Tear Glucose Analysis for the Noninvasive Detection and Monitoring of Diabetes Mellitus

JUSTIN T. BACA, PHD,[1] DAVID N. FINEGOLD, MD,[2] AND SANFORD A. ASHER, PHD[1,2]

ABSTRACT One approach to the noninvasive monitoring of blood glucose concentration is to monitor glucose concentrations in tear fluid. While several methods for sensing glucose in tear fluid have been proposed, controversy remains as to the precise concentrations of tear glucose in normal and diabetic subjects and as to whether tear fluid glucose concentrations correlate with blood glucose concentrations. This review covers the present understanding of the physiology of glucose transport in tears, the regulation of the aqueous tear fraction, and studies of tear glucose concentration over the last 80 years. The various tear collection methods employed greatly influence the measured tear glucose concentrations. Studies that involve mechanical irritation of the conjunctiva during sampling measure the highest tear glucose concentrations, while studies that avoid tear stimulation measure the lowest concentrations. Attempts to monitor tear glucose concentration in situ by using contact lens-based sensing devices are discussed, and new observations are presented of tear glucose concentration obtained by a method designed to avoid tear stimulation. These studies indicate the importance of the sampling method in determining tear glucose concentrations. On the basis of these results, we discuss the future of in vivo tear glucose sensing and outline the studies needed to resolve the remaining questions about the relationship between tear and blood glucose concentrations.

Accepted for publication August 2007.

From the [1]Department of Chemistry, Chevron Science Center, University of Pittsburgh, and [2]Department of Human Genetics, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

Supported by the National Institutes of Health fellowship 1 F31 EB004181-01A1 to Justin T. Baca, and grant 2 R01 EB004132-04 to Sanford A. Asher.

Sanford A. Asher is the scientific founder of Glucose Sensing Technologies LLC, a company developing PCCA sensors for glucose sensing. The authors gratefully acknowledge the assistance of Christopher R. Taormina in the mass spectral analysis of tear samples.

Single-copy reprint requests to Prof. Sanford A. Asher, PhD (address below).

Corresponding author: Prof. Sanford A. Asher, PhD, Department of Chemistry, 701 Chevron Science Center, 219 Parkman Avenue, Pittsburgh, PA 15260. Tel: 412-624-8570. Fax: 412-624-0588. Email: asher@pitt.edu.

KEY WORDS conjunctiva, contact lens sensors, diabetes mellitus, glucose sensing, glucose transport, in situ tear analysis, tear collection, tear glucose, tear production, tear stimulation

I. INTRODUCTION

According to current statistics, approximately 20.8 million people in the United States, or about 7% of the population, has diabetes mellitus.[1] Worldwide, over 180 million people have diabetes mellitus, and the prevalence of this disease is expected to double by the year 2030.[2] The economic cost of diabetes mellitus in the United States alone is about $132 billion, representing about 10% of healthcare expenditures.[3]

The Diabetes Control and Complications Trial clearly demonstrated that tight glycemic control is critical in managing diabetes mellitus and preventing complications such as retinopathy, nephropathy, and neuropathy.[4] To control blood glucose levels, current standards of care require self-monitoring of glucose several times a day, with increased frequency for patients receiving insulin.[5] Currently, self-monitoring of blood glucose requires a finger-stick blood sample for direct measurement of glucose. Many noninvasive approaches to blood glucose monitoring have been investigated, but none have been able to replace the direct measurement of glucose.[6] Implantable, continuous glucose sensors have shown promise for improving glycemic control.[7,8] However, currently approved devices must be calibrated at least twice a day with a direct blood measurement and must be replaced after 3-7 days.

Noninvasive glucose monitoring techniques under development include infrared (**IR**) spectroscopy,[9,10] Raman spectroscopy,[11,12] measurement of optical polarization rotation,[13] photoacoustic phenomena,[14] optical coherence tomography,[15] fluorescence measurements,[16,17] surface-plasmon resonance in nanoparticles,[18] and electrical impedance measurements.[19] A single noninvasive device for monitoring blood glucose, the GlucoWatch (Cygnus, Redwood City, CA), has been approved by the U.S. Food and Drug Administration for supplementary blood glucose monitoring. However, results from the GlucoWatch must be frequently checked against direct blood glucose measurements. Additionally, recent studies have found that adding

OUTLINE

GlucoWatch monitoring to standard self-monitoring of blood glucose did not result in improved glycemic control.[20]

One approach to noninvasive blood glucose monitoring is to measure the concentration of glucose in an accessible surrogate fluid, such as tears. Tear glucose in diabetic patients has been studied for over 80 years,[21] and the now widespread use of contact lenses has motivated ideas for their use in tear glucose analysis to detect and monitor glycemic control in patients with diabetes mellitus. At least three independent groups have developed glucose sensors that can be incorporated into contact lenses.[22-25] The safety of daily wear soft contact lenses in diabetic patients has recently been demonstrated,[26,27] suggesting that contact lens monitoring is potentially a viable supplement or alternative to blood glucose monitoring. For this approach to be successful, it is necessary to understand how blood and tear glucose concentrations correlate and to know the underlying physiology of glucose secretion into tears. Currently, significant disagreement exists as to the absolute concentrations of tear glucose in normal and diabetic subjects, as well as to whether tear glucose concentrations correlate with blood glucose concentrations.

Reported values for tear glucose in normal individuals range from 0 to 3.6 mM (65 mg/dL),[28] whereas concentrations as high as 84 mg/dL (4.7 mM) have been reported for patients with diabetes mellitus.[21] The reported tear glucose concentrations are generally lower when the analytical techniques require smaller tear volumes. Recently, median glucose concentrations of 89 μM have been measured in 5 μL tear samples,[29] whereas concentrations of 28 μM have been measured in 1 μL tear samples,[30] all from fasting, nondiabetic individuals. Although older studies generally measured glucose in larger volumes of chemically or mechanically stimulated tears, most recent studies specifically try to avoid conjunctival irritation and tear stimulation (Figure 1).

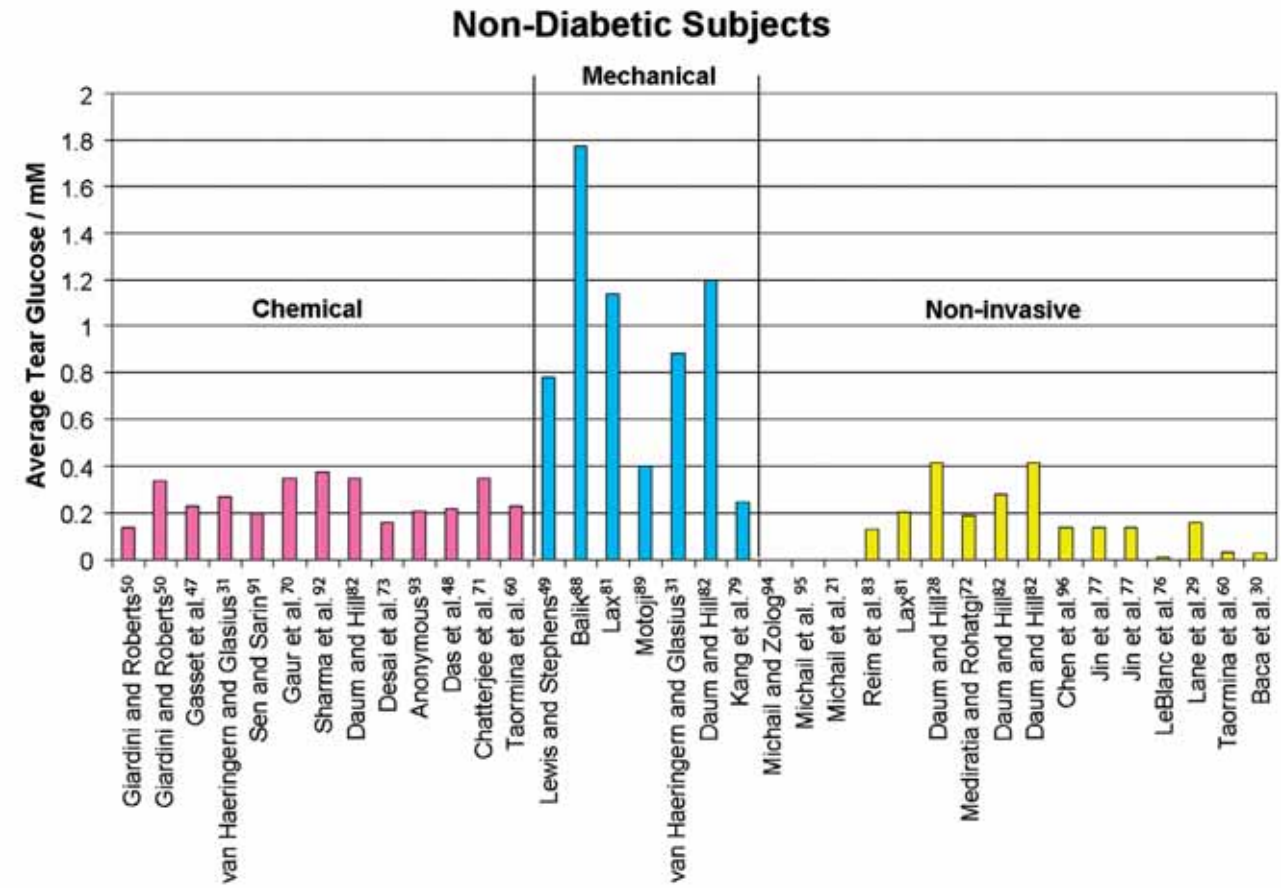

FIGURE 1 Summary of average tear glucose concentrations found in nondiabetic subjects from 1930 to present. Studies are grouped by the type of stimulation used to induce tearing (chemical, mechanical, and noninvasive). These are arranged in chronological order from left to right within each section. The details of each study are discussed in the tables and text. Note that studies employing mechanical stimulation measure the highest tear glucose concentrations.

Although the effect of the collection method on the tear glucose concentration has been considered,[31] there has been little clarification of the discrepancies in reported tear glucose concentrations. The recent interest in developing in situ tear glucose monitors has motivated this review of the current state of glucose sensing in tear fluid.

II. TEAR PRODUCTION AND GLUCOSE TRANSPORT

The tear glucose concentrations measured appear to be determined by both the amount of glucose present and the volume of the aqueous tear fraction collected. Changes in the transport of water in and out of the tear film have as much or greater effect on the tear glucose concentration as changes in glucose transport into the tear film. The structure and function of the tear film, especially as these relate to the regulation of the aqueous tear volume, is central to the determination of tear glucose concentration.

The tear film on the surface of the eye is composed of several layers. Most superficially is a lipid layer that is less than 100 nm thick and serves several functions, including preventing evaporation of the underlying aqueous layer and providing a smooth optical surface over the cornea.[32] This layer is comprised of sterol esters, wax esters, and many other minor lipid components.[33] These are secreted from the meibomian glands located on the margins of the eyelids, just posterior to the eyelashes. Dysfunction of these glands can lead to increased evaporation of tears from the eye, causing an increased tear osmolarity and clinical dry eye.[34] The lipid layer is compromised when contact lenses are worn; this layer may be completely absent over rigid contact lenses.[33]

Just below the lipid layer is the aqueous fraction. Measurements of the thickness of this layer over the cornea vary from 2.7 µm to 46 µm, with the most recently reported value being 3.3 µm.[35] One difficulty in measuring the thickness of this layer is that it changes immediately after a blink, thinning as it distributes over the surface of the eye.[35] In the presence of a contact lens, the aqueous tear film can be measured both in front of the lens (pre-lens) and between the lens and the cornea (post-lens). Both the pre-lens and post-lens tear films are approximately 3 µm thick.[35] With or without a contact lens, the tear film is considerably thicker near the margins of the eyelids, where a meniscus forms. The total volume of the aqueous tear film is about 7 µL.[36] Its production and elimination are discussed below.

Below the aqueous layer of the tear film is a mucin layer, consisting of glycoproteins, which lubricate the surfaces of the eye. At least 20 different mucins are present in this layer. They provide a hydrophilic surface, over which the aqueous fraction can rapidly flow.[37,38] This layer is approximately 30 µm thick,[32] and its components are produced by both the cornea and conjunctiva.[39] The mucin layer moves freely over the glycocalyx, which is comprised of membrane-associated mucins bound to the cornea and conjunctiva.

A. Production and Elimination of Aqueous Tear Fluid

The rate of tear production can vary by a factor of over 100 between basal tear production and active tearing.[40] By studying the clearance of fluorescein, Mishima et al estimated the average rate of tear fluid production to range between 0.5 and 2.2 µL/min (with an average of 1.2 µL/min) at baseline.[36] After the introduction of fluorescein, a rapid decrease in fluorescence was observed that was ascribed to reflex tearing. After about 5 minutes, the fluorescein clearance rate decreased to about 17% per minute. Calculation of the "unstimulated" tear production was based on the lower fluorescein clearance rates seen at later times.[36]

Although estimates of the rate of baseline tear fluid production have not varied significantly since the 1966 report of Mishima et al, the relative contributions of different sources to the aqueous tear fraction continue to be debated. Traditionally, aqueous tear fluid was thought to be almost entirely produced by the main lacrimal gland with very minor contributions from the accessory lacrimal glands and goblet cells in the conjuctiva.[41] Recent studies, however, have shown that the rate of water flow across the conjunctiva can be as high as 1-2 µL/min and may account for a large proportion of basal tear production.[42] Although estimates of the contribution of conjunctival secretion to basal tear fluid production vary widely, recent models of tear production suggest that 25% of tear fluid is produced by the conjunctiva in the absence of reflex tearing.[43] The aqueous fraction in stimulated tears is derived primarily from the lacrimal glands, but the aqueous fraction in unstimulated tears may have a significant conjunctival source.

Elimination of the aqueous fraction of tears can also influence the concentration of tear fluid analytes. The major routes of elimination for aqueous tears are drainage through the lacrimal canaliculi to the nasolacrimal duct and evaporation.[41] Under certain conditions, conjunctival or corneal absorption of water can contribute to the elimination of aqueous tears.[43] During a blink, tears are swept from the lateral canthus toward the medial canthus.[44] Tears then pass into the lacrimal canaliculi, which connect to the lacrimal sac. The tears then drain by gravity through the nasolacrimal duct and into the inferior nasal meatus.[44] Blockage of this drainage system by cautery or with plugs increases the basal tear volume, and is a common treatment for dry eyes.[34]

The rate of tear film evaporation is proportional to the surface area of the eye exposed to the environment.[45] The palpebral fissure, ie, the distance between the upper and lower eyelids, varies between subjects. It can also be increased by lid surgery or by disease states, such as hyperthyroidism.[34]

Disturbances of the lipid tear film can lead to dramatically increased rates of evaporation. When the lipid layer is washed from the eye or if the meibomian glands are occluded, the rate of evaporation increases from 0.1 µL/min to 1.7 µL/min.[32] Rates of evaporation also depend on the ambient temperature and humidity.[32] As noted above, the lipid layer can be compromised by contact lenses, with increased tear evaporation during contact lens use.[40] This increase in evaporation does not appear to vary significantly with contact lens composition or level of hydration.[46] The average increase in the rate of evaporation during contact lens wear is about 35%; however, the rate of evaporation may vary significantly between subjects.[46]

B. Glucose Transport in Tear Fluid

The source of glucose in tears remains unclear. Explanations for the mechanisms of glucose transport into the tear fluid have changed as different tear glucose concentrations were observed. The studies that used mechanically irritative methods to obtain tear fluid found the highest glucose concentrations. Those studies also found a correlation between blood and tear glucose concentrations.[31,47-49] Mechanical irritation abrades the conjunctiva and results in the leakage of glucose from epithelial cells or the interstitial space directly into the tear fluid.[31,41]

Studies that attempt to avoid abrasion of the cornea and stimulation of tearing measure the lowest glucose concentrations.[29-31] It has been suggested that exclusion of glucose from tear fluid aids in preventing bacterial infections of the exterior eye.[50] Many of the studies of chemically stimulated tears find very low concentrations of glucose, suggesting that very little, if any, glucose comes from the lacrimal glands.[31,48,50]

There is only limited evidence for glucose transporters in the tear glands, the conjunctiva, and the cornea. The constitutive glucose transporter, GLUT-1, is present in the apical corneal epithelium,[51] but absent in the lacrimal glands and conjunctiva.[52] Expression of GLUT-1 in the corneal epithelium is increased after corneal abrasion and appears to have a role in corneal wound healing.[53] A sodium/glucose cotransporter, SGLT-1, is present on the apical side of the bulbar and palpebral conjunctiva.[54,55] This transporter operates in both directions, allowing both secretion and absorption of glucose, depending on sodium and glucose concentrations.[56] Although this transporter removes glucose from the tear fluid under physiological conditions,[57] it can add glucose to the tear fluid during the hypo-osmotic stress that occurs when the eye is rinsed with water or after swimming.[28,43,56,57]

Recent models of electrolyte and metabolite transport in the tear fluid suggest that a paracellular transport mechanism is required to fully explain observed electrolyte concentrations.[43] Studies of polyethylene glycol oligomer permeability in the cornea and conjunctiva of rabbits suggest that there are paracellular pores with diameters of ~4 nm and ~2 nm in the conjunctiva and cornea, respectively.[58] Although glucose should be able to pass though these pores and into the tear fluid, its transit has not been directly measured. The distribution and regulation of glucose transporters affecting the tear glucose concentration is not yet fully characterized, but glucose transport across the conjunctiva appears to be the major determinant of tear glucose concentration in the absence of reflex tearing.

C. Stimulated Tears

Increased aqueous tear volumes can be caused by increased tear production (lacrimation) or by decreased tear elimination, which may be caused by obstruction of the tear drainage system or paralysis of the muscles responsible for sweeping tears towards the puncta.[44] Some authors have questioned the existence of a true basal tear production rate, suggesting that all tears are "stimulated."[59] This argument is based on the fact that the rate of tear production decreases dramatically with the administration of anesthesia. However, although tear production from the lacrimal gland may require stimulation, the epithelial cells of the conjunctiva continuously secrete water and ions in response to electrolyte and metabolite concentration gradients.[57] The rate of aqueous tear production can increase over 100-fold in response to a variety of physical or chemical stimuli; the changes in aqueous tear production may alter the chemical composition of tear fluid.[40]

In a 1981 review of tear biochemistry, Van Haeringen recounted several methods of inducing tearing in order to increase the sample volume.[41] Chemical stimulants of lacrimation include onion vapors, ammonia, formalin, benzyl bromide, chloroacetophenone, and bromoacetone.[41] Cigarette smoke and onion vapors have also been used to stimulate tearing.[41,60] Mechanical stimulation of the conjunctiva or cornea with absorbent materials, such as filter paper, cellulose sponges, and cotton thread, is also commonly used to increase tear production.[41,47,49] Because these methods can abrade the conjunctiva, which could alter analyte concentrations, other methods of stimulating lacrimation that do not directly contact the conjunctiva have been investigated. These include stimulating the nasal mucosa, inducing a gag reflex with a tongue depressor, having subjects look into the sun, and inducing emotional tearing.[40,41,59]

Lacrimation in response to physical and chemical stimulation of the ocular surfaces and photo stimulation of the optic nerve is mediated by a reflex arc. Activation of afferent sensory nerves in the conjunctiva, cornea, nasal mucosa, or optic nerve leads to reflexive stimulation of efferent sympathetic and parasympathetic nerves innervating the lacrimal gland.[61,62] Although neurotransmitters from the autonomic efferent nerves, such as acetylcholine and norepinephrine, are the most potent stimulants of tearing, many other minor neuromediators have been identified and recently reviewed.[62,63] Recent immunohistochemical studies suggest that there is direct parasympathetic enervation of goblet cells in the conjunctiva and direct sympathetic enervation of stratified squamous cells in the conjunctiva.[39] Therefore, transport of fluid and mucins directly across the conjunctiva may also lead to increased tear volume and mucin secretion after mechanical stimulation of the conjunctiva.

Clearly, reflex tearing must be avoided if basal tear glucose concentration is to be accurately measured. In practice, this is very difficult, because only small volumes of basal tear fluid are available. Individuals may respond differently to even the most benign collection techniques; the mere discussion of tear fluid collection is sufficient to stimulate tearing in some subjects. There may also be uncontrolled and unrecognized variables affecting tear stimulation in different subjects, such as changes in room temperature or humidity from day to day. In addition to all of these factors, contact lens use can cause irritation and stimulate tear production.[40] This could alter the relevant basal tear glucose concentration measured by a contact lens-based glucose sensor. As contact lens wearers adapt to their lenses, however, this irritation, as measured by blink frequency, decreases.[40]

D. Effects of Diabetes Mellitus on Tear Glucose Transport

Most studies of tear physiology have been conducted on nondiabetic animals and humans. If tear glucose analysis is to be used to detect or monitor diabetes mellitus, we must consider the effects of this disease on aqueous tear production and glucose transport in tear fluid. While the relative importance of different molecular mechanisms in diabetes mellitus pathogenesis is debated, the increased intravascular concentration of glucose that results from diabetes mellitus ultimately leads to microvascular and nerve damage.[64,65] In light of the above discussion of aqueous tear film production, damage to either the vasculature supplying blood to the eye or the nerves of the lacrimal reflex arc might be expected to alter tear production.

Keratoconjunctivitis sicca, or dry eye, can be caused by decreased tear production or alterations in the tear fluid, such as loss of the lipid layer, leading to increased tear osmolarity. Dry eye is more common in diabetic patients, and it correlates with poor glycemic control as measured by mean annual HbA1c levels.[66] When measured by fluorescent dye clearance, basal tear secretion rates are indistinguishable in control and diabetic subjects.[67,68] Reflex tearing, as measured by a Schirmer test without anesthesia, is decreased significantly in diabetic subjects.[67] This decrease in reflex tearing may be due to a decreased sensitivity of the conjunctiva resulting from neuropathy.[69] During episodes of hyperglycemia, increased osmolarity in the extracellular fluid may also impede aqueous tear flux across the conjunctiva or into the lacrimal gland.[66] Other differences noted in the eyes of diabetic subjects include a decreased tear break-up time, loss of mucin-secreting goblet cells, and squamous metaplasia of the conjunctiva, indicating chronic irritation.[69] Overall, diabetes mellitus can affect tear composition and the structure and function of the tissues that contribute to aqueous tear production.

Increased tear glucose concentration in diabetic subjects has been repeatedly demonstrated.[21,29,47-49,70-73] However, many of these studies used filter paper to collect the tear sample, and the observed high glucose levels may be due to intercellular fluid leaking through the abraded conjunctiva.[31] A recent study of tear glucose concentration in 50 nondiabetic subjects and 33 diabetic subjects specifically tried to avoid chemical or mechanical stimulation by collecting tear samples with a glass microcapillary.[29] That study found overlapping ranges of tear glucose concentration in fasting normal and diabetic subjects, but found a statistically significant increase in average tear fluid concentrations in the diabetic subjects (89 μM for normal and 150 μM for diabetic subjects).

Although tear glucose concentrations are clearly increased in diabetic subjects, the precise mechanism remains unclear. Studies using sampling methods that cause mechanical stimulation of the conjunctiva are likely to be simply measuring analytes in direct equilibrium with intercellular fluid. Studies of nonstimulated tears may measure increased glucose due to paracellular glucose transport in the conjunctiva. It is also possible that the reported studies were not entirely successful in avoiding contact with the eye during sampling with glass microcapillaries over the required 5-minute sampling periods.[29,31]

III. MEASUREMENT OF TEARS IN EXTRACTED TEAR FLUID

The chemical components of tears were first studied by Fourcroy and Vauquelin over 200 years ago.[74] The first quantitative report of glucose in tear fluid appears to be from 1930, when Ridley reported tear glucose concentrations of 3.6 mmol/L (65 mg/dL).[75] Although the method of glucose analysis was not described in this study, the tear volume studied was 0.2 mL, suggesting that tearing was induced to collect such large volumes. Reported values of tear glucose concentration have steadily decreased as the analytic methods required less sample volume.

At the opposite extreme, LeBlanc et al recently reported an average tear glucose concentration of 7.25 ± 5.47 μmol/L in five patients in an intensive care unit.[76] Glucose was measured using high performance liquid chromatography with pulsed amperometric detection. The sample volumes studied were variable and not individually reported, but were presumably less than 1 μL.[77,78] The fact that the reported values of tear glucose concentrations have varied by 1000-fold between studies demonstrates the need for careful consideration and control of experimental parameters, such as collection method, analysis method, and selection of the clinical population.

A. Mechanical Stimulation

Because the tear fluid collection method seems to have the greatest influence on the reported tear glucose concentrations, we have grouped the previous studies of tear glucose concentration by collection methodology. As noted above, tear collection methods that mechanically stimulate, and presumably abrade the conjunctiva, measure the highest levels of glucose (Table 1).

These studies generally use a filter paper or Schirmer test strip to collect the tear sample. Glucose is then extracted from the filter paper and measured enzymatically,[31,79] or transferred to a glucose oxidase reagent strip and measured "semiquantitatively" by comparing the color of the strip to a calibrated color chart.[47] More recent investigations have instilled reagent strips in the lower conjunctival sac to more directly measure glucose concentration.[48,80] A limitation of these studies is that color comparison can be subjective, and the subjects could only be stratified into four or five arbitrarily defined groups.[48,49,71,72]

Van Haeringen and Glasius compared glucose concentrations in the chemically and mechanically stimulated tears of normal and diabetic subjects.[31] They first stimulated tearing with 2-chloracetophenone and collected 20 μL tear samples with a capillary tube. They then collected a second tear sample using filter paper strips. The tear glucose concentration determined using filter paper collection was higher for all subjects. The increase was between 0.1 and 1.5 mmol/L for subjects with blood glucose below 10 mmol/L (180 mg/dL) and as high as ~9 mmol/L for

Table 1. Mechanical Stimulation in the Study of Tear Glucose Concentration

Year	Reference	Sample Collection Method	Glucose Measurement Method	Volume of Sample [mL]	NORMAL				DIABETES MELLITUS			
					Range [mM]	Average ± std [mM]	Patients	n†	Range [mM]	Average ± std [mM]	Patients	n†
1958	Lewis and Stephens[49]	Clinistix	King method	0.1	0–2.22	0.78 ± 0.50	13	18	0.56–8.30	2.90 ± 1.90	15	29
1965	Balik[88]	filter paper strips	reduction	0.1	0.25–3.3							
1969	Lax[81]	filter paper strips	enzymatic	NA		1.141 ± 0.159	32	32				
1971	Motoji[89]	filter paper strips	glucose oxidase	NA		0.40 ± 0.09	32	32		0.80 ± 0.24	169	169
1977	van Haeringern and Glasius[31]	filter paper strips	glucose dehydrogenase	NA	0.1–1.5*	0.88	10	10	2–10*	5.36	5	5
1984	Daum and Hill[82]	capillary[a]	glucose dehydrogenase	0.005	1.1–1.4*	1.2*	3	6				
1988	Kang et al[79]	Schirmer strips	glucose oxidase	0.01	0.15–0.45	0.25 ± 0.10	30	30				
1993	Haeckel et al[90]	Schirmer strips	NA	NA		0.056						

* Estimated from graph
[a] Mechanical rubbing of bulbar conjunctiva
† Number of samples collected

extremely hyperglycemic subjects with blood glucose concentrations of ~20 mmol/L (360 mg/dL). A 1969 dissertation by Lax, which is cited by Van Haeringen and Glasius, found glucose concentrations of 0.206 ± 0.027 mmol/L (mean ± SD) using capillary collection and 1.141 ± 0.159 mmol/L using filter paper collection in non-diabetic subjects.[31,81]

Daum and Hill used capillaries to collect 5 µL tear samples at different times from nondiabetic subjects after mechanical stimulation of the conjunctiva with a cotton applicator.[82] They measured an increase in tear glucose concentration from ~0.28 mmol/L (~5 mg/dL) before stimulation to a peak value of ~2.5 mmol/L (~45 mg/dL) 10 minutes after stimulation. Tear glucose concentrations remained elevated at 30 minutes, but returned to baseline at 60 minutes. This time course suggests that caution must be exercised in planning and interpreting tear glucose studies. Unrecognized conjunctival stimulation early in a time course study of tear glucose may affect many subsequent measurements. A similar increased tear glucose concentration after corneal or conjunctival irritation has also been observed in rabbits.[83,84]

Daum and Hill also observed an increase in tear glucose after hypo-osmotic stress induced by immersion of the eye in distilled water for 60 seconds. It is not immediately clear whether this should be considered mechanical or chemical stimulation of tearing. However, the tear glucose response seems similar to that of mechanically stimulated tears.

Studies of mechanically stimulated tears generally find a correlation between the tear and blood glucose. This is not surprising, as the glucose measured in these studies likely comes directly from the interstitial space in the conjunctiva. Rabbit studies suggest that short-term contact lens use increases tear glucose concentrations in a manner similar to other means of mechanical stimulation.[40,84] However, it should be noted that our recent study found no evidence of increased tear glucose concentrations in fasting subjects wearing contact lenses.[30]

B. Chemical and Noncontact Stimulation

Early analytical techniques used to study tear glucose concentration required analyte volumes that would take hours to collect at basal rates of tear secretion. Hence, many studies have used a chemical lachrymator to induce tearing and speed sample collection (Table 2).

The concentration of many analytes is known to vary with tear secretion rate. Protein and calcium concentrations decrease as the flow rate increases, whereas potassium concentrations increase with the flow rate.[41] In a 1984 study,

Daum and Hill collected 5 µL tear samples every 20 seconds for 2 minutes after nondiabetic subjects were exposed to raw onion (Allium Cepa) vapors for 30 seconds.[82] Tear glucose concentration decreased monotonically from 0.3 mmol/L (6 mg/dL) before tearing to 0.1 mmol/L (2 mg/dL) at 2 minutes after exposure.

Comparison between studies that used chemically stimulated tears is difficult, as different lachrymators are used and the precise timing of tear collection is rarely reported. Some of these agents have been shown to cause corneal and conjunctival edema and epithelial erosion or ulceration.[85] Hence, long-term exposure to lachrymators may cause a significant *increase* in glucose concentration as the physical barriers of the ocular surface are compromised. Use of lachrymators is also likely to cause subjects to rub their eyes, which we have anecdotally found to correlate with increased tear glucose concentrations in our recent studies.[30]

Overall, the average tear glucose concentrations measured in studies of chemically stimulated tears fall within the range of tear glucose concentrations measured in nonstimulated tears. Many studies of chemically stimulated tears did not find a general correlation between tear and blood glucose for all subjects.[50] However, the ability to broadly classify subjects as diabetic or nondiabetic through tear glucose measurement was often demonstrated in these studies, especially when postprandial samples were considered.[48,71]

C. Nonstimulated Tears

As more sensitive analytical methods have been developed, investigators have attempted to study glucose concentrations in nonstimulated or basal tears (Table 3). Nonstimulated tear samples are generally collected with a capillary by gently touching it to the tear film meniscus. Daum and Hill trained subjects to collect their own tears throughout the day,[28,82] but most investigators have conducted their investigations by collecting tear samples in a clinical setting.

One of the first large studies of nonstimulated tears measured an average tear glucose concentration of 0.42 ± 0.356 mmol/L in 875 tear samples from 12 nondiabetic subjects.[28] This average tear glucose concentration was somewhat higher than more recent measurements of chemically stimulated tears. Although studies using lachrymators could be measuring artificially low tear glucose concentrations, it is also possible that some mechanical stimulation was involved in the collection of basal tears in this study. The glucose dehydrogenase method used in this study required sample volumes of 5 µL, which would have required sample collection times of at least 5 minutes. Even if mechanical stimulation were avoided during sampling, increased evaporation due to the prolonged suppression of blinking could alter the glucose concentration. One of the interesting results of this study was the observation of diurnal variations in tear glucose that roughly track changes in blood glucose.

One of the largest and most recent studies of tear glucose concentration was reported by Lane et al in 2006.[29] This study monitored tear glucose concentration in 73 normal subjects and 48 diabetic subjects before and after an oral glucose bolus. These groups were further divided into fasting and nonfasting groups. The 5 µL tear samples collected at each time point were analyzed with a liquid chromatography-pulsed amperometric detection method. Average tear glucose concentration in normal subjects was 0.16 ± 0.03 mmol/L, whereas average tear glucose concentration in diabetic subjects was 0.35 ± 0.04 mmol/L (mean ± standard error). Individual glucose determinations, however, varied from below the limit of detection to over 9.1 mmol/L. Lane et al were able to show a modest correlation between average tear and blood glucose concentrations at the five time points in the study. Unfortunately, only results averaged over the subject population were reported. Thus, there is no indication of how well tear and blood glucose concentrations correlate within individual subjects. The use of 5 µL tear samples may have precluded the study of truly nonstimulated tears for the reasons noted previously.

A few recent studies analyzed microliter or submicroliter volumes of tears.[30,60,76,77,86] The previously mentioned study of critically ill patients attempted to assess the feasibility of monitoring tear glucose instead of blood glucose in the intensive care unit.[76] The investigators obtained 44 simultaneous blood and tear samples from five sedated subjects who were receiving insulin, two of whom had a history of diabetes mellitus. This study measured the lowest average tear glucose concentration (7.25 ± 5.47 µmol/L) of any study where glucose was detected in tears. Despite a wide range of blood glucose concentrations, the study did not detect a clinically useful correlation between these values. These results in critically ill patients are of little use in predicting whether tear fluid analysis can be used to monitor glucose in healthy people. However, it is clear that tear glucose monitoring with this method in the intensive care unit is not a feasible replacement for blood glucose monitoring.

A recent study in our laboratory found a median (range) tear glucose concentration of 28 (7–161) µmol/L or 0.50 (0.13–2.90) mg/dL in 25 fasting normal subjects.[30] The mean (standard deviation) tear glucose concentration was 37 ± 37 µmol/L. We found a highly skewed distribution of tear glucose values; tear glucose concentrations were less than 42 µmol/L in 80% of subjects. We found no statistically significant difference between contact lens wearers and nonwearers. Linear regression showed a modest correlation between tear and blood glucose concentrations (R = 0.5). We compared tear glucose concentrations within subjects over 30 minutes and did not see any significant trend with time, suggesting that conjunctival irritation was minimized or eliminated. In our study, glucose concentrations were measured with liquid chromatography and electrospray ionization mass spectrometry. Because we collected only 1 µL tear samples, studied nondiabetic fasting subjects, and observed no evidence of conjunctival stimulation, we believe this to be one of the most precise studies of baseline tear glucose.

As described in detail above, there is very little reliable information on whether glucose concentrations in unstimulated tears track blood glucose concentrations. While previous studies showed correlations between

Table 2. Chemical Stimulation in the Study of Tear Glucose Concentration

Year	Reference	Inducing agent	Sample Collection Method	Glucose Measurement Method	Volume of Sample [mL]	NORMAL†				DISEASE STATE†			
						Range [mM]	Average ± std [mM]	Patients	n¶	Range [mM]	Ave ± std [mM]	Patients	n¶
1950	Giardini and Roberts[50]	tear gas	pyrex tubes	Hagedorn-Jensen	0.22	0.16–0.43	0.34 ± 0.07	12	25				
				fermentation methods	0.22	0.056–0.28	0.14 ± 0.07	12	25				
1968	Gasset et al[47]	spirit of ammonia	capillary	enzymatic	0.005 – 0.020		0.23 ± 0.17	28	50		1.53 ± 0.8†	26	26
1977	van Haeringern and Glasius[31]	2-chloraceto-phenone	capillary	glucose dehydrogenase	.020	0.1–1.0*	0.27	10	10	0.5–1.0*	0.76	5	5
1980	Sen and Sarin[91]	reflex	NA	glucose oxidase	0.1 – 0.2		0.20 ± 0.14	50	50		0.92 ± 0.52[d]	50	50
1982	Gaur et al[70]	spirit of ammonia	capillary	NA	NA		0.35 ± 0.06	50	50		0.74 ± 0.16[e]	25	25
											1.20 ± 0.08	50	50
1983	Sharma et al[92]	spirit of ammonia	capillary	King and Asatoor	0.05	0.18–0.67	0.38 ± 0.14	35	35				
							0.43 ± 0.04c+	25	25		1.05 ± 0.19c	25	25
							0.39 ± 0.07a+	25	25		0.90 ± 0.20[a]	25	25
1984	Daum and Hill[82]	allium cepa, onion	capillary	glucose dehydrogenase	0.005	0.3–0.4*	0.35*	3	10				
1987	Desai et al[73]	ammonium hydroxide	steel cannula	glucose oxidase	> 0.2	0–0.28	0.16 ± 0.11	25	25	0 – 1.6	0.67 ± 0.05	40	40
1988	Anonymous[93]	ammonium hydroxide	NA	glucose oxidase	0.13	0.15–0.26	0.21 ± 0.04	15	15	0.39 – 1.22	0.75 ± 0.23	10	10
1995	Das et al[48]	spirit of ammonia	capillary	glucose oxidase	NA		0.22 ± 0.04				1.00 ± 0.22		
2003	Chatterjee et al[71]	spirit of ammonia	dextrostix	glucose oxidase	NA	0.056–1.00	0.35 ± 0.24	76	76	1.2 – 1.8	1.58 ± 0.17	52	52
										2.1 – 3.8	2.68 ± 0.57	44	44
										4.2 – 5.3	4.67 ± 0.39	16	16
										7.0 – 7.9	7.44 ± 0.67	8	8
										9.4 – 10.2	9.78 ± 0.44	4	4
2007	Taormina et al[60]	allium cepa, onion	capillary	ESI-MS	0.001		0.211 ± 0.008	1	6				

† All disease states reflect diabetes and all normal states reflect normal health, except "a" signifies acute conjunctivitis, "a+" signifies treatment for acute conjunctivitis; "c" signifies corneal ulcer, and "c+" signifies treatment for a corneal ulcer.

† Data listed for highest level of glucose observed; [d] Chemical diabetes; [e] Uncontrolled diabetes; * Estimated from graph

¶ Number of tear samples collected

Table 3. The Study of Glucose Concentration in Nonstimulated Tears

Year	Reference	Sample Collection Method	Glucose Measurement Method	Volume of Sample [mL]	NORMAL				DIABETES MELLITUS			
					Range [mM]	Average ± std [mM]	Patients	n¶	Range [mM]	Average ± std [mM]	Patient	n¶
1937	Michail and Zolog[94]	capillary	NA	NA	0	0	8	8				
1937	Michail et al[95]	capillary	Hagedorn-Jensen	NA	0	0	8	8				
1937	Michail et al[21]	capillary	Hagedorn-Jensen	NA	0	0	5	5	1.78-4.89	3.311 ± 0.906	12	12
1967	Reim et al[83]	capillary	enzymatic	NA		0.13						
1969	Lax[81]	capillary	enzymatic	NA		0.206 ± 0.027	43	43				
1982	Daum and Hill[28]	capillary	glucose dehydrogenase	0.005	0–3.43	0.42 ± 0.36	12	875				
1983	Mediratia and Rohatgi[72]	capillary	glucose oxidase	0.013	0.11–0.33†	0.19 ± 0.04†	30	30	0.67-1.60	1.10 ± 0.26	10	10
1984	Daum and Hill[82]	capillary	glucose dehydrogenase	0.005	0.06–0.90*	0.28[a]	8	48				
						0.42[b]	8	548				
1996	Chen et al[96]	capillary	glucose oxidase	0.0001–0.0005	0.128–0.166	0.139 ± 0.014	6	6				
1997	Jin et al[77]	capillary	CE-LIF	0.0002–0.0005		0.137 ± 0.013[c]; 0.139 ± 0.003[d]	1	1				
2005	LeBlanc et al[76]	capillary	HPLC-pulse amperometry	NA					0.0008–0.021*	0.00725 ± 0.005‡	5[e]	44
2006	Lane et al[29]	capillary	HPLC-pulse amperometry	0.005	0–5.7	0.16 ± 0.03‡	50	50	0-9.1	0.35 ± 0.04‡	33	33
2007	Taormina et al[60]	capillary	ESI-MS	0.001	0.013–0.051	0.032	1	12				
2007	Baca et al[30]	capillary	ESI-MS	0.001	0.007–0.161	0.028[f]	25	25				

† Average concentration and range for all age groups. ‡ Mean given. *Estimated from graph.

[a] Prolonged closed eyes, [b]Open eyes, [c]On column, [d]Precolumn, [e]Critically ill patients receiving insulin, where two had a history of diabetes, [f]Median given.

¶ Number of tear samples collected

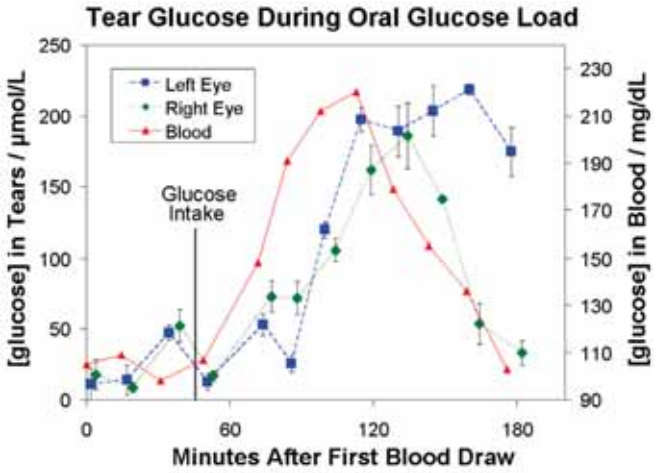

FIGURE 2 Tear and blood glucose concentrations in a non-diabetic, male subject. Blood glucose concentration doubles by ~70 minutes after glucose ingestion.

averaged tear and blood glucose concentrations, there is little direct information about the existence of such a correlation within individual subjects. We recently used our mass spectrometry method to investigate the relationship between blood and tear glucose concentrations in subjects during a glucose tolerance test (oral administration of 75 grams of glucose). Tear samples of 1 µL were collected and analyzed as previously reported, and great care was taken to avoid abrasion of the conjunctiva.[60]

Figure 2 shows the blood and tear glucose concentrations over time for a nondiabetic subject. Tear glucose concentrations appear to track blood glucose concentrations in this subject with a lag time of ~20 minutes. The tear glucose concentration in the left eye remains elevated even after the blood glucose concentration decreases. Although this may be the true physiological response for this subject, it could also be explained by an unrecognized irritation of the left conjunctiva during the latter half of the experiment (140-160 min). An essential point is that while the blood glucose concentration increases ~2-fold, the left

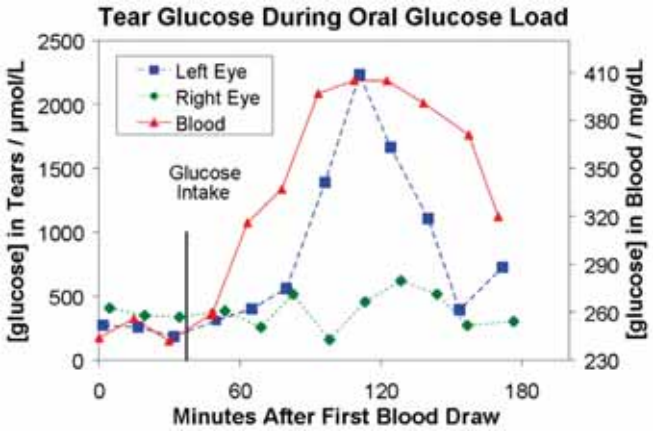

FIGURE 3 Tear and blood glucose concentrations in a diabetic, female subject. Blood glucose concentration peaks at ~80 minutes after glucose ingestion with a ~60% increase.

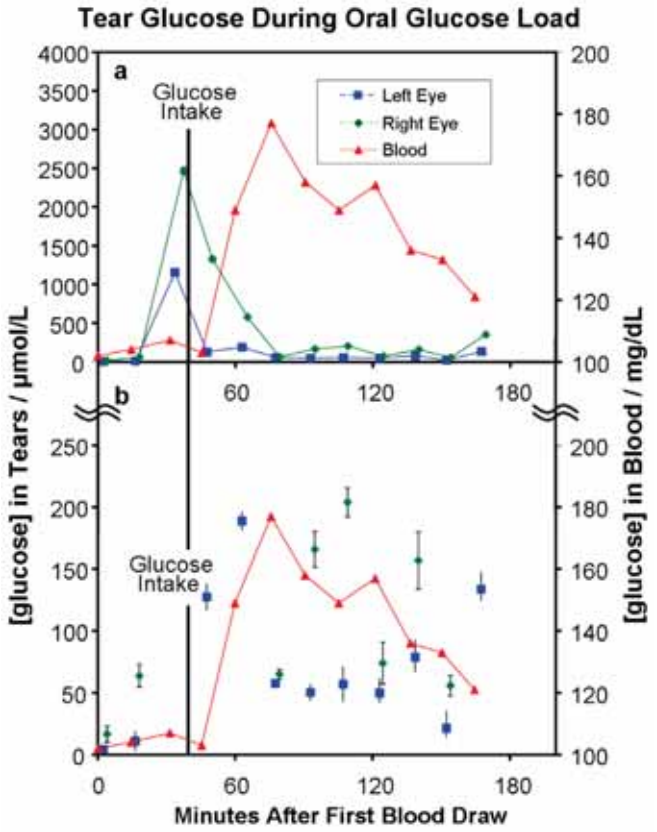

FIGURE 4 Tear and blood glucose concentrations in a replicate of the study shown in Figure 2. a) The tear glucose concentration scale must be expanded to show the large and abrupt increase in tear glucose before glucose intake. Blood glucose concentration peaks at ~40 minutes after glucose ingestion with a ~80% increase. b) Plotting the tear glucose on the same scale as in Figure 2 highlights that the tear glucose concentration appears to track the blood glucose concentration except for the early spike in tear glucose concentrations.

and right eye tear glucose concentrations increase ~7-fold. This demonstrates a complex relationship between tear and blood glucose concentrations for this subject.

Figure 3 shows a similar plot of blood and tear glucose concentrations during a glucose tolerance test for a diabetic subject. Blood and tear glucose concentrations are clearly higher for the diabetic subject at all times. Prior to glucose ingestion, the blood glucose concentration in this subject was about twice as high as for the non-diabetic subject of Figure 2. However, the basal tear glucose concentration of the diabetic subject is about ten times higher than for the nondiabetic subject.

The extraordinary ~5-fold increase in tear glucose concentration in the left eye differs completely from the change in the right eye, where (excepting a single point at ~95 minutes) the ~60% increase in tear glucose concentration is roughly proportional to the increase in blood glucose concentration. It is possible that the dramatic increase in left eye tear glucose concentration results from unrecognized conjunctival irritation, causing interstitial glucose to leak into the tear fluid.

A replicate of the glucose tolerance test shown in Figure 2, with the same nondiabetic subject, demonstrates the challenge of determining a correlation between tear and blood glucose concentration (Figure 4.) Identical sampling

and tear glucose determination procedures were followed, and the same glucose load was given. Before glucose ingestion, the blood glucose concentrations are similar for both experiments (~105 mg/dL). Basal tear glucose concentrations at the earliest times are also similar (~20 μmol/L). In contrast to the first study, we see an abrupt increase in the tear glucose concentration before glucose intake. The right eye tear glucose concentration increases by ~100 fold, while the left eye tear glucose concentration increases by ~50 fold, suggesting nonphysiologic transport of glucose into the tear fluid. The timing and magnitude of this spike in tear glucose concentration suggest that the increase may be due to a perturbation during sampling. Although we attempted to exclude this possibility, the increased glucose concentration in both eyes might suggest that the subject rubbed his eyes after slight conjunctival irritation.

The tear glucose concentrations appear to return to baseline after about 60 minutes. This time scale agrees with the time that Daum and Hill measured for tear glucose to return to baseline after the conjunctiva was stimulated by a cotton applicator.[82] When the results of the replicate study are plotted on the same scale as Figure 2, we see that the tear glucose concentrations at later times (after 60 minutes) appear to track changes in the blood glucose concentration. However, the relative tear glucose elevation (~5-fold) in the left eye is much less than observed in Figure 2, whereas the relative increase in tear glucose concentration in the right eye is comparable.

Most of our studies show that before glucose intake, the blood and tear glucose concentrations remain relatively constant. Repeat measurements of tear glucose concentration in the presence of a constant blood glucose concentration can be used to define the baseline relationship between tear and blood glucose. After glucose ingestion, the blood and tear glucose concentrations generally increase together, with an apparent 20–30-minute delay between increases in blood glucose concentration and in tear glucose concentration.

In our other studies of tear glucose concentration in subjects undergoing a glucose tolerance test, we see only a few instances of the 50-100-fold spikes in tear glucose concentration seen in Figure 4. In general, these abrupt changes do not seem to correlate between the different eyes of the same subject. In future studies it would be desirable to use a hyperglycemic clamp[87] to maintain blood glucose at a constant, elevated concentration while tear glucose is measured repeatedly. This would clarify the relationship between tear and blood glucose concentrations in the presence of elevated blood glucose concentration.

Although most studies of tear and blood glucose correlation collect and analyze tears from a single eye, we measured glucose concentrations in tears from both eyes. Our preliminary results suggest that tear glucose may differ between the left and right eye of a single subject. While we previously showed a general correlation between the tear glucose in the right and left eyes of fasting subjects,[30] we occasionally observe significant differences in glucose concentration between eyes. Future studies that analyze tear glucose in both eyes could help to better define the correlation in tear glucose concentration between left and right eyes.

Although we do not have enough data to specify the precise relationship between tear and blood glucose concentrations over time and its variation between subjects, we believe that our tear collection method, which specifically attempts to avoid mechanical stimulation of the conjunctiva, can answer some of the outstanding questions regarding the utility of tear glucose sensing for monitoring or detecting diabetes mellitus. We have shown that we can track changes in tear and blood glucose concentrations over time. However, our results to date indicate that repeated tear sampling can affect glucose concentrations; it may be extraordinarily difficult to completely and accurately characterize daily tear glucose dynamics with a method that requires the collection of tear samples.

IV. IN SITU TEAR GLUCOSE MEASUREMENTS

There are limited reports of in situ tear glucose determinations using contact lens-based devices. March et al developed and reported the first clinical trial of a contact lens tear glucose sensor.[24] This sensor uses fluorescence to report the tear glucose concentration using a competitive binding mechanism. As the glucose concentration increases in the modified contact lens, quenching groups are displaced and the fluorescent signal increases. In this study, the absolute fluorescent signal was not calibrated to allow the determination of absolute glucose concentrations. Rather, fluorescence intensity changes reported relative changes in tear glucose concentrations. This group also developed a hand-held photofluorometer to monitor the fluorescent signal. March et al reported a glucose tolerance test for five diabetic subjects wearing the sensors. While the fluorescent signal appeared to track the blood glucose concentration, the scale had to be changed for each subject in order for the fluorescence signal to fit the blood glucose concentration profile. This study clearly showed that changes in tear and blood glucose concentration correlate. However, this approach would be unable to predict blood glucose levels in a patient without extensive calibration of the sensor response and of the correlation between tear and blood glucose concentration each time a new contact lens was inserted.

Domschke et al developed a holographic, glucose-sensitive contact lens and tested it in a single subject.[25] The wavelength of light diffracted from the contact lens changed as the holographic spacing changed in response to glucose binding. A red-shift in diffracted wavelength indicated an increase in glucose concentration. This sensor motif eliminates the challenge of measuring absolute fluorescence intensities. This sensor also detects relative changes in tear glucose concentration. Domschke et al did not show a calibration curve for the diffracted wavelength dependence on glucose, and they only reported the peak diffraction wavelength as a function of time. The peak diffraction wavelength appears to track the increasing blood glucose concentration with little or no delay. These results,

although clearly preliminary, indicate the potential utility of monitoring tear glucose concentrations in order to determine blood glucose concentrations. Larger studies are needed to evaluate the possibility of long-term tear glucose monitoring with these contact lens sensors.

V. SUMMARY AND CONCLUSIONS

Any future studies that measure ex vivo tear glucose concentrations must use nonstimulating or minimally stimulating collection methods. As reviewed here, the literature clearly demonstrates spuriously high tear glucose determinations with use of paper strip collection methods that contact the conjunctiva. Although it is clear that measured tear glucose concentration can vary throughout the day[28] and from eye to eye,[30] it is still not clear to what extent these variations are biological and to what extent they are introduced by the sampling method. Further studies of conjunctiva and lacrimal gland immunohistochemistry along with the cell signaling mechanisms behind glucose transport in these tissues may help to better discriminate between biological and sampling variations.

Although several large studies have found evidence for a correlation between averaged tear and blood glucose concentrations, the nature of this correlation within individuals is not yet fully characterized. The limited studies of critically ill patients, which showed little blood-tear glucose correlation within individuals, contrasts with the clinical studies of contact lens-based glucose sensors, which showed significant correlations between blood and tear glucose concentrations.

There is clearly enough evidence of a correlation between tear and blood glucose to justify continued efforts to develop contact lens glucose sensors. Due to the difficulties of collecting tear samples without altering the glucose concentration, it may be difficult to predict how well these sensors will work on the basis of ex vivo glucose analysis alone. Only well-designed in vivo animal studies or further clinical trials of contact lens sensors in humans will be able to determine the potential utility of glucose-sensing contact lens sensors to help achieve glycemic control through noninvasive glucose monitoring.

REFERENCES

1. National Institute of Diabetes and Digestive and Kidney Diseases. *National Diabetes Statistics fact sheet: general information and national estimates on diabetes in the United States.* http://diabetes.niddk.nih.gov/dm/pubs/statistics/index.htm#7 (Accessed 6/14/2007)
2. World Health Organization. *Fact sheet N°312.* http://www.who.int/mediacentre/factsheets/fs312/en/ (Accessed 6/14/2007)
3. American Diabetes Association. Economic Costs of Diabetes in the U.S. in 2002. *Diabetes Care* 2003;26:917-32
4. Shamoon H, Duffy H, Fleischer N, et al. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes-mellitus. *N Engl J Med* 1993;329:977-86
5. American Diabetes Association. Standards of medical care in diabetes. *Diabetes Care* 2004;27:15S-35S
6. Khalil OS. Noninvasive glucose measurement technologies: an update from 1999 to the dawn of the new millennium. *Diabetes Technol Ther* 2004;6:660-97
7. Garg S, Jovanovic L. Relationship of fasting and hourly blood glucose levels to HbA1c values: safety, accuracy, and improvements in glucose profiles obtained using a 7-day continuous glucose sensor. *Diabetes Care* 2006;29:2644-9
8. Deiss D, Bolinder J, Riveline J-P, et al. Improved glycemic control in poorly controlled patients with type 1 diabetes using real-time continuous glucose monitoring. *Diabetes Care* 2006;29:2730-2
9. Malin SF, Ruchti TL, Blank TB, et al. Noninvasive prediction of glucose by near-infrared diffuse reflectance spectroscopy. *Clin Chem* 1999;45:1651-8
10. Gabriely I, Wozniak R, Mevorach M, et al. Transcutaneous glucose measurement using near-infrared spectroscopy during hypoglycemia. *Diabetes Care* 1999;22:2026-32
11. Dou XM, Yamaguchi Y, Yamamoto H, et al. Biological applications of anti-Stokes Raman spectroscopy: Quantitative analysis of glucose in plasma and serum by a highly sensitive multichannel Raman spectrometer. *Appl Spectrosc* 1996;50:1301-6
12. Shafer-Peltier K, Haynes C, Glucksberg M, Van Duyne R. Toward a glucose biosensor based on surface-enhanced Raman scattering. *J Am Chem Soc* 2003;125:588-93
13. March WF, Rabinovitch B, Adams RL. Noninvasive glucose monitoring of the aqueous-humor of the eye. Part II. Animal studies and the scleral lens. *Diabetes Care* 1982;5:259-65
14. MacKenzie H, Ashton H, Spiers S, et al. Advances in photoacoustic noninvasive glucose testing. *Clin Chem* 1999;45:1587-95
15. Larin K, Eledrisi M, Motamedi M, Esenaliev R. Noninvasive blood glucose monitoring with optical coherence tomography -- a pilot study in human subjects. *Diabetes Care* 2002;25:2263-7
16. Russell R, Pishko M, Gefrides C, et al. A fluorescence-based glucose biosensor using concanavalin A and dextran encapsulated in a poly(ethylene glycol) hydrogel. *Anal Chem* 1999;71:3126-32
17. Badugu R, Lakowicz JR, Geddes CD. Boronic acid fluorescent sensors for monosaccharide signaling based on the 6-methoxyquinolinium heterocyclic nucleus: progress toward noninvasive and continuous glucose monitoring. *Bioorgan Med Chem* 2005;13:113-9
18. Aslan K, Lakowicz JR, Geddes CD. Nanogold-plasmon-resonance-based glucose sensing. *Anal Biochem* 2004;330:145-55
19. Caduff A, Hirt E, Feldman Y, et al. First human experiments with a novel noninvasive, non-optical continuous glucose monitoring system. *Biosens Bioelectron* 2003;19:209-17
20. The Diabetes Research in Children Network Study Group. A Randomized Multicenter Trial Comparing the GlucoWatch Biographer With Standard Glucose Monitoring in Children With Type 1 Diabetes. *Diabetes Care* 2005;28:1101-6
21. Michail D, Vancea P, Zolog N. Lacrimal elimination of glucose in diabetic patients. *C R Soc Biol Paris* 1937;125:194-5
22. Alexeev VL, Das S, Finegold DN, Asher SA. Photonic crystal glucose-sensing material for noninvasive monitoring of glucose in tear fluid. *Clin Chem* 2004;50:2353-60
23. Badugu R, Lakowicz J, Geddes C. Ophthalmic glucose monitoring using disposable contact lenses—a review. *J Fluoresc* 2004;14:617-33
24. March WF, Mueller A, Herbrechtsmeier P. Clinical trial of a noninvasive contact lens glucose sensor. *Diabetes Technol Ther*

2004;6:782-9
25. Domschke A, March WF, Kabilan S, Lowe C. Initial clinical testing of a holographic noninvasive contact lens glucose sensor. *Diabetes Technol Ther* 2006;8:89-93
26. O'Donnell C, Efron N, Boulton AJ. A prospective study of contact lens wear in diabetes mellitus. *Ophthal Physiol Opt* 2001;21:127-38
27. March W, Long B, Hofmann W, et al. Safety of contact lenses in patients with diabetes. *Diabetes Technol Ther* 2004;6:49-52
28. Daum KM, Hill RM. Human tear glucose. *Invest Ophthalmol Vis Sci* 1982;22:509-14
29. Lane JD, Krumholz DM, Sack RA, Morris C. Tear glucose dynamics in diabetes mellitus. *Curr Eye Res* 2006;31:895-901
30. Baca JT, Taormina CR, Feingold E, et al. Mass spectral determination of fasting tear glucose concentrations in nondiabetic volunteers. *Clin Chem* 2007;53:1370-2
31. Van Haeringen NJ, Glasius E. Collection method-dependent concentrations of some metabolites in human tear fluid, with special reference to glucose in hyperglycemic conditions. *Albrecht von Graefes Arch Klin Exp Ophthalmol* 1977;202:1-7
32. Bron AJ, Tiffany JM, Gouveia SM, et al. Functional aspects of the tear film lipid layer. *Exp Eye Res* 2004;78:347-60
33. Driver PJ, Lemp MA. Meibomian gland dysfunction. *Surv Ophthalmol* 1996;40:343-67
34. Gilbard JP. The scientific context and basis of the pharmacologic management of dry eyes. *Ophthalmol Clin N Am* 2005;18:475-84
35. King-Smith PE, Fink BA, Hill RM, et al. The thickness of the tear film. *Curr Eye Res* 2004;29:357–68
36. Mishima S, Gasset A, Klyce S. Determination of tear volume and tear flow. *Invest Ophthalmol* 1966;5:264–76
37. Ohashi Y, Dogru M, Tsubota K. Laboratory findings in tear fluid analysis. *Clin Chim Acta* 2006;369:17-28
38. Argueso P, Gipson IK. Epithelial mucins of the ocular surface: structure, biosynthesis and function. *Exp Eye Res* 2001;73:281-9
39. Dartt DA. Regulation of mucin and fluid secretion by conjunctival epithelial cells. *Prog Retin Eye Res* 2002;21:555-76
40. Farris RL. Tear analysis in contact lens wearers. *Trans Am Ophthalmol Soc* 1985;83:501-45
41. Van Haeringen NJ. Clinical biochemistry of tears. *Surv Ophthalmol* 1981;26:84-96
42. Li Y, Kuang K, Yerxa B, et al. Rabbit conjunctival epithelium transports fluid, and P2Y22 receptor agonists stimulate Cl{-} and fluid secretion. *Am J Physiol Cell Physiol* 2001;281:C595-602
43. Zhu H, Chauhan A. Tear dynamics model. *Curr Eye Res* 2007;32:177-97
44. Crick RP, Khaw PT. *Textbook of clinical ophthalmology: a practical guide to disorders of the eyes and their management*. River Edge, NJ, World Scientific Pub, 2003, ed 3
45. Rolando M, Refojo MF. Tear evaporimeter for measuring water evaporation rate from the tear film under controlled conditions in humans. *Exp Eye Res* 1983;36:25-33
46. Thai LC, Tomlinson A, Doane M. Effect of contact lens materials on tear physiology. *Optom Vis Sci* 2004;81:194-204
47. Gasset AR, Braverman LE, Fleming MC, et al. Tear glucose detection of hyperglycemia. *Am J Ophthalmol* 1968;65:414-20
48. Das BN, Sengupta S, Das BK, Goswami NR. Tear glucose estimation--an alternative to blood glucose estimation. *J Indian Med Assoc* 1995;93:127-8
49. Lewis JG, Stephens PJ. Tear glucose in diabetes. *Br J Ophthalmol* 1958;42:754-8
50. Giardini A, Roberts JR. Concentration of glucose and total chloride in tears. *Br J Ophthalmol* 1950;34:737-43
51. Kumagai AK, Glasgow BJ, Pardridge WM. GLUT1 glucose transporter expression in the diabetic and nondiabetic human eye. *Invest Ophthalmol Vis Sci* 1994;35:2887-94
52. Harik SI, Kalaria RN, Whitney PM, et al. Glucose transporters are abundant in cells with "occluding" junctions at the blood-eye barriers. *Proc Natl Acad Sci USA* 1990;87:4261-4
53. Lu L, Reinach PS, Kao WW. Corneal epithelial wound healing. *Exp Biol Med* 2001;226:653-64
54. Turner HC, Alvarez LJ, Bildin VN, Candia OA. Immunolocalization of Na-K-ATPase, Na-K-Cl and Na-glucose cotransporters in the conjunctival epithelium. *Curr Eye Res* 2000;21:843-50
55. Hosoya K, Lee VH, Kim KJ. Roles of the conjunctiva in ocular drug delivery: a review of conjunctival transport mechanisms and their regulation. *Eur J Pharm Biopharm* 2005;60:227-40
56. Eskandari S, Wright EM, Loo DD. Kinetics of the reverse mode of the Na+/Glucose cotransporter. *J Membrane Biol* 2005;2005:23-32
57. Candia OA. Electrolyte and fluid transport across corneal, conjunctival and lens epithelia. *Exp Eye Res* 2004;78:527-35
58. Hamalainen KM, Kananen K, Auriola S, et al. Characterization of paracellular and aqueous penetration routes in cornea, conjunctiva, and sclera. *Invest Ophthalmol Vis Sci* 1997;38:627-34
59. Jordan A, Baum J. Basic tear flow. Does it exist? *Ophthalmology* 1980;87:920-30
60. Taormina CR, Baca JT, Asher SA, et al. Analysis of tear glucose concentration with electrospray ionization mass spectrometry. *J Am Soc Mass Spectrom* 2007;18:332-6
61. Zoukhri D. Effect of inflammation on lacrimal gland function. *Exp Eye Res* 2006;82:885-98
62. Dartt D. Interaction of EGF family growth factors and neurotransmitters in regulating lacrimal gland secretion. *Exp Eye Res* 2004;78:337-45
63. Hodges RR, Dartt DA. Regulatory pathways in lacrimal gland epithelium. *Int Rev Cytol* 2003;231:129-96
64. Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus. *JAMA* 2002;287:2563-9
65. King GL, Brownlee M. The cellular and molecular mechanisms of diabetic compliations. *Endocrinol Metab Clin North* 1996;25:255-70.
66. Kaiserman I, Kaiserman N, Nakar S, Vinker S. Dry eye in diabetic patients. *Am J Ophthalmol* 2005;139:498-503
67. Goebbels M. Tear secretion and tear film function in insulin dependent diabetics. *Br J Ophthalmol* 2000;84:19-21
68. Stolwijk TR, van Best JA, Lemkes HH, et al. Determination of basal tear turnover in insulin-dependent diabetes mellitus patients by fluorophotometry. *Int Ophthalmol* 1991;15:337-82
69. Dogru M, Katakami C, Inoue M. Tear function and ocular surface changes in noninsulin-dependent diabetes mellitus. *Ophthalmology* 2001;108:586-92
70. Gaur MS, Sharma GK, Kabra SG, et al. Tear glucose in uncontrolled and chemical diabetics. *Indian J Ophthalmol* 1982;30:367-9
71. Chatterjee PR, De S, Datta H, et al. Estimation of tear glucose level

and its role as a prompt indicator of blood sugar level. *J Indian Med Assoc* 2003;101:481-3
72. Mediratia RK, Rohatgi JN. Glucose-estimation in tear fluid--its diagnostic significance--a preliminary study. *Indian J Ophthalmol* 1983;31:635-8
73. Desai BM, Desai MT, Lavingia BC. Tear and blood glucose levels in diabetes mellitus. *J Assoc Physicians India* 1987;35:576-7
74. Fourcroy A, Vauquelin L. Examen Chimique des Larmes et de l'Humeur des Narines. *Annales de Chimie* 1791;10:113-30
75. Ridley F. The intraocular pressure and drainage of the aqueous humor. *Br J Exp Pathol* 1930;11:217-40
76. LeBlanc JM, Haas CE, Vicente G, Colon LA. Evaluation of lacrimal fluid as an alternative for monitoring glucose in critically ill patients. *Intensive Care Med* 2005;31:1442-5
77. Jin Z, Chen R, Colon LA. Determination of glucose in submicroliter samples by CE-LIF using precolumn or on-column enzymatic reactions. *Anal Chem* 1997;69:1326-31
78. Lee YC. Carbohydrate analyses with high-performance anion-exchange chromatography. *J Chromatogr A* 1996;720:137-49
79. Kang J, Fulop G, Friedman AH. Tear urea nitrogen and creatinine levels in renal patients. Acta Ophthalmol 1988;66:407-12
80. Romano A, Rolant F. A noninvasive method of blood glucose evaluation by tear glucose measurement, for the detection and control of diabetic states. *Metab Pediatr Syst Ophthalmol* 1988;11:78-80
81. Lax F. *Uber die Spiegel einiger Metaboliten in der Tranenflussigkeit, Inagural-Dissertation [Inaugural-Dissertation]:* Marburg, 1969
82. Daum KM, Hill RM. Human tears: glucose instabilities. *Acta Ophthalmol* 1984;62:530-6
83. Reim M, Lax F, Lichte H, Turss R. Steady state levels of glucose in the different layers of the cornea, aqueous humor, blood and tears in vivo. *Ophthalmologica* 1967;154:39-50
84. Kilp H, Heisig B. Glucose and lactate concentration in tears of rabbits following mechanical stress and wearing of contact lenses. *Albrecht von Graefes Arch Klin Exp Ophthalmol* 1975;193:259-67
85. Olajos EJ, Salem H. Riot control agents: pharmacology, toxicology, biochemistry and chemistry. *J Appl Toxicol* 2001;21:355-91
86. Perez SA, Colon LA. Determination of carbohydrates as their dansylhydrazine derivatives by capillary electrophoresis with laser-induced fluorescence detection. *Electrophoresis* 1996;17:352-8
87. Elahi D. In praise of the hyperglycemic clamp. A method for assessment of beta-cell sensitivity and insulin resistance. *Diabetes Care* 1996;19:278-86
88. Balik J. Method for the biochemical study of tears. *Acta Ophthalmol* 1965;43:190-8
89. Motoji K. The glucose content of tear fluid in normal and diabetic subjects. *Jpn J Clin Ophthalmol* 1971;25:1945-50
90. Haeckel R, Hanecke P. The application of saliva, sweat and tear fluid for diagnostic purposes. *Ann Biol Clin* 1993;51:903-10
91. Sen DK, Sarin GS. Tear gluocose levels in normal people and diabetic patients. *Br J Ophthalmol* 1980;64:693-5
92. Sharma GK, Kabra SG, Sharma PK, et al. Tear glucose in ocular inflammations and its enzymatic lysis as a possible therapeutic adjunct in treatment of ocular infections. *Indian J Ophthalmol* 1983;31:563-5
93. Anonymous. Tear glucose level in diabetes mellitus--a preliminary study. *J Assoc Physicians India* 1988;36:183-4
94. Michail D, Zolog N. Lacrimal elimination of glucose during alimentary hyperglycemia. *CR Soc Biol Paris* 1937;126:1042-3.
95. Michail D, Vancea P, Zolog N. Lachrymal elimination of glucose during adrenaline hyperglucemia. *CR Soc Biol Paris* 1937;125:1095-6
96. Chen R, Jin Z, Colon LA. Analysis of tear fluid by CE/LIF: A non-invasive approach for glucose monitoring. *J Capillary Electrophor* 1996;3:243-8.