Mass Spectral Determination of Fasting Tear Glucose Concentrations in Nondiabetic Volunteers, Justin T. Baca,1 Christopher R. Taormina,1 Eleanor Feingold,3 David N. Finegold,2 Joseph J. Grabowski,4 and Sanford A. Asher1 (1 Department of Chemistry, University of Pittsburgh, Pittsburgh, PA; 2 Department of Pediatrics, University of Pittsburgh School of Medicine, Children's Hospital of Pittsburgh, Pittsburgh, PA; 3 Department of Human Genetics and Department of Biostatistics, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA; * address correspondence to this author at: Department of Chemistry, Chevron Science Center, 219 Parkman Ave., Pittsburgh, PA 15260; fax 412-624-0588, e-mail asher@pitt.edu)

**Background:** There is considerable disagreement regarding the concentration of glucose in tears and its relationship to the concentration in blood. Improved sampling and analysis methods may resolve these discrepancies and possibly provide a basis for in situ tear glucose sensors.

**Methods:** We used liquid chromatography (LC) with electrospray ionization mass spectrometry (ESI-MS) to determine glucose in 1-μL tear fluid samples obtained from 25 fasting study participants. Tear fluid was collected with microcapillaries and a slitlamp microscope.

**Results:** The median (range) of fasting tear glucose concentrations was 28 (7–161) μmol/L or 0.50 (0.13–2.90) mg/dL. The SD of tear glucose measurements for individuals varied linearly with the mean tear glucose concentration and was approximately half of the mean. We found no significant difference in tear glucose concentrations between contact lens users and nonusers (P = 0.715). We observed significant correlations between fasting blood and tear glucose concentrations (R = 0.50, P = 0.01).

**Conclusions:** Our tear fluid collection and analysis method enables reliable measurement of equilibrium, fasting tear glucose concentrations. These concentrations are lower than those previously reported for nondiabetic persons. Larger population studies are required to determine correlations between blood and tear glucose concentrations and to determine the utility of contact lens–based sensors for the monitoring of diabetics. Our methods are applicable for study of other tear fluid analytes and may prove useful for monitoring other disease states.

© 2007 American Association for Clinical Chemistry

Glucose has been a recognized component of tear fluid since the early 1900s, but disagreement continues regarding its concentration in tear fluid and its correlation with blood glucose concentration (1–6). Literature reports of normal tear glucose concentrations range between 0 and 9.1 mmol/L (164 mg/dL), with median values of 110–280 μmol/L (1.98 and 5.04 mg/dL) (1, 7). In a recent study of 121 persons, tear glucose concentrations ranged from below the limit of detection to 9.1 mmol/L (164 mg/dL). Much of the difference in reported tear glucose concentrations is likely from the use of different tear collection techniques (8). Collection techniques causing severe eye irritation [such as filter paper collection (6)] are associated with the highest tear glucose concentrations, whereas less irritating techniques (such as glass capillary collection) are associated with the lowest (2, 3). Chemically stimulated tears have increased tear glucose (8, 9). Reliable tear sampling may also be confounded by individual differences in tolerance to real or expected eye stimulation during sampling. (see the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol53/issue7 for an extensive review of the tear glucose literature.)

Improved tear fluid collection, and the ability to analyze very low volumes of tear fluid, may dramatically improve measurement of tear fluid glucose concentrations and help resolve the reported discrepancies in basal tear glucose concentrations. Improved methods would also enable the study of physiologic glucose transport in the eye and advance the use of tear fluid as a surrogate for blood in measuring other clinically important analytes.

Some groups have tried to use tear glucose to diagnose diabetes (4, 5), and others have proposed continuous monitoring of blood glucose concentrations by use of contact lenses with glucose sensors (10–13). We recently reported a photonic crystal glucose-sensing material for noninvasive monitoring of glucose in tear fluid (10). Detailed understanding of tear glucose concentration and its regulation is critical to developing noninvasive glucose sensors.

We recently developed an electrospray ionization mass spectrometry (ESI-MS) method for measuring glucose in 1-μL samples of collected tear fluid (9). With this method, we studied basal tear glucose concentrations in healthy persons without diabetes.

We recruited volunteers (age 18–60 years) within and around the University of Pittsburgh. Persons with a history of diabetes were excluded. Blood and tear samples were obtained after participants had fasted overnight for at least 8 h. The samples were always collected in the same order: capillary blood from the finger pad, tear fluid from the left eye, and then tear fluid from the right eye. This sequence was repeated 3 times for each study participant, with at least 10 min between successive blood sample collections. Glucose in capillary blood was measured with an Accu-Chek® Compact glucometer (Roche), according to the manufacturer’s protocol.

A total of 26 volunteers completed the study; 11 wore their usual contact lenses at the time of the study, and 15 did not wear contact lenses. The type of contact lenses worn were daily disposable (n = 1), daily wear (n = 6), extended wear (n = 1), and silicone hydrogel lenses (n = 2). Of the 15 non-contact lens wearers, 1 had a history of contact lens use, but did not wear contacts on the day of
the study. For 1 contact lens wearer, the collected tear samples were lost because of vial breakage before analysis. For 2 participants (1 from the contact lens group and 1 from the non-contact lens group), 1 of the 6 tear glucose samples was lost because of instrument failure. The tear glucose concentrations for these 2 participants were determined from only 5 samples. The University of Pittsburgh School of Medicine Institutional Review Board approved all clinical procedures, and all participants signed a detailed informed consent form.

Tear fluid samples of 1 μL were collected and analyzed by liquid chromatography ESI-MS as previously reported (9). Three aliquots of each tear sample were injected into the analyzer to determine the mean glucose concentration. Our study found much lower tear fluid glucose concentrations for healthy individuals than did previous studies (see Fig. 1 in the online Data Supplement).

The population median (range) of the tear glucose concentrations were 28 (7–161) μmol/L [0.50 (0.13–2.90) mg/dL]. The distribution of mean tear glucose concentrations was highly skewed; <28 μmol/L (0.50 mg/dL) in 50% of the study participants and <42 μmol/L (0.76 mg/dL) in 80% of the study participants. Two individuals were observed rubbing their eyes during the course of the study, and they had the highest mean (SD) tear glucose concentrations: 128 (75) and 161 (71) μmol/L or 2.31 (1.35) and 2.90 (1.28) mg/dL.

Because the SD of the mean tear glucose concentration for each participant was proportional to the mean for each participant, a natural log transformation was applied to the tear glucose concentration values (see Figs. 2 and 3 in the online Data Supplement). After transformation, standard statistical methods were applied.

Contact lens use did not affect mean tear glucose (P = 0.715). Transformed tear glucose concentrations were significantly correlated with mean blood glucose (R = 0.50, P = 0.01, Fig. 1A). This correlation for fasting tear and blood glucose is similar to that reported by Daum and Hill of R = 0.53 for blood and tear glucose measurement variations throughout the day. However, they reported a mean (SD) population tear fluid glucose concentration of 420 (355) μmol/L or 7.57 (6.40) mg/dL.

The glucose concentrations in the right and left eyes were highly correlated within individuals (Fig. 1B). There was no evidence that eye-to-eye variation in individual study participants differed significantly from variation in a single eye over time. Furthermore, we observed no evidence that variation in tear glucose concentration between individuals with similar blood glucose concentrations was greater than variation in tear glucose concentration within an individual.

The correlation between the transformed tear glucose value and the average blood glucose concentration appears to be stronger for non-contact lens wearers than for participants wearing contact lenses (R = 0.70 vs R = 0.22).

However, analysis of covariance does not indicate a significant difference (P = 0.63).

The ESI-MS method used here enables reliable determination of basal tear glucose concentrations in fasting individuals. Study participants had fasted overnight, and had stable blood glucose concentrations over the brief course of the study. We observed much lower basal tear glucose concentrations than previously reported for healthy individuals. This difference is likely a result of our sampling methods, which were less irritating than earlier methods that stimulated tear production chemically or with filter paper (8). If our collection method had caused significant irritation, we would have expected tear glucose concentrations to increase over the course of the

---

**Fig. 1.** (A) Correlation (P = 0.01) between the mean ln(tear glucose concentration) and the mean blood glucose concentration. A linear regression for all participants gives y = 0.80x – 1.07 (R = 0.50). Linear regression for the subpopulations of contact wearers and non-contact wearers gives y = 0.570x + 0.332 (R = 0.22) for contact lens wearers and y = 0.961x – 2.079 (R = 0.70) for non-contact wearers. (b), paired tear glucose observations within study participants. No significant differences were observed between right and left eyes (P = 0.76, two-tailed paired t-test), but results may differ at any given time. The left and right tear glucose determinations that are closest in time are plotted against each other. There are hence 3 data points for each study participant.
study. We observed no evidence of such an increase (see Fig. 4 in the online Data Supplement).

We observed significant variation in tear glucose concentrations between the 2 eyes of individual study participants, as well as over time within a single eye. During this study, we were cognizant of potential confounding events such as yawning and eye rubbing. Although the 2 individuals who rubbed their eyes during the study had the highest glucose concentrations, events like these do not explain the large within-individual differences observed.

Variations in tear glucose concentrations within a single individual must derive from the sum of the biological variance and any variances associated with sampling and measurement. The error in our tear fluid collection volumes was negligible (9); the SD observed for an individual was ~3 times the SD of the 3 replicate mass spectral measurements of a single tear fluid sample. The relative SDs of these 3 replicates varied somewhat with glucose concentration but had a median value of 14%. Thus, the observed SD in tear glucose measurements derive mainly from actual variations of the glucose concentration in the different tear fluid samples.

We did not observe a systematic increase over time in glucose concentration variations that could result from the effect of tear depletion during repeated measurements. Tear glucose concentration appeared to vary randomly over the repeated sampling events.

We observed variations in tear glucose concentrations among fasting individuals and a significant correlation between ln(tear glucose concentration) and blood glucose concentration. Mean fasting tear glucose concentrations did not differ significantly in relation to contact lens use. Further studies are needed to investigate the apparent difference in the correlation between tear glucose and blood in these subpopulations.

The extremely low glucose concentrations in tear fluid, more than 100 times lower than in blood, raise questions about the physiologic role of tear glucose. Future studies are needed to address the correlation between tear and blood glucose in hypoglycemic and hyperglycemic states and in the presence of diabetes.

Financial Disclosures: S.A.A. is the scientific cofounder of Glucose Sensing Technologies LLC, a company developing glucose-sensing contact lenses.

Acknowledgements: We thank Drs. Gary Foulks and Cholappadi Sundar-Raj for helpful discussions and critical reviews of the manuscript.

References

Previously published online at DOI: 10.1373/clinchem.2006.078543

Grant funding/support: This research was supported by the National Institutes of Health Grant DK-55348 (to S.A.A.).