



Association between tear and blood glucose concentrations: Random intercept model adjusted with confounders in tear samples negative for occult blood

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Keywords

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ABSTRACT

Aims/Introduction: To prevent diabetic complications, strict glucose control and frequent monitoring of blood glucose levels with invasive methods are necessary. We considered the monitoring of tear glucose levels might be a possible method for non-invasive glucose monitoring. To develop tear glucose monitoring for clinical application, we investigated the precise correlation between the blood and tear glucose concentrations.

Materials and Methods: A total of 10 participants and 20 participants with diabetes were admitted, and blood and tear samples were collected. Before statistical analysis, we eliminated tear samples contaminated with blood. We observed the daily blood and tear glucose dynamics, and carried out a random intercept model analysis to examine the association between the blood and tear glucose concentrations.

Results: Tear occult blood tests showed that the tear glucose concentrations and their variation increased in both participants with and without diabetes as contamination of blood increased. In both participants with and without diabetes, fluctuations of the plasma glucose concentrations were observed depending on the timing of collection of the samples, and the dynamics of the tear glucose concentrations paralleled those of the plasma glucose concentrations. The random intercept model analysis showed a significant association between the plasma and tear glucose concentrations in participants with diabetes ($P < 0.001$). This association still existed even after adjusting for the glycated hemoglobin levels and the prandial state ($P < 0.001$).

Conclusions: It is important to eliminate the tear samples contaminated with blood. Tear glucose monitoring might be a reliable and non-invasive substitute method for monitoring the blood glucose concentrations for diabetes patients, irrespective of glycated hemoglobin levels and timing of sample collection.

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INTRODUCTION

According to an estimate of the International Diabetes Federation, in 2017, there were 425 million diabetes patients aged 20–79 years, and 4 million deaths were caused by diabetes worldwide. It is predicted that by 2045, the number of diabetes patients would increase to 629 million¹.

Clinical trials, such as the Diabetes Control and Complications Trial and United Kingdom Prospective Diabetes Study 33, have shown that strict glucose control is important to minimize the risk of microvascular complications, such as retinopathy, nephropathy and neuropathy^{2,3}. The United Kingdom Prospective Diabetes Study 80 showed that intensive glucose control reduces the risk of macrovascular complications, such as myocardial infarction and diabetes-related death, in the follow-up period⁴.

To achieve strict glucose control, it is necessary for diabetes patients to measure their blood glucose levels frequently⁵. However, all currently available methods for blood glucose monitoring, including the finger prick method, are invasive. Therefore, non-invasive blood glucose monitoring approaches have been investigated, but to date, none has been found to be a suitable substitute for direct glucose measurement in blood samples⁶. For example, one of the currently commercially available continuous glucose monitoring systems, which allows continuous measurement of the interstitial fluid glucose concentration, requires needling to place the sensor under the skin. In addition, for some of these systems, calibration to ensure the accuracy of the sensors necessitates measurement of the blood glucose levels (with invasive finger pricks) at least four times a day. Salivary glucose monitoring has been reported⁷; however, the association between the blood glucose concentrations and salivary glucose concentrations in humans has not yet been sufficiently investigated, and further work is required to establish evidence of the association between blood glucose concentrations and salivary glucose concentrations.

Another possible non-invasive glucose monitoring approach is tear glucose monitoring. Tear glucose concentrations in diabetes patients have been studied since 1937⁸, and many studies have reported that tear glucose concentrations are higher in diabetes patients than in participants with normal glucose tolerance^{9–13}. In 1959, Lewis *et al.*⁹ showed higher tear glucose concentrations in diabetes patients with hyperglycemia using enzymatic urine glucose test strips. In the 1980s, some studies used quantitative enzymatic methods to show the correlation between the blood glucose concentrations and tear glucose concentrations^{11,12}. The dynamics of the tear glucose concentrations within a single day have been reported, with the trend shown to be the same as that of the blood glucose concentrations^{12,13}.

One of the problems is that the order of the tear glucose concentrations varies widely among reported studies. For example, while the mean tear glucose concentration in participants with normal glucose tolerance was reported to be 0.032 mmol/L in one study¹⁴, another study reported a corresponding value

of 0.160 mmol/L¹¹. One possible reason is the difference in the type of stimulation (chemical, mechanical or non-invasive) used among studies to induce tearing¹⁵. It is possible that the discrepancies in the tear glucose levels are derived from the differences in the methods of glucose measurement. In particular, the possibility of blood contamination is higher for tear samples with high glucose concentrations. It could also be attributable to the insufficient standardization of the conditions for tear sample collection, such as the time of day and the prandial state of the participants.

Some studies have shown linear regression formulas between blood glucose concentrations and tear glucose concentrations^{13,16}. The correlations were statistically significant; however, the precision was insufficient to predict the blood glucose concentrations from tear glucose concentrations.

For clinical development of tear glucose monitoring as a tool for predicting the blood glucose concentrations, it is important to investigate the precise correlation between the blood glucose concentrations and tear glucose concentrations. In the present study, 10 participants without diabetes and 20 participants with diabetes were admitted to the University of Tokyo Hospital, Tokyo, Japan, overnight on two occasions, and blood and tear samples were collected (Figure 1). We observed the daily blood and tear glucose dynamics, and examined the association between the blood and tear glucose concentrations by random intercept model analysis with adjusting for confounders, using tear samples negative for occult blood.

METHODS

This observational study was carried out with the approval of the research ethics committee of the University of Tokyo Hospital (11374-(1)), and in accordance with the principles of the Declaration of Helsinki. Written informed consent to this study was obtained from all participants after explanation.

Participants

We recruited men without diabetes and men with type 2 diabetes mellitus aged ≥ 20 years for this study. The exclusion criteria, overall, included wearing of contact lenses, presence of ocular disorders, history of using eye drops, carriers of syphilis, hepatitis B virus, hepatitis C virus or human immunodeficiency virus and difficulty in the collection of tear samples. The exclusion criteria for the participants without diabetes included participants previously diagnosed as having diabetes and fasting blood glucose levels of ≥ 7 mmol/L. The exclusion criteria for the participants with diabetes included glycated hemoglobin (HbA1c) levels of < 42 mmol/mol (6.0%) or ≥ 75 mmol/mol (9.0%), a history of receiving insulin and the presence of severe diabetic complications (Table S1). The reason for limiting our participants to those with HbA1c levels of < 75 mmol/mol (9.0%) was that we were concerned about the safety of study participants with severe hyperglycemia during hospitalization. HbA1c (International Federation of Clinical Chemistry and

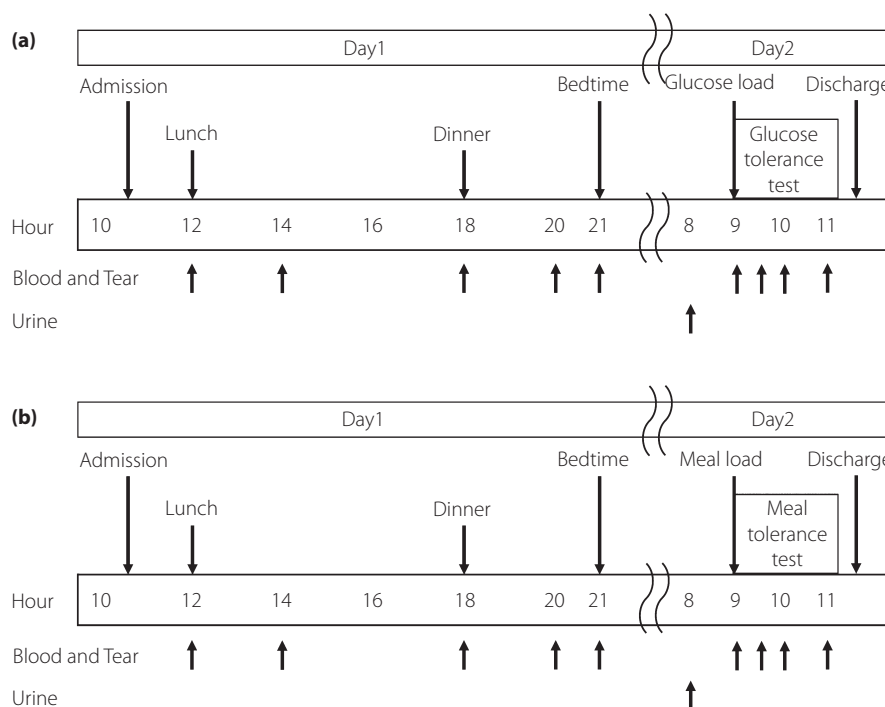


Figure 1 | Schedule of sample collection. (a) Participants without diabetes. (b) Participants with diabetes. All participants were admitted to the hospital on two occasions according to this schedule.

Laboratory Medicine [IFCC]) was converted from HbA1c (National Glycohemoglobin Standardization Program) by $IFCC = (10.93 \times NGSP) - 23.50$.

Schedule of sample collection

All samples were collected at the Phase 1 Unit, the University of Tokyo Hospital. The time course of sample collection for and participants with and without diabetes is shown in Figure 1. Both the participants with and without diabetes were admitted for 1 night to our hospital on two occasions within 1 month. On the day of admission, the participants without diabetes came to the hospital after fasting overnight, and the participants with diabetes took breakfast and their regular oral hypoglycemic medication(s) in the morning. Tear and blood samples were obtained before lunch, 2 h after lunch, before dinner, 2 h after dinner and before bedtime. On day 2 of admission, an oral glucose tolerance test was carried out in the participants without diabetes, and a meal tolerance test in the participants with diabetes, after overnight fasting. Tear and blood samples were collected before the glucose/meal load, and 30, 60 and 120 min after the load. Urine samples were also collected early in the morning before the glucose/meal load on day 2 of admission.

Meals during the admissions

The participants without diabetes consumed a normal diet, whereas the participants with diabetes consumed a diet restricted in calories to 7,531 kJ/day. All the participants were

prohibited from having any food(s) other than that provided by the hospital. The meals in the second admission were the same as those in the first admission.

Blood and urine sampling and measurement

Blood samples were collected in vacuum-sealed blood collection tubes. The glucose, HbA1c, insulin and creatinine concentrations in the blood samples were measured by SRL, Inc. (Tokyo, Japan). The urinary albumin and creatinine concentrations were also measured by SRL, Inc.

Tear sampling and measurement

Tear samples were collected with Microcaps micro capillaries (Drummond Scientific Co., Broomall, PA, USA). The tear fluid meniscus in the lower eyelid was gently touched with the micro capillary, and the tear fluid was drawn in by capillary action. Tear samples were collected from either the left or right eye. Chemical or mechanical tear stimulation was not used. We collected the tear samples immediately (within 10 min) after collecting the blood samples. All the tear samples were frozen at -80°C until laboratory measurement. A high-pressure liquid chromatography method (e2695 Separations Module; Water Co., Tokyo, Japan) was used to measure the tear fluid glucose concentrations. The same standard glucose solutions were measured by both the methods used to measure the blood glucose concentrations (SRL, Inc.) and the tear glucose concentrations (high-pressure liquid chromatography) to confirm the

correlations of the results between the methods (Figure S1). If the volume of the tear samples was insufficient for measurement, samples were diluted to 20 μL with water before measurements. All the samples were divided into six portions, and the measurements were carried out on 6 days. On each day, calibration curves were drawn with the standard solutions before and after the measurements, to confirm the intraday and interday accuracy (Figure S2a–f).

Tear occult blood test

In many previous studies, it is known that the tear glucose concentrations vary widely^{9–13}. Considering the possibility of blood contamination, we tested the samples for occult blood. To verify the validity of the application of urine test strips to tears, we used artificial tears (Artificial Tear Mytear Ophthalmic Solution; Senju Pharmaceutical Co., Ltd., Osaka, Japan) and hemoglobin standard substances (Interference Check A plus; Sysmex Corporation, Hyogo, Japan; Figure S3). The tear samples were first tested for occult blood using urine test strips (Terumo Uriace KC; Terumo Corp., Tokyo, Japan) and classified into five groups (occult blood –, +, ++, +++ and unmeasurable due to a shortage of samples; 56.3, 11.7, 20.0, 7.4 and 4.6%, respectively). The tear samples negative for occult blood were used for statistical analyses.

Statistical analysis

Age and laboratory data on admission, except overt proteinuria, were expressed as the mean \pm standard deviation, and statistically compared between participants with and without diabetes by Welch's *t*-test, because the Kolmogorov–Smirnov test showed that some variables did not follow normal distribution (Table S2). We carried out Student's *t*-test for comparison between preprandial and 2-h postprandial plasma and tear glucose levels on the first day between participants with and without diabetes. The daily plasma and tear glucose dynamics are shown, with the data expressed as the mean \pm standard error of the mean. Receiver operating characteristic (ROC) analysis was carried out to verify its applicability to screening for hyperglycemia, which was defined as plasma glucose concentrations of >10 mmol/L in the present study. In this study, as 18 blood and tear samples were collected from each participant, the data showed a nested structure (i.e., the observations in each participant were not mutually independent). To properly analyze such nested data for evaluating the associations between the tear glucose and blood glucose concentrations, we used a random intercept model and random slope model (also called multilevel model, random effects model). The random intercept model decomposes the total variance of outcome into within-participant fluctuations (i.e., residual variances) and between-participant differences (i.e., random intercepts), whereas the random slope model decomposes the same variance into within-participant fluctuations (i.e., residual variances) and between-participant differences in terms of slopes (random slopes). Furthermore, confounders (HbA1c level and prandial state

[preprandial/postprandial] of the participants) of the association between the plasma and tear glucose concentrations were included to the random intercept model. All the statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS) version 22.0 (IBM Corp., Chicago, IL, USA). A significance level of $\alpha = 0.05$ was set for all the analyses.

RESULTS

Enrollment and outcomes

From 1 November 2016 to 31 January 2017, 10 participants without diabetes and 20 participants with diabetes were enrolled in the present study. The main exclusion criteria were unacceptably low or unacceptably high HbA1c levels (11 participants with diabetes), difficulty in the collection of tear samples (three participants without diabetes and three participants with diabetes), and withdrawal of consent by the participant (three participants with diabetes; Figure 2). All the participants completed the study without dropouts.

Characteristics of the participants

The age and laboratory data of the participants are shown in Table 1. The mean age at study entry of the participants without diabetes was 31.2 years, whereas that of the participants with diabetes was 52.0 years ($P < 0.001$). The mean HbA1c level at the first admission of the participants without diabetes was 31.7 mmol/mol (5.1%), whereas that of the participants with diabetes was 54.1 mmol/mol (7.1%). The mean plasma insulin level of participants with diabetes was significantly higher than that of participants without diabetes ($P < 0.05$). Although it might be due to aging, the mean plasma albumin level of participants with diabetes was significantly lower than that of participants without diabetes ($P < 0.05$). The renal functions were not statistically different between participants with and without diabetes.

Tear occult blood test

In many previous studies, it is known that the tear glucose concentrations vary widely^{9–13}. Considering the possibility of blood contamination, we tested the samples for occult blood. The tear samples were first tested for occult blood using urine test strips and classified into five groups (occult blood –, +, ++, +++ and unmeasurable due to shortage of samples; 56.3, 11.7, 20.0, 7.4 and 4.6%, respectively). As contamination of blood increased, the tear glucose concentrations and their variation increased in both participants with and without diabetes (Figures 3a,b; S6A–D). Thus, we eliminated the tear samples positive for occult blood for the analyses, and used the tear samples negative for occult blood.

Comparison of the plasma and tear glucose concentrations between participants with and without diabetes

The preprandial (before lunch and dinner on day 1 of both admissions) plasma glucose concentrations and tear glucose concentrations were significantly higher in the participants with

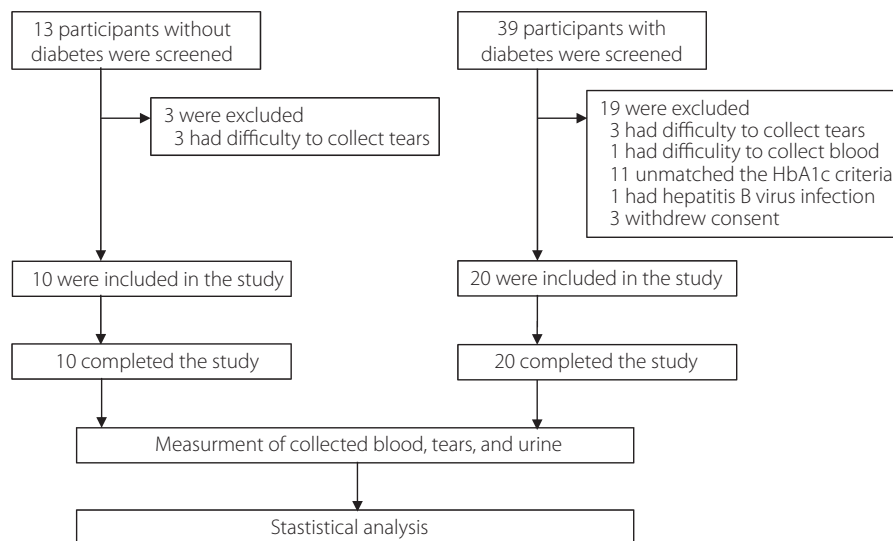


Figure 2 | Participant enrollment and outcomes. HbA1c, glycated hemoglobin.

Table 1 | Age and laboratory data on admission

	Participants without diabetes	Participants with diabetes	<i>P</i>
<i>n</i>	10	20	
Age (years)	31.2 ± 0.4	52.0 ± 1.3	<0.001
HbA1c (mmol/mol)	31.7 ± 3.4	54.1 ± 6.8	<0.001
HbA1c (%)	5.1 ± 0.3	7.1 ± 0.6	<0.001
Plasma insulin (pmol/L)	27.9 ± 15.7	107.6 ± 104.6	0.024
Plasma albumin (g/dL)	4.9 ± 0.3	4.5 ± 0.3	0.001
Plasma creatinine (μmol/L)	76.3 ± 7.3	82.9 ± 53.6	0.702
Overt proteinuria, <i>n</i> (%)	0 (0%)	5 (25%)	0.083

Each value, except overt proteinuria, shows the mean ± standard deviation. Plasma insulin levels were measured with blood samples before lunch on the day of admission. Overt proteinuria was determined by the urine albumin-to-creatinine ratio calculated by urine albumin and urine creatinine levels on the second day. HbA1c, glycated hemoglobin.

diabetes than in the participants without diabetes ($P < 0.001$ and $P < 0.05$, respectively; Figure 3c,d). In addition, the 2-h postprandial (2-h after lunch and dinner on day 1 of both admissions) plasma glucose concentrations and tear glucose concentrations were also significantly higher in the participants with diabetes than in the participants without diabetes ($P < 0.001$ and $P < 0.05$, respectively; Figure 3e,f). The mean ± standard deviation of the overall value in the entire participant population and values in individuals of the tear glucose concentrations-to-plasma glucose concentrations ratio are shown in Figure S4.

Daily tear and blood glucose dynamics

The plasma and tear glucose dynamics are shown in Figure 3g–j. In both participants with and without diabetes, the plasma glucose concentrations fluctuated according to the timing of the sample collection, such as whether the samples were

collected in the preprandial state, postprandial state or glucose-loaded state of the participants. The dynamics of the tear glucose concentrations generally paralleled those of the plasma glucose concentrations.

ROC analysis

As the area under the curve of the ROC curve was 0.777, we determined that tear glucose measurement could detect plasma glucose concentrations of >10 mmol/L (Figure S5).

Random intercept model and random slope model analysis for examining the association between the plasma glucose concentrations and tear glucose concentrations

The random intercept model analysis showed that there was no association between the plasma glucose concentrations and tear glucose concentrations in participants without diabetes (Figure 4a), but there was a tendency of association between the

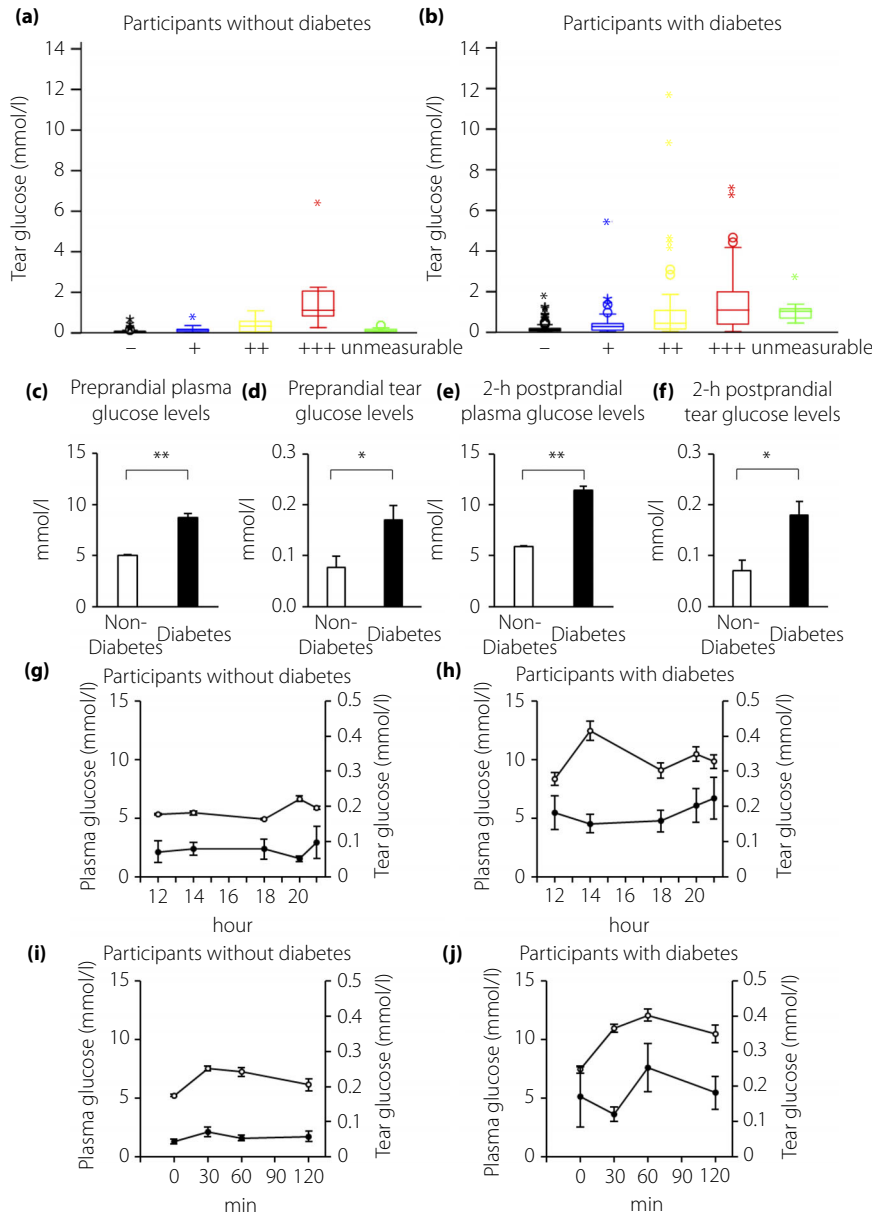


Figure 3 | (a,b) The box plots show the tear glucose concentrations in the five groups with occult blood testing. Open circles stand for outliers that are >1.5 and ≤ 3 box lengths above the 75% tile, and stars stand for outliers that are >3 box lengths above the 75% tile. (a) Participants without diabetes (occult blood -, +, ++, +++; $n = 112, 17, 29, 7, 15$, respectively). (b) Participants with diabetes (occult blood -, +, ++, +++; $n = 192, 46, 79, 33, 10$, respectively). (c–f) Comparison of the plasma and tear glucose concentrations between participants without diabetes (open columns) and participants with diabetes (closed columns). Data are expressed as the mean \pm standard error of the mean. (c) Preprandial plasma glucose concentrations at 12 and 18 o'clock on the day of admission (samples collected from participants without diabetes, $n = 22$; samples collected from participants with diabetes, $n = 47$). (d) Preprandial tear glucose concentrations at 12 and 18 o'clock on the day of admission (samples collected from participants without diabetes, $n = 22$; samples collected from participants with diabetes, $n = 47$). (e) The 2-h postprandial plasma glucose concentrations at 14 and 20 o'clock on the day of admission (samples collected from participants without diabetes, $n = 28$; samples collected from participants with diabetes, $n = 41$). (f) The 2-h postprandial plasma glucose concentrations at 14 and 20 o'clock on the day of admission (samples collected from participants without diabetes, $n = 28$; samples collected from participants with diabetes, $n = 41$). (g–j) Plasma (open circles) and tear (closed circles) glucose dynamics (samples collected from participants without diabetes, $n = 7$ –18; samples collected from participants with diabetes, $n = 19$ –24). Data are expressed as the mean \pm standard error of the mean. Data are expressed as the mean \pm standard error of the mean. (g) Participants without diabetes on the day of admission. (h) Participants with diabetes on the day of admission. (i) Participants without diabetes on day 2 of admission. (j) Participants with diabetes on day 2 of admission. AD, after dinner; AL, after lunch; BB, before bedtime; BD, before dinner; BL, before lunch.

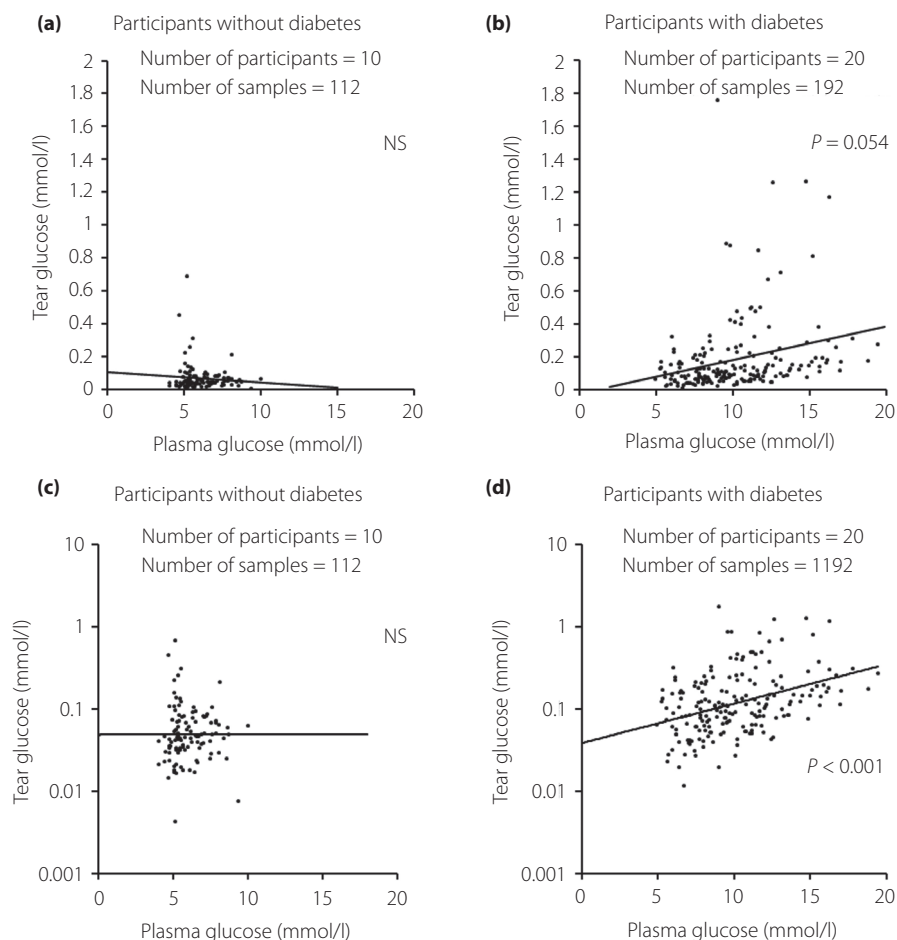


Figure 4 | (a,b) Correlation charts and regression lines between the plasma and tear glucose concentrations. (a) Samples collected from participants without diabetes; $n = 112$. (b) Samples collected from participants with diabetes; $n = 192$. (c,d) The correlation charts and regression lines between plasma glucose concentrations and logarithmically transformed values of the tear glucose concentrations. (c) Samples collected from participants without diabetes; $n = 112$. (d) Samples collected from participants with diabetes; $n = 192$. All regression lines and P -values were calculated by random intercept model analysis. NS, not significant.

plasma glucose concentrations and tear glucose concentrations in participants with diabetes ($P = 0.054$; Figure 4b; Table 2). There was also no association between the plasma glucose concentrations and tear glucose concentrations in participants without diabetes after logarithmic transformation of the tear glucose concentrations (Figure 2c), but there was a significant association between the plasma glucose concentrations and tear glucose concentrations in participants with diabetes ($P < 0.001$, Figure 4d; Table 2). In addition, there was a significant association between the plasma glucose concentrations and tear glucose concentrations, as well as between the plasma glucose concentrations and logarithmically transformed values of the tear glucose concentrations in participants with diabetes by random slope model analysis (Table 2). These results suggest that the association between the tear glucose concentrations and plasma glucose concentrations were stronger in the participants with diabetes whose plasma glucose concentrations were high.

The random intercept model with the confounders (HbA1c level, prandial state [preprandial/postprandial] of the participants) to determine the association between the plasma glucose concentrations and logarithmically transformed values of the tear glucose concentrations showed that the association remained statistically significant even after adjustments for such confounders ($P = 0.002$), and the random effects of intercepts were significantly different among participants with diabetes ($P = 0.015$; Table 3).

DISCUSSION

In the present study, we showed the existence of a significant association between the plasma glucose concentrations and tear glucose concentrations, with the association remaining significant even after adjustments for the HbA1c level and prandial state (preprandial/postprandial) of the participants by random intercept model analysis, which can evaluate both interpersonal and

Table 2 | Random intercept model and a random slope model for the tear glucose concentrations to examine its association with the plasma glucose concentrations in participants with diabetes

Response variable	Tear glucose concentration				log ₁₀ (Tear glucose concentration)			
Random intercept model								
Fixed effect	Estimate	<i>t</i>	<i>P</i>	95% CI	Estimate	<i>t</i>	<i>P</i>	95% CI
Intercept	0.096	1.289	0.201	-0.052, 0.243	-1.289	-11.592	<0.001	-1.509, -1.068
Plasma glucose (mmol/L)	0.012	0.006	0.054	-0.000, 0.024	0.041	4.468	<0.001	0.023, 0.059
Random effect	Estimate	<i>z</i>	<i>P</i>	95% CI	Estimate	<i>z</i>	<i>P</i>	95% CI
Residual (level 1)	0.036	9.183	<0.001	0.029, 0.035	0.079	9.205	<0.001	0.064, 0.098
Intercept (level 2)	0.026	2.403	0.016	0.011, 0.058	0.597	2.476	0.013	0.027, 0.132
Random slope model								
Fixed effect	Estimate	<i>t</i>	<i>P</i>	95% CI	Estimate	<i>t</i>	<i>P</i>	95% CI
Intercept	0.047	0.728	0.467	-0.080, 0.173	-1.375	-14.026	<0.001	-1.569, -1.182
Plasma glucose (mmol/L)	0.016	2.265	0.026	0.002, 0.030	0.049	4.431	<0.001	0.027, 0.071
Random effect	Estimate	<i>z</i>	<i>P</i>	95% CI	Estimate	<i>z</i>	<i>P</i>	95% CI
Residual (level 1)	0.037	9.146	<0.001	0.030, 0.046	0.084	9.127	<0.001	0.068, 0.104
Slope (level 2)	0.000	2.182	0.029	0.000, 0.000	0.001	2.239	0.025	0.000, 0.001

Fixed effect: means of the intercept and regression coefficients. Random effect: variances of the intercept and regression coefficients. Fixed effect: means of the intercept and regression coefficients. Random effect: variances of the slope and regression coefficients. CI, confidence interval.

Table 3 | Random intercept model for logarithmically transformed values of the tear glucose concentrations to examine its association with the plasma glucose concentrations in participants with diabetes by including glycated hemoglobin and the prandial state (preprandial/postprandial) of the participants as confounders

Fixed effect	Estimate	<i>t</i>	<i>P</i>	95% CI
Intercept	-1.299	-2.600	0.018	-2.347, -0.251
Plasma glucose (mmol/L)	0.038	3.194	0.002	0.015, 0.062
HbA1c (mmol/mol)	0.000	0.031	0.976	-0.019, 0.020
Preprandial (0) or Postprandial (1)	0.113	0.418	0.677	-0.042, 0.064
Random effect	Estimate	<i>z</i>	<i>P</i>	95% CI
Residual (level 1)	0.080	9.172	<0.001	0.064, 0.099
Intercept (level 2)	0.065	2.429	0.015	0.029, 0.146

Fixed effect: means of the intercept and regression coefficients. Random effect: variances of the intercept and regression coefficients. CI, confidence interval; HbA1c, glycated hemoglobin.

intrapersonal association. These results suggest that tear glucose measurements might be useful, irrespective of the characteristics of the population surveyed (e.g., diabetes control status) and the timing of sample collection (e.g., preprandial/postprandial).

The strength of the present study lies in the fact that it is the first to use random intercept model analysis to determine the association between the plasma glucose concentrations and tear glucose concentrations using tear samples not contaminated with blood. Furthermore, we carried out the measurements in one of the largest number of samples (*n* = 304) recorded to date, using a sensitive and reliable high-pressure liquid chromatography method.

The limitations of the present study were that all of the participants were men, and the influence of sex on the association

between the plasma and tear glucose concentrations was not investigated. Furthermore, the mean age of the participants with diabetes in the present study was significantly higher than that of the participants without, and it is possible that some of the relevant variables were influenced by age.

The association between the tear glucose concentrations and blood glucose concentrations were known to be insufficient to allow tear glucose monitoring to be used as a substitute for the invasive blood glucose monitoring. Various reasons for the insufficient association are speculated, including those related to the method of tear collection, method of stimulation of tearing, contamination of blood, method of measurement of the tear glucose concentrations and the effects of diabetes itself¹⁷. In this study, we used not only a non-invasive tear collection method

without stimulation of tears, but also the first tear occult blood testing to eliminate the tear samples positive for occult blood.

In addition to the methodological issues described above, the effects of diabetes itself should be considered. Hyperglycemia in diabetes patients causes microvascular damage and nerve damage^{18,19}, and such damage to the vasculature supplying blood to the eyes and the nerves of the lacrimal reflex arc could alter tear production. For example, dry eyes can result because of reduced tear production. In fact, the prevalence of dry eyes is known to increase with HbA1c levels among participants with diabetes²⁰. To rule out the effects of diabetes severity and prandial state, we used random intercept model analysis to eliminate the confounding effects of HbA1c and prandial state (preprandial/postprandial). The random effect of intercepts was significantly different among participants with diabetes. This result suggests that the associations between plasma and tear glucose concentrations vary by participants, and a tear glucose monitoring device requires calibrations to predict blood glucose levels properly.

The random intercept model and charts (Figure 4a–d) showed that the correlation between the tear glucose concentrations and plasma glucose concentrations were stronger in hyperglycemia states. In contrast, the correlation was not very good in normoglycemia states. In addition, the ROC analysis showed that tear glucose measurement could detect hyperglycemia without the need for calibrations. Therefore, when used for screening tests, tear glucose measurement can be expected, as a non-invasive test that does not require calibrations. In contrast, for the purpose of daily blood glucose control in diabetes patients, there is a possibility of monitoring the blood glucose levels through non-invasive tear glucose measurements without calibrations, if calibrations for correcting individual differences are carried out first. This is different from the case of continuous glucose monitoring systems, which require calibrations for correcting deterioration of the sensors throughout the measurements. In future studies, we propose to improve the accuracy of tear glucose measurements by devising better methods for collection and calibration.

Furthermore, we created models incorporating logarithmically transformed values of the tear glucose concentrations, which have scarcely been reported before. We transformed the data of tear glucose concentrations to account for normality assumption in applying the random intercept and slope model (Figure 4a–d; Table 2). The correlation charts and regression lines (Figure 4a–d) of the logarithm models appeared to be better.

The source of glucose in tears remains unclear. There are few reports about the existence of glucose transporters in the tear glands. The constitutive glucose transporter, glucose transporter 1, has been reported to be expressed in the apical corneal epithelium²¹. The sodium–glucose cotransporter, sodium–glucose cotransporter 1, has been reported to be expressed on the apical side of the bulbar and palpebral conjunctiva^{22,23}. Although the roles of these transporters are still unclear, leakage

of glucose from the interstitial space into the tear fluid cannot reach an equilibrium state, because tear fluid always flows out, contrary to the case for leakage of glucose from the blood vessels into the interstitial fluid.

Recently, it has been reported that tear fluid contains many kinds of metabolites, including glucose^{24,25}. Some of these metabolites could serve as biomarkers of diseases (e.g., Alzheimer's disease), paving the way for the development of a non-invasive method for the diagnosis of these diseases^{26,27}. Thus, tears appear to represent a very attractive material for diagnosis. In the future, we propose to measure other biomarkers associated with diabetes in tear samples.

In contrast, an ophthalmic lens that can measure the glucose concentrations in tears has been reported. This is a very attractive device, because it can measure the glucose concentrations continuously while eliminating the effects of the collecting method used^{28–33}, and is expected to be an alternative device of continuous glucose monitoring systems, measuring interstitial fluid glucose concentrations. Although the sensitivity of these sensors is such that even glucose concentrations as low as those in tears can be measured, they are not yet suitable for use in clinical studies at this stage.

It has been known for a long time that there is an association between blood glucose and tear glucose concentrations^{8–17}, but the present study revealed three new findings. The first is that there are individual differences in the association between blood glucose and tear glucose concentrations. The second is that this association still existed after adjusting for the HbA1c levels and the prandial state. Finally, contamination of the tear samples with blood led to a false elevation of the tear glucose concentration. The present results suggested the feasibility of measurement of biomarkers in tears, which could be collected non-invasively. In the future, investigating biomarkers, including glucose in tears, is thought to be required toward the realization of non-invasive measuring.

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DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 | Correlation charts between the glucose concentrations of standard solutions measured by both the methods used to measure the blood glucose concentrations (SRL, Inc.) and tear glucose concentrations (high-pressure liquid chromatography).

Figure S2 | Calibration curves of high-pressure liquid chromatography measurement before measurement (black line) and after measurement (red line). (a) Day 1, (b) day 2, (c) day 3, (d) day 4, (e) day 5 and (f) day 6.

Figure S3 | Discoloration of test strips depending on the hemoglobin concentration (0, 0.06, 0.15 and 0.75).

Figure S4 | The mean \pm standard deviation of the total value in the entire participant population and values in individuals of the tear glucose concentrations-to-plasma glucose concentrations ratio.

Figure S5 | Receiver operating characteristic curve to detect plasma glucose concentrations of >10 mol/L. The area under the curve for this discrimination was 0.777.

Figure S6 | (a,b) Correlation charts between the plasma and tear glucose concentrations before excluding samples with occult blood (black, blue, yellow, red and green circles stand for $-$, $+$, $++$, $+++$ and unmeasurable, respectively). (a) Samples collected from participants without diabetes ($n = 180$) and (b) samples collected from participants with diabetes ($n = 360$). (c,d) The correlation charts between plasma glucose concentrations and logarithmically transformed values of the tear glucose concentrations before excluding samples with occult blood (black, blue, yellow, red and green circles stand for $-$, $+$, $++$, $+++$ and unmeasurable, respectively). (c) Samples collected from participants without diabetes ($n = 180$) and (d) samples collected from participants with diabetes ($n = 360$).

Table S1 | Exclusion criteria.

Table S2 | Kolmogorov–Smirnov test for validation of normal distribution.