as an alternative CGM site.

Translational Relevance: The study provides the basis for developing an intraocular continuous glucose sensor that can overcome limitations of current CGM systems.

Introduction

Diabetes mellitus is a disease characterized by chronic hyperglycemia. It is associated with numerous serious complications that can be fatal, including myocardial infarctions, cerebrovascular accidents, and renal failure, as well as many comorbidities such as sight loss and peripheral polyneuropathy. As there is no cure for diabetes, the mainstay of treatment aims to reduce the risk of its complications by closely monitoring and managing blood glucose levels.¹,²

Continuous glucose monitors (CGMs) have many advantages over the traditional discrete glucose measurement devices. The repeated testing needed with discrete glucose testing strips is inconvenient, causes discomfort, and may lead to long-term tissue damage.³,⁴ CGM sensors give continuous glucose level readings, and reports have shown fewer episodes of hyper- and hypoglycemia when using CGM devices.⁵–⁹ Most commercially available CGM systems measure glucose levels in the interstitial fluids. These CGM sensors are equipped with a microneedle that penetrates the skin in the arm or the abdomen to access the subdermal space.⁹,¹⁰ The interstitial fluid meets two important criteria for CGM systems—namely, the glucose concentrations in interstitial fluid correlate well with glucose concentrations in blood,¹¹ and the delay between the change in blood glucose as compared to interstitial fluid glucose concentration is low.¹²,¹³ However, these CGM systems also have their drawbacks since inflammation and scarring are inevitable around the sensor when introducing a foreign body underneath the skin, and most CGM
devices rely on glucose oxidase reactions for measuring glucose concentrations, which consumes the enzyme, thereby limiting their lifetime.14,15

Recent studies investigated whether other physiologic fluids can be used to measure systemic glucose levels. Sweat, tears, aqueous, and saliva have been studied,16–19 and while some of them show promise, many have proven difficult to measure continuously20 or have only a weak correlation to blood glucose levels.21,22 Aqueous glucose concentrations have been shown to closely correlate with blood glucose concentrations, and previous attempts at making an aqueous CGM system using optical polarimetry and Raman spectroscopy have been reported.23–25 The ocular media are clear, which makes the aqueous a suitable medium to analyze noninvasively.

There are, however, conflicting reports on how fast the glucose concentrations in aqueous rise following an increase in blood glucose.26,27 In a study by Cameron et al.,26 aqueous was sampled from rabbits’ anterior chamber every 2 weeks at varying time points after using xylazine to elevate blood glucose levels. Their results showed a delay of 3.4 minutes for aqueous glucose levels to rise as compared to blood glucose levels. Smith27 calculated the time delay from intravenous glucose injection to a rise in aqueous glucose concentration based on the flow rate of aqueous and estimated the delay to be around 40 minutes. Using a noninvasive optical heterodyne technique, Chou and Lin28 estimated the delay to be around 30 minutes. Another noninvasive technique for measuring aqueous glucose levels using optical rotation estimated the time delay to be more than 2 hours between changes in blood glucose concentration and the aqueous.29 The large variability in the results of these studies can be due to the different techniques used for increasing systemic glucose levels as well as the method for measuring aqueous glucose concentrations. In our study, we used the standardized intravenous glucose tolerance test with direct sampling of aqueous from the posterior and anterior chamber of the eye. The results were compared to the time delay measured using a commercially available CGM device that measures the glucose concentrations in the interstitial fluid. The aim of the study was to determine if aqueous glucose levels rise in a comparable time frame to interstitial fluid and could therefore be suitable for CGM monitoring.

Materials and Methods

Animals

This study was conducted following approval from the Animal Experimentation Ethics Committee of the Chinese University of Hong Kong (CUHK). The study complied with the Guidelines of the Association for Research in Vision and Ophthalmology (ARVO) Statement on the Use of Animals in Ophthalmic and Vision Research.

Five New Zealand white rabbits (2.3–3.1 kg in weight and aged 3 to 5 months) were bred at the Laboratory Animal Services Centre of the CUHK and housed in the animal laboratory at the Department of Ophthalmology and Visual Sciences of CUHK. Rabbits were fasted for 8 to 15 hours with free access to water before the experiment.

Glucose Assay

A commercially available glucometer (Contour Plus ONE; Ascensia Diabetes Care, Basel, Switzerland) was used to measure glucose levels in both blood and aqueous humor. Glucose concentration in the interstitial fluid was monitored continuously using a commercially available CGM system (FreeStyle Libre; Abbott Diabetes Care, CA, Alameda, USA). The sensor of the FreeStyle CGM system was implanted between the scapulae of the rabbit 1 hour before the start of the experiment for the sensor to fully calibrate. A smartphone was used to take measurements from the sensor using the Freestyle app via near-field communication.

Sampling Techniques

Rabbits were first sedated by isoflurane and a catheter was inserted at a marginal ear vein for intravenous injection. Pentobarbital at 30 mg/kg was first injected via the catheter to anesthetize the rabbit followed by heparin solution to prevent clotting in the catheter. Further pentobarbital at 17 mg/kg was injected per hour throughout the entire experiment to keep the rabbit anesthetized. Then, a 34-gauge 1/2-in. needle was inserted 1.5 to 2 mm posterior to the limbus for posterior segment sampling and another 34-gauge needle was inserted through the peripheral cornea. The aqueous that accumulated passively inside the needle was drawn out by pipette. Blood samples were taken using a syringe and 23-gauge needle from a vein in the ear.

The glucose tolerance test was performed by administering a bolus injection of 40% glucose (0.5 g glucose/kg body weight) into a vein in the ear of the rabbit over 60 seconds.30 Sampling was performed at 2-minute intervals for the first 40 minutes and thereafter every 5 minutes for the remaining 50 minutes of the experiment. All aqueous humor within the gauge of the needle was evacuated using a suction pipette 1 minute before sampling. This aqueous was discarded, allowing “fresh” aqueous to reaccumulate inside the
gauge of the needle. The flow rate of aqueous humor is around 2 μL/min, and the minimum required volume of sample for the glucometer is 0.6 μL. The aqueous was allowed to reaccumulate for 1 minute, after which the sample was taken (again using a suction pipette). The time point recorded for the sample collection was at the moment when fluid was extracted from the 34-gauge needle. The aqueous was immediately analyzed using the glucometer (Contour Plus ONE; Ascensia Diabetes Care). The measurements from the Freestyle Libre CGM sensor were taken using a smartphone.

Statistics and Modeling

All data were analyzed and plotted using MATLAB (MathWorks, Natick, MA, USA) software. A compartment model (Fig. 1) was constructed to illustrate the relationship of glucose concentration between blood, interstitial fluid, and aqueous humor based on published reports. The movement of glucose between blood and interstitial fluid is represented as transfer coefficients $k_{12}$ and $k_{21}$, while $k_{10}$ and $k_{20}$ represented the clearance of glucose from interstitial fluid and blood, respectively. Glucose was transported during the secretion of aqueous humor into the posterior chamber and is represented as $k_{sec,PC}$. Thereafter, glucose diffused into the anterior chamber by flow from the posterior chamber and diffusion from iris blood vessels ($k_{flow,PC}$ and $k_{diff,AC}$) and left the anterior chamber by flow ($k_{flow,AC}$). $k_{sec,PC}$ represents the kinetics of aqueous secretion. This involves the transportation of glucose across the blood–ocular barrier through facilitated diffusion. It is assumed that the concentration of glucose in blood has an effect on the rate of increase in posterior chamber glucose concentration. $k_{flow,PC}$ describes the flow of aqueous humor from the posterior to anterior chamber. This is modeled as a flow that is constant and proportional to the production of aqueous. The production of aqueous is referred to $k_{sec,PC}$. $k_{flow,AC}$ represents the aqueous humor entering through the pupil and leaving the anterior chamber through the trabecular meshwork and the uveoscleral pathway. The rate of glucose concentration change in the anterior chamber is therefore affected by both the posterior chamber flow and the current concentration in the anterior chamber. $k_{diff,AC}$ represents the diffusion of glucose from the iris blood vessels directly into the anterior chamber, which has been reported in the literature. The diffusion gradient is estimated by the difference between the glucose concentration in blood and the anterior chamber.

From the compartment model (Fig. 1), several differential equations were used to describe the glucose concentration in each compartment. First, the changes in glucose levels in blood after intravenous injection were assumed to increase rigorously in the first 2 minutes and then decrease according to simple first-order kinetics, as shown in Equation (1):

$$\frac{dC_{Bl,d}}{dt} = k_{10}C_{Bl,d}$$

$C_{Bl,d}$ = glucose concentration in blood
$k_{10}$ = elimination coefficient from blood

Immediately after the bolus injection of intravenous glucose, the blood glucose concentrations rose rapidly. After reaching a peak, the glucose levels decreased according to Equation (1). The first sampling time point was at 2 minutes, and this was the first data point that could be used to fit the model. The relationship of glucose levels between blood and interstitial fluid is
Glucose Time Delay in Different Compartments described in Equation (2)\(^{31,33}\):

\[
\frac{dC_{IF}}{dt} = -(k_{21} + k_{20})C_{IF} + k_{12}C_{Bld}
\]

\(^{(2)}\)

\(C_{Bld}\) = initial glucose concentration in blood
\(C_{IF}\) = initial glucose concentration in interstitial fluid
\(k_{12}\) = diffusion coefficient from blood to interstitial fluid
\(k_{21}\) = diffusion coefficient from interstitial fluid to blood
\(k_{20}\) = elimination coefficient from interstitial fluid

The glucose kinetics in aqueous humor are described in Equations (3) and (4)\(^{32}\):

\[
\frac{dC_{PC}}{dt} = k_{sec,PC}C_{Bld} - k_{flow,PC}C_{PC}
\]

\(^{(3)}\)

\[
\frac{dC_{AC}}{dt} = k_{flow,AC}C_{PC} + k_{diff,AC}(C_{Bld} - C_{AC}) - k_{flow,AC}C_{AC}
\]

\(^{(4)}\)

\(C_{Bld}\) = glucose concentration in blood
\(C_{PC}\) = glucose concentration in aqueous humor in posterior chamber
\(C_{AC}\) = glucose concentration in aqueous humor in anterior chamber
\(k_{sec,PC}\) = coefficient of transfer by secretion from blood to posterior chamber
\(k_{flow,PC}\) = coefficient of transfer by flow between posterior and anterior chamber
\(k_{flow,AC}\) = coefficient of transfer by flow into and out of anterior chamber
\(k_{diff,AC}\) = coefficient of transfer by diffusion between blood and anterior chamber

The observed data from the experiment were fitted to this compartment model to find the glucose response curve using a nonlinear least squares technique. The compartment model developed in this study is a simplified version of a complex physiologic system. For this model, we selected only the flow, diffusion, and secretion of glucose within the compartments (as detailed above). Other factors such as the metabolism of glucose, intraocular pressure, pH, and integrity of the blood–ocular barrier (to name a few) were not included. The model uses the trust region reflective algorithm (fmincon), which is one of the constrained nonlinear optimization algorithms in MATLAB. In this compartment model, all coefficients \(k\) and concentrations in all compartments were assumed to be always greater than zero, and all coefficients \(k\) were first initially randomly assigned and then adjusted until the sum of squares (SS) reached a minimum.

\[
SS = \sum (C_{predicted} - C_{measured})^2
\]

\(^{(5)}\)

\(C_{predicted}\) = Concentration calculated using compartment models at each time point
\(C_{measured}\) = Concentration recorded in the experiment at each time point

SS calculated the differences between the regression model and observed data and therefore determined the goodness of fit for the model. This fitted model was then used to calculate glucose response curves for each rabbit. The time for glucose levels to reach a maximum after glucose injection was derived from the glucose response curves. The mean values of each compartment among all five rabbits were calculated and compared using Kruskal–Wallis test. The rate of glucose response change in each compartment was also calculated and compared using the Kruskal–Wallis test.

\[
Rate\ of\ glucose\ response = \frac{Maximum\ glucose\ level - initial\ glucose\ level}{Time\ taken\ to\ reach\ maximum}
\]

\(^{(6)}\)

Apart from the time delay between compartments, absolute differences between the baseline and maximum glucose levels of each compartment were determined from the results. This value would be vital for the future development of a glucose monitoring system as it sets the requirement for the range and sensitivity of the glucose sensor.

### Results

A glucose tolerance test was performed in five rabbits, with the mean ± standard deviation of the fasting glucose level in four types of fluid sample (blood, posterior aqueous humor, anterior humor, and interstitial fluid) being 5.18 ± 0.40, 12.28 ± 1.05, 9.84 ± 0.54, and 7.27 ± 0.40 mmol/L, respectively. A compartment model was applied to the glucose response data collected during the intravenous glucose tolerance test in MATLAB. The final transfer coefficients of the fitted model in each rabbit are summarized in Table 1. The corresponding program-generated curve fits for four types of fluid compartments in each rabbit are shown in Figure 2. In addition, the averaged response for each compartment is shown in Figure 3. The time for glucose levels to reach a maximum after intravenous glucose injection was determined from the fitted models, and their correlation between observed and predicted values for each fluid compartment in
Table 1. Transfer Coefficients of the Fitted Models in Each Animal

| Rabbit | $k_{10}$ | $k_{SC\_PC}$ | $k_{flow\_PC}$ | $k_{diff\_AC}$ | $k_{12}$ | $k_{21} + k_{20}$ | $k_{flow\_AC}$ |
|--------|----------|----------------|-----------------|----------------|----------|------------------|--------------|
| 1      | 0.0131   | 0.1891         | 0.1275          | 0.0488         | 0.1637   | 0.1224           | 0.0841       |
| 2      | 0.0133   | 0.0350         | 0.0234          | 0.0465         | 0.0563   | 0.0419           | 0.0514       |
| 3      | 0.0164   | 0.0829         | 0.0432          | 0.1036         | 0.1067   | 0.0719           | 0.1960       |
| 4      | 0.0094   | 0.0883         | 0.0481          | 0.0880         | 0.1453   | 0.1059           | 0.1973       |
| 5      | 0.0143   | 0.1103         | 0.0657          | 0.0351         | 0.0782   | 0.0619           | 0.0691       |

Table 2. Spearman’s Correlation Coefficients Between Observed and Predicted Data From the Fitted Model

| Rabbit Blood | Posterior Chamber | Anterior Chamber | Interstitial Fluid | Spearman’s $\rho$ | MSE (min) |
|--------------|-------------------|------------------|--------------------|-------------------|-----------|
| 1            | 0.988             | 0.842            | 0.873              | 0.986             | 6.6       |
| 2            | 0.990             | 0.816            | 0.962              | 0.883             | 9.72      |
| 3            | 0.991             | 0.885            | 0.927              | 0.974             | 6.2       |
| 4            | 0.991             | 0.807            | 0.909              | 0.943             | 4.24      |
| 5            | 0.979             | 0.885            | 0.886              | 0.984             | 6.59      |

The MSE shows the goodness of fit of the models to the measured values.

Table 3. Time for Glucose Levels to Reach a Maximum After Intravenous Glucose Injection in Blood, Posterior Chamber, Anterior Chamber, and Interstitial Fluid

| Rabbit | Blood | Posterior Chamber | Anterior Chamber | Interstitial Fluid | Time (min) |
|--------|-------|-------------------|------------------|--------------------|------------|
| 1      | 2     | 16                | 20               | 18                 | 2.047     |
| 2      | 2     | 22                | 26               | 34                 | 2.047     |
| 3      | 2     | 28                | 26               | 22                 | 2.047     |
| 4      | 2     | 32                | 32               | 22                 | 2.047     |
| 5      | 2     | 22                | 26               | 26                 | 2.047     |
| Mean   | 2     | 24                | 27.2             | 24.4               | 2.047     |
| SD     | 0.16  | 5.02              | 6.07             |                    | 2.047     |

Table 4. Rate of Glucose Response to Reach Maximum After Glucose Injection in Posterior Chamber, Anterior Chamber, and Interstitial Fluid

| Rabbit | Posterior Chamber | Anterior Chamber | Interstitial Fluid | Rate (mmol/L Per Minute) |
|--------|-------------------|------------------|--------------------|--------------------------|
| 1      | 0.62              | 0.38             | 0.60               |                          |
| 2      | 0.32              | 0.24             | 0.24               |                          |
| 3      | 0.24              | 0.24             | 0.24               |                          |
| 4      | 0.28              | 0.27             | 0.45               |                          |
| 5      | 0.48              | 0.30             | 0.35               |                          |
| Mean   | 0.39              | 0.29             | 0.41               |                          |
| SD     | 0.16              | 0.06             | 0.13               |                          |

each rabbit and mean squared error (MSE) of the fitted model in each rabbit are shown in Table 2 for measuring the goodness of fit of the best-fit curves. All the correlation coefficients between observed and predicted values were greater than 0.8 (Table 2), suggesting that the data fit the model well for four fluid compartments among the five rabbits. The mean of MSE of all five rabbits was 6.67 minutes with a coefficient of variation of 0.29, which showed a low level of dispersion around the mean value and suggested all the fitted models had a similar MSE.

The mean times for glucose levels in blood, interstitial fluid, aqueous humor in the posterior chamber, and aqueous humor in the anterior chamber to reach maximum were 2, 24.4, 24, and 27.2 minutes, respectively (Table 3). A Kruskal–Wallis test comparing the mean peak times for interstitial fluid, posterior aqueous humor, and anterior aqueous humor showed no statistically significant difference between the groups ($P = 0.68$). Thus, the time delay seen between blood and interstitial fluid is comparable to the time delay between blood and aqueous humor.

Table 4 shows the rate of glucose response to reach maximum from the baseline in each compartment. The calculated mean values were 0.41, 0.39, and 0.29 mmol/L per minute for interstitial fluid, posterior chamber aqueous, and anterior chamber aqueous, respectively (Table 4). The mean rates of response were compared using the Kruskal–Wallis test. No statistically significant difference was found between the groups ($P = 0.26$).
Figure 2. Results of glucose tolerance test in five rabbits. The glucose response curves of blood, posterior chamber, anterior chamber, and interstitial fluid are shown for each rabbit. Solid line shows the nonlinear regression model of each result, and crosses represent the values measured for each sample.

Table 5 shows the absolute difference between baseline and maximum glucose levels in each compartment for each rabbit. The calculated mean values were 9.46, 8.65, and 7.66 mmol/L for interstitial fluid, posterior aqueous, and anterior aqueous, respectively (Table 5). Results of the Kruskal–Wallis test showed no statistically significant difference in mean absolute differences between the compartments ($P = 0.18$).
Figure 3. Averaged results of glucose concentrations in blood, posterior chamber, anterior chamber, and interstitial fluid from five rabbits. The error bars show the standard deviation at each time point. The time point with maximum glucose concentrations is shown with a bold error bar.

Table 5. Absolute Difference Between Baseline and Maximum Glucose Level in Each Compartment

| Rabbit | Blood   | Posterior Chamber | Anterior Chamber | Interstitial Fluid |
|--------|---------|-------------------|------------------|--------------------|
| 1      | 11.88   | 9.90              | 7.66             | 10.78              |
| 2      | 12.47   | 7.02              | 6.16             | 8.24               |
| 3      | 11.54   | 6.71              | 6.28             | 9.26               |
| 4      | 10.77   | 9.12              | 8.64             | 10.01              |
| 5      | 13.65   | 10.50             | 9.54             | 8.99               |
| Mean   | 12.06   | 8.65              | 7.66             | 9.46               |
| SD     | 1.08    | 1.70              | 1.47             | 0.97               |

Discussion

To the best of our knowledge, the real-time changes in glucose concentrations in the posterior and anterior chamber of the eye during a glucose tolerance test have not been previously reported. In this study, the results were compared to the changes in glucose concentration in blood and interstitial fluid. The interstitial fluid is commonly used by CGM devices that measure the concentration of glucose using either glucose oxidase or fluorescence spectroscopy. Previous reports have shown a close correlation between glucose concentration in aqueous with the glucose concentration in blood in both humans and rabbits. Likewise, the close correlation between interstitial fluid and blood has also been previously shown. This study compared the time delay for the aqueous and interstitial fluid to respond to acute changes in blood glucose levels.

The mechanism of glucose transportation into aqueous and interstitial fluid is different, since aqueous receives glucose via facilitated diffusion across the blood–ocular barrier and interstitial fluid receives glucose through passive diffusion from the blood plasma. The facilitated diffusion of glucose in the eye is predominantly via the GLUT1 receptors. Previous studies have shown a closer correlation of blood glucose with aqueous glucose concentrations in diabetic patients compared to nondiabetic individuals, which was attributed to a breakdown of the blood–
ocular barrier in diabetics. Ideally, CGM systems should show a change in the glucose concentration within 10 minutes after the blood glucose levels rise. This can be achieved in interstitial fluid, and the results from this study have shown an equivalent response time in the aqueous of the anterior chamber and an even faster response in the posterior chamber of the eye. The reason for this is due to the anatomic location of the ciliary body where aqueous is produced. By the time the aqueous fluid reaches the anterior chamber, there is a delay due to the dilution that takes place into the larger volume of the anterior chamber.

Even though positive results have been obtained supporting the aqueous environment being suitable for the development of a CGM system, the challenge still remains to develop a sensor that is small enough to be implantable inside the eye or to develop an external sensor that can accurately measure the aqueous glucose concentrations noninvasively. Previous attempts at recording glucose concentrations in the aqueous have involved optical polarimetry and Raman spectroscopy, which measure glucose concentration of aqueous humor in the anterior chamber. There have been no reported attempts to record continuous glucose concentrations in the posterior chamber due to the technical challenges of making such an implantable glucose sensor.

The study was limited by the small sample size. While the experimental results were reproducible between animals, only five rabbits were used in this study. Despite the small number, the standard deviations of our modeled results were 6.16, 5.02, and 6.07 minutes for posterior chamber, anterior chamber, and interstitial fluid, respectively, which is similar to previously published similar studies. Repeating the experiments in more animals may improve the statistical validity of the results, but the main outcome of the experiment would likely not change. Namely, the increase in glucose following intravenous injection of glucose is comparable in time between the posterior segment of the eye and interstitial fluid.

Despite this limitation, the results provide preliminary data on the rate of change in glucose levels inside an eye. The results should be replicated in human eyes, of both diabetic patients and normal controls. However, this is difficult as repetitive sampling of aqueous from the posterior chamber is invasive. It may also be worth designing a new CGM system since the commercially available system used in this study uses a compensatory mechanism when showing the peak of glucose concentration in the interstitial fluid. This makes the exact changes in glucose concentration more difficult to interpret.

In conclusion, this study shows no time lag or rate changes in glucose concentrations between blood and interstitial fluids or aqueous humor in rabbit eyes. The results show aqueous humor can be used as an alternative continuous glucose monitoring site, but the sensor design has to be optimized and validation is needed in studies on human eyes.

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