Comparison of Glucose Concentration in Interstitial Fluid, and Capillary and Venous Blood During Rapid Changes in Blood Glucose Levels

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ABSTRACT

The relationship between glucose concentrations in interstitial fluid (ISF) and blood has generated great interest due to its importance in minimally invasive and noninvasive techniques for measuring blood glucose. The relationship between glucose levels in dermal ISF, and capillary and venous blood was studied with the dermal ISF samples obtained using the suction blister technique. The study was conducted with intensely managed diabetics whose blood glucose levels were manipulated so as to induce rapid changes in blood glucose levels. Glucose levels in the three compartments exhibited high correlations both when individual subjects were considered separately and when data from all subjects were combined. No significant time lag during glucose excursions was observed among the ISF, and capillary and venous glucose levels.

INTRODUCTION

Efforts at developing noninvasive methods of monitoring blood glucose levels have been greatly motivated by the outcomes from the Diabetes Controls and Complications Trial (DCCT), which showed the advantage in controlling blood glucose levels in diabetic patients at close to normal levels.¹² The pain and inconvenience associated with glucose measurements using finger sticks have been a major factor in preventing diabetic patients from making frequent measurements to facilitate proper control of blood glucose levels. Therefore, complete elimination or reduction of pain has been the focus of development of new products for the measurement of glucose. There have been attempts to develop minimally invasive techniques wherein a small quantity of interstitial fluid (ISF) is used to make the measurement.³⁴ However, there is concern that the concentration of glucose in the ISF does not accurately reflect blood glucose levels due to the possibility of a significant time lag.⁵⁶

Several groups have reported on the development of spectroscopic methods using near-infrared (NIR) light for noninvasive blood glucose measurements.⁷⁸ Many of these methods involve the extraction of the glucose signal from light that has passed through skin. While the target analyte is the capillary blood in the dermis, the glucose “seen” by the light consists of glucose in both the ISF and the capillary blood. This signal is then used to build a cali-
ibration model using capillary blood glucose concentrations as reference values. As a result, any difference in glucose levels between the ISF and capillary blood will lead to a “reference” error, which will depend on the relationship between blood and ISF glucose concentrations. If this “reference” error is large, then the error in the noninvasive estimation of glucose will be dominated by it. Therefore, an understanding of the relationship between blood and ISF glucose levels will help quantify this error, and may even be useful in accounting for part or all of the difference between the two compartments, thus leading to an improved estimation of blood glucose.

Several studies have been conducted to quantify the relationship between ISF and blood glucose using ISF sampled from either subcutaneous tissue or the dermis by different techniques such as microdialysis, microperfusion, or suction blister techniques. Lönnroth et al. sampled ISF from subcutaneous tissue using a microdialysis technique and found that venous plasma glucose was identical to ISF glucose under steady-state conditions in healthy subjects. Jansson et al. used the same technique to study responses during oral glucose tolerance tests (OGTT) and hyperglycemic conditions. They found that the time lag between ISF and plasma glucose depended on the rate of glucose infusion. At high infusion rates, the lag was greater than 8 min. Bolinder et al. found a time lag of 10 min between blood glucose and subcutaneous ISF. Petersen et al. used microdialysis to sample ISF glucose in skin. They reported that, for nondiabetic subjects, there was no significant difference between ISF and venous glucose. Shaupp et al. used open-flow microperfusion technique to sample ISF from the adipose tissue. Under basal conditions, they concluded that the difference between the ISF glucose in the adipose tissue and plasma was about 23 mg/dL. In hyperglycemic clamp experiments, the difference was found to be around 47 mg/dL. Petersen reported a lag of 30 min during OGTT between ISF and venous plasma glucose levels with ISF collected from skin using microdialysis. At steady state, glucose levels in the two compartments were reported to be identical. Summers et al. used the microdialysis technique to collect subcutaneous ISF during a three-stage glucose clamp. Blood glucose was kept at 90 mg/dL, then raised to 180 mg/dL, and then down again to 90 mg/dL, with the blood glucose concentration being maintained at each of these levels for 60 min. A lag of 15–20 min during the second phase was reported. In the third phase, the ISF glucose concentration was measured to be higher than the plasma glucose levels. Bantle and Thomas investigated the dermal ISF using the microneedle technique on subjects with type I diabetes and found no significant difference between dermal ISF and plasma glucose levels during a 5-h pre- and postprandial period. Jensen et al. used the suction blister technique to sample ISF from type I diabetic subjects. From glucose clamp experiments, they concluded that the ISF glucose levels are significantly lower than venous plasma levels.

From this brief review of the literature, it can be seen that the methods, experimental designs, and the ISF collection sites (dermal or adipose layer) used for investigating the relationship between ISF and blood glucose are varied and so are the results obtained from them. The aim of our investigation is to understand the ISF–blood glucose relationship with the goal of ultimately quantifying the effect on NIR measurement of glucose by diffuse reflectance. Since the major contribution to the glucose information present in NIR reflectance spectra can be expected to be from the dermal layer (from glucose present in the ISF and the capillaries), our interest lies primarily with the behavior of dermal ISF with changes in blood glucose. We investigate the relationship among dermal ISF, and capillary and venous plasma glucose when rapid changes are induced in blood glucose levels.

**MATERIALS AND METHODS**

The study was conducted on eight subjects with diabetes (four males and four females), whose daily treatment included three or more shots of fast-acting insulin (e.g., Humalog). This inclusion criterion was set to ensure the achievement of rapidly changing glucose profiles. Each subject had an initial screening visit
with a physician to assess their health status, and all subjects gave prior written informed consent to the protocol, which was approved by the Allensdale Internal Review Board.

The experiment was conducted over 3 consecutive days consisting of two 8-h and one 4-h data collection periods. During the first 2 days, the subject’s glucose levels were manipulated through carbohydrate ingestion and insulin injections to achieve target glucose profiles ranging from 50 to 350 mg/dL. The target profiles, shown in Figure 1, were selected to simulate a moderate (day 1) and rapid (day 2) rate of change in the subject’s blood glucose level. The third day was used to test a baseline profile, wherein it was attempted to keep the glucose level constant over 4 h. Though the target profiles were not always achieved, the rate of change of blood glucose levels varied between the first 2 days.

Twenty sample sets were collected on each of the first 2 days, and 10 sample sets were collected on the third day. Each sample set consists of a venous and capillary blood draw and an ISF sample. The sample sets were collected at 24-min intervals and included a 6-min period for the ISF extraction. The venous and capillary blood draws were taken while the ISF was being collected.

Dermal ISF was extracted using the suction blister technique, which has been used widely since the 1960s.\textsuperscript{18–21} The advantage of using suction to form the blisters is that the dermis and epidermis will cleanly separate without damaging either layer, and it has been shown that the suction blister fluid is representative of ISF.\textsuperscript{22} A set of three blisters was created at the onset of the first day of experimentation on the volar aspect of each forearm by applying mild suction. A PTC 330 Vac vacuum pump (Ventipress Oy) and modified 3-cc syringes were used to form the blisters. The syringes were modified for use as suction cups by expanding the luer end to fit the rubber tubing connecting the vacuum pump and rounding the sharp edges of the syringe opening to reduce injury to the tissue. Velcro straps were used to hold the syringes securely to the arm during formation.

Prior to blister formation, the subject’s arms were shaved, if necessary, and cleaned with Ivory\textsuperscript{TM} soap and water. Three suction cups were then attached to each arm, and suction was started. Once the target negative pressure was reached, heating pads were applied to the arm in order to reduce the blister formation time. A moderate level of negative pressure over an extended period of time was used to ensure that the tissue was not damaged or the blister fluid contaminated with blood (blisters were formed in approximately 75 min). Further care was taken to ensure that the blisters were not overdeveloped since this could also lead to contamination of the blister fluid with blood.

The newly formed blisters were aspirated and the epidermis excised. The open wounds were covered with Tegaderm\textsuperscript{TM} (3M) patches to ensure their viability for the study duration and provide a means to repeatedly extract the ISF. A different set of three blisters was cyclically sampled on each of the first 2 days, and on the third day, the arm with the blisters in the best condition was used. The 4-μL ISF sample volume used for glucose analysis was extracted through the following procedure. The old patch covering the blister was removed, and a new patch was secured over it before applying suction to the patch for 6 min, during which period the ISF was accumulated. Then the suction was stopped, and the patch was pierced using a 20-gauge hypodermic needle. The ISF under the patch was pushed out.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Plot of target glucose profiles for the first and second day of a subject’s visit. These profiles were chosen to ensure that the rate of change of glucose concentration was different for the 2 days.}
\end{figure}
through the hole created by the needle. From the droplet of ISF formed outside the patch, 4 μL was sampled using a digital micropipette.

Venous and capillary blood samples of at least 40 μL were taken from the arm not used for collecting ISF and were accumulated in an ethylenediamine-tetraacetic acid (EDTA)–lined microcontainer. Venous blood samples were drawn through an intravenous (IV) catheter placed in the upper forearm or back of the hand. Finger sticks were used to collect the capillary blood samples. All samples (ISF, and venous and capillary blood) were immediately analyzed using a Yellow Springs Instrument (YSI) glucose analyzer. The blood samples were analyzed by following the normal operating procedure established by YSI for the sipper arm sampling station. The small-volume ISF samples were directly injected into the YSI sample chamber using a micropipette, and the resulting glucose values were volume corrected by a factor of 6.25 ([normal sample size (25 μL)]/[injected sample size (4 μL)]) to compensate for the reduced sample size.

The venous and capillary whole blood glucose values measured by the YSI analyzer were converted to plasma values by using the average hematocrit levels measured from venous blood samples taken at the beginning and end of each day. The subjects were required to

![Graphs showing glucose profiles for subject 1 over 3 days of data collection.](image)

**FIG. 2.** Plot of glucose profiles for subject 1 over 3 days of data collection. Venous and capillary blood glucose values were converted to plasma values by using hematocrit readings taken at the beginning and end of each day.
drink at least 8 oz of water each hour to circumvent possible dehydration resulting from the high glucose levels.

RESULTS

Two subjects (both males) were dropped from the analysis because sufficient quantities of ISF could not be extracted. In other subjects, some data points were rejected before beginning the analysis due to insufficient sample volume or other known problems with sampling ISF, or capillary or venous blood samples. Representative data collected on two of the subjects are shown in Figures 2 and 3.

The relationships among ISF, and capillary and venous plasma glucose levels were studied using scatterplots and linear regression. Figure 4 shows plots of these relationships for a single subject. Tables 1–3 contain a summary of the regression analysis. It can be seen that the relationships are linear and there is a high correlation between the glucose levels in the three compartments, as indicated by the $R^2$ values. Examination of the root mean square error (RMSE) reveals that, in the case of ISF versus capillary and ISF versus venous glucose, the errors are higher than in the case of capillary versus venous glucose. This increase in error is probably due to the lower precision in the measurement of glucose in ISF samples because of the procedure used for measuring the small quantity of sample.

FIG. 3. Plot of glucose profiles for subject 5 over 3 days of data collection. Venous and capillary blood glucose values were converted to plasma values by using hematocrit readings taken at the beginning and end of each day.
A rough estimate of the precision in the measurement of ISF glucose was obtained by preparing glucose–water solutions at different concentrations. The concentrations were measured by the standard method of using the YSI (with the sipper arm) and then with 4 μL of solution injected directly into the sample chamber using a micropipette. The average standard deviation of the measurements by the standard method was 1.2 mg/dL and for the micropipette method it was 8.7 mg/dL. This difference in precision between the standard method and the micropipette method would explain the higher error in regression involving ISF glucose concentrations.

In Table 1, the intercepts provide an estimate of bias in the ISF glucose compared to capillary glucose and are observed to vary significantly from subject to subject. Whether this variation in bias is truly a subject-to-subject variation or the result of the differences in the glucose profiles achieved by each subject could not be determined. This inconsistent variation in bias is also exhibited when ISF glucose is compared to venous glucose. In the case of capillary versus venous plasma glucose, the bias was negligible.
GLUCOSE IN ISF AND CAPILLARY AND VENOUS BLOOD

for all subjects except subject 4. The reason for this large bias in subject 4 is not known.

Regression analysis was also performed to examine the correlation between the glucose concentrations of the three compartments over all subjects. Figure 5 shows the regression results through scatterplots. Comparing these results with the analyses summarized in Tables 1–3, it is observed that the error in the regression over all subjects is equivalent to the regression error determined for each individual subject. Also, the correlation between the glucose concentrations in the three compartments is similar to the data from each individual subject. This suggests the possibility of obtaining a global calibration model, which can be used to relate the glucose concentrations in the three compartments.

Finally, an examination of the ISF, and capillary and venous glucose profiles of the six subjects showed no observable lag or shift in the peak ISF glucose concentrations compared to capillary or venous plasma glucose levels. This observation is based on the fact that if a large lag exists, the concentration of glucose in the ISF will be lower on one side of the critical point (maximum or minimum) and higher on the other side, which is not the case with the data collected in this study.

CONCLUSION

The results presented here show no observable lag among ISF, and capillary and venous plasma glucose levels when blood glucose levels change rapidly. The extent of error introduced in estimating capillary blood glucose through measurement of glucose in dermal ISF can be estimated from the regression analysis presented here. We argued that the higher RMS errors in regression of capillary versus ISF glucose and venous versus ISF glucose compared to the RMS error in regression of venous versus capillary glucose were due to the low precision in measurement of glucose in ISF. It is then reasonable to assume that the RMS error is the same in all three cases. Under this assumption, the error in estimating capillary plasma glucose concentration using ISF glucose concentration is the same as the error in estimating capillary glucose concentration using venous glucose concentration from a linear regression model (about 13 mg/dL, as shown

| Table 1. Summary of Regression Analysis Using Data on Individual Subjects for Capillary versus Dermal Interstitial Fluid Glucose Concentrations |
|---|---|---|---|---|---|
| Subject | No. of points | Slope | Intercept | R² | RMS error (mg/dL) | Percentage error |
| 1 | 37 | 1.06 ± 0.064 | −9.6 ± 2.5 | 0.9697 | 15.8 | 5.6 |
| 2 | 35 | 0.96 ± 0.098 | 10.7 ± 3.9 | 0.9238 | 26.7 | 10.2 |
| 3 | 38 | 1.07 ± 0.064 | −11.9 ± 2.7 | 0.9691 | 19.7 | 7.9 |
| 4 | 39 | 0.95 ± 0.091 | 0.99 ± 3.2 | 0.9255 | 22.6 | 8.5 |
| 5 | 32 | 1.04 ± 0.05 | −0.83 ± 2.3 | 0.9822 | 15.1 | 6.6 |

| Table 2. Summary of Regression Analysis Using Data on Individual Subjects for Venous versus Dermal Interstitial Fluid Glucose Concentrations |
|---|---|---|---|---|---|
| Subject | No. of points | Slope | Intercept | R² | RMS error (mg/dL) | Percentage error |
| 1 | 40 | 1.14 ± 0.084 | −11.9 ± 3.0 | 0.9524 | 19.3 | 6.8 |
| 2 | 37 | 1.11 ± 0.063 | −9.7 ± 2.3 | 0.9730 | 17.8 | 6.8 |
| 3 | 38 | 1.07 ± 0.081 | 1.6 ± 2.8 | 0.9524 | 20.8 | 7.6 |
| 4 | 39 | 1.22 ± 0.083 | −29.0 ± 3.1 | 0.9598 | 22.2 | 7.8 |
| 5 | 42 | 0.98 ± 0.082 | 2.3 ± 2.6 | 0.9361 | 20.3 | 7.8 |
| 6 | 35 | 1.11 ± 0.062 | 3.89 ± 2.4 | 0.9758 | 17.5 | 8.6 |
in Figure 5). This line of reasoning assumes that ISF glucose can be measured accurately and provides a lower bound in the error between capillary and interstitial glucose concentrations.

<table>
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<th>Subject</th>
<th>No. of points</th>
<th>Slope</th>
<th>Intercept</th>
<th>$R^2$</th>
<th>RMS error (mg/dL)</th>
<th>Percentage error</th>
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<td>1</td>
<td>46</td>
<td>1.06 ± 0.054</td>
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<td>0.9726</td>
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<td>2.01 ± 1.41</td>
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ACKNOWLEDGMENTS

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FIG. 5. Regression analysis using data from all six subjects comparing venous, capillary, and dermal interstitial fluid glucose concentrations.
REFERENCES


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