Time Lag of Glucose from Intravascular to Interstitial Compartment in Humans

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Abstract:

The accuracy of continuous interstitial fluid glucose sensing is an essential component of current and emerging open and closed loop systems for type 1 diabetes. An important determinant of sensor accuracy is the physiological time lag of glucose transport from the vascular to the interstitial space. We have performed the first direct measurement of this phenomenon in 8 healthy subjects under overnight fasted condition. Microdialysis catheters were inserted into the abdominal subcutaneous space. After intravenous bolus administrations of glucose tracers, timed samples of plasma and interstitial fluid were collected sequentially and analyzed for tracer enrichments. After accounting for catheter dead space and assay noise, the mean time lag of appearance of tracer into the interstitial space after intravenous bolus was 5.3 to 6.2 minutes. We conclude that in the overnight fasted state in healthy adults, the physiological delay of glucose transport from the vascular to the interstitial space is 5-6 minutes. Physiological delay between blood glucose and interstitial fluid glucose should, therefore, not be an obstacle to sensor accuracy in overnight or fasting state closed loop systems of insulin delivery or open loop therapy assessment for type 1 diabetes.
Introduction

There has been rapid development of prototype artificial endocrine pancreas (AEP) systems for the automated management of type 1 diabetes (1). A necessary prerequisite for this is accurate continuous glucose sensing accomplished by continuous glucose monitors (CGM) that measure interstitial fluid (ISF) glucose concentrations through subcutaneously placed glucose sensing probes. A recent critique of the AEP cited large lag times between plasma and ISF glucose as an important limiting factor in the ability of closed loop control (CLC) systems to successfully manage type 1 diabetes (2).

Studies that have attempted to examine the temporal relationship between changes in plasma glucose to ISF glucose concentrations in subjects with and without diabetes suggest a wide time lag of 4-50 minutes (3-11). If the intrinsic physiological delay between blood and interstitial glucose were as high as some have hypothesized, it might be challenging to develop CGM systems with sufficient accuracy to permit CLC. However, if this delay were smaller, e.g. < 10 minutes, CGM accuracy might be sufficient to permit the development of safe and effective CLC algorithms. In addition, a modest intrinsic physiological delay might also permit development of specialized algorithms to compensate for the physiologic time-lag that is purported to occur across compartmental barriers (5; 8).

To our knowledge, there have been no previous studies reported regarding direct measurement of the transport of glucose from the vascular compartment to the ISF. Smith and co-workers used a fluorescein tracer to measure the kinetics of blood to interstitial transport, but the results were obtained using fluorescein dye as a surrogate for glucose rather than fluorescent or tracer glucose
We used a systematic approach to understand physiology of glucose transport combining glucose isotope dilution methodology with microdialysis technique in overnight fasted healthy adults. Following sequential intravenous boluses of glucose tracers, we simultaneously assessed plasma and microdialysis samples for the appearance and decay of glucose tracers over time. The time to appearance of glucose tracers is indicative of the time delay of glucose transport between intravascular and interstitial compartments since the glucose tracers have no isotopic effect (premise of isotope dilution technique) and are handled similarly as glucose.

**Research Design and Methods:**

The study was approved by the Mayo Institutional Review Board. Following informed consent, screening tests were performed at Mayo CTSA-Clinical Research Unit (CRU) to ensure subjects were healthy, not pregnant and met enrollment criteria. Subjects with past history of diabetes, glucose intolerance or family history of diabetes in first degree members were excluded. All medications except stable thyroid and hormone replacement therapy were exclusionary.

Participants were admitted to the CRU at ~1600 hours, consumed a standard 10 kcal/kg evening meal at ~1700 hours and remained NPO except water for remainder of the study. At ~0600 next morning, heated hand vein method was utilized to periodically draw arterialized venous blood for glucose and tracer concentrations (13). A catheter was inserted into a forearm vein for infusion of tracer boluses.

Experimental design is shown in Figure 1. Four microdialysis catheters (CMA 63, 20 kDa M Dialysis Inc, North Chelmsford, MA) were inserted, under local anesthesia and aseptic
precautions into subcutaneous abdominal fat, two on each side of the anterior abdomen and were infused with CMA perfusion fluid via CMA 107 microdialysis pump at a constant rate of ~1 µl/min for the study period. At periodic intervals, timed-pooled microdialysate effluent and blood samples were collected for glucose and tracer measurements simultaneously. Four microdialysis catheters were necessary to collect adequate sample volume to enable measurement of glucose tracers on pooled samples from all four catheters for analyses at the end of each collection point. Following insertion of the last of four microdialysis catheters, at least 1 hour was provided to allow catheters to stabilize and reach steady state. At ~8 am (0 min), an intravenous bolus of [1-13C] glucose was administered over 10 seconds. Starting 4 min prior to the [1-13C] glucose bolus, microdialysate samples were collected every 5 min for the next 30 min and periodically thereafter until 9:57 am (117 min). Subsequent tracer glucose boluses and sequential timing of microdialysate and blood sample collections are as depicted (Figure 1).

Doses of the stable isotopes (1-13C, 6,6-2H2, 2-13C glucose) were estimated to achieve plasma enrichment of ~4% and [3-3H] glucose (100 µCi) was also used as the fourth tracer. Subjects 1-4 were infused with all 4 tracers and subjects 5-8 were only infused with stable tracers eliminating the [3-3H] glucose and replacing with saline in a different order.

Analytical Techniques: Samples were placed on ice, aliquoted and stored at -20°C until assayed. Plasma glucose concentration was measured using a YSI 2300 (Yellow Springs Instrument, Yellow Springs, OH) (14). [3-3H] glucose counts were analyzed as previously described (15). Microdialysis and plasma samples for stable tracer enrichment were analyzed by GCMS. Selected Ion Monitoring was used to monitor fragments with mass/charge ratio (m/z) of 160 and

**Statistical Considerations:** The analysis of the tracer concentrations occurred in two steps. First, the timings of sample collection were re-indexed to represent time from infusion to appearance at the microdialysis catheter based on infusion setting and tubing volume to account for the lag-time taken to cover the catheter dead space. It was determined that a 6.2 min transit time correction factor had to be applied. The next step was to provide descriptive statistics and concentration profiles and use a Kaplan-Meier product limit curve to estimate the time to detectable levels in the ISF that was defined as enrichment molar ratios (MR) >0.3%. This corresponds to three times the upper limit of the mass spectroscopy assay noise (± 0.1 MR %). In the context of the time-to-event analysis, the time from infusion to appearance at the catheter with an enrichment of at least 0.3% was used in the modeling. The upper limit for 95% confidence interval of the 75$^{th}$ percentile of the failure distribution (i.e., the estimated time for which 75% of the study subjects had detectable isotope levels beyond MR>0.3%) was used as a conservative estimate of the time required for appearance in the ISF. Spearman’s rank order correlation was used to explore the association of time to appearance, appearance being quantified as the time from bolus to the time of highest observed concentration, with anthropometric measurements.

[2-$^{13}$C] glucose data was not used due to interference from the [1-$^{13}$C] glucose given at 8:00 am. The glucose derivative uses a fragment with m/z of 160 and 161 for determination of [13C] glucose enrichment that contains both C1 and C2 carbons of glucose. [1-$^{13}$C] glucose tracer
enrichments were still above the baseline value at the time the $[2^{-13}\text{C}]$ glucose tracer was administered, indicating that the $[1^{-13}\text{C}]$ glucose was not totally cleared in 360 min. Therefore we did not use the $[2^{-13}\text{C}]$ glucose data. Statistical analyses were conducted using the SAS System (version 9.3, Cary, NC).

**Results:**

**Subject Demographics (Table 1):** Table shows the demographic characteristics of the study participants.

**Plasma glucose concentrations (Figure 2):** Plasma glucose concentrations were normal prior to start of study ($5.15 \pm 0.45 \text{ mM}$) and remained unchanged thereafter for the duration of the study.

**Microdialysate tracer glucose Molar Ratio (Figure 3 left and right panel):** The left panel illustrates the subject-specific profiles for $[1^{-13}\text{C}]$ glucose and $[6,6^{-2}\text{H}_2]$ glucose for the participants over the entire sampling period for each isotope and the right panel the subject-specific profiles for the same tracers between 0-10 min.

The right panel illustrates the same data as above with more resolution over the first 10 minutes after isotope infusion. The timing shown is the time to appearance in the interstitial catheter, with estimated transit time subtracted from the time at which the sample was collected. From the above figures, the time from isotope bolus infusion to appearance in the ISF beyond MR > 0.3% appeared to be 4 to 6 minutes for $[6,6^{-2}\text{H}_2]$ glucose and slightly longer for $[1^{-13}\text{C}]$ glucose. From the Kaplan Meier survival model, the mean (standard error) time to appearance for $[6,6^{-2}\text{H}_2]$
glucose and [1-\textsuperscript{13}C] glucose were 5.4 (0.6) and 6.2 (1.2) minutes respectively. The 95% confidence intervals for the 75\textsuperscript{th} percentiles of the time-to-appearance distributions were 6.8 (5.8 to 6.8) and 9.8 (4.8 to 9.8) minutes, respectively. Note, the upper limits of the confidence intervals equal the point estimates since all participants had detectable values by 6.8 and 9.8 minutes for [6,6\textsuperscript{2}H\textsubscript{2}] glucose and [1-\textsuperscript{13}C] glucose, respectively. Thus, 9.8 minutes provides a conservative estimate of the maximum overall time to appearance at the ISF.

Plasma tracer glucose Molar Ratio (Figure 4, left and right panels): The left panel illustrates the subject-specific profiles for [1-\textsuperscript{13}C] glucose and [6,6\textsuperscript{2}H\textsubscript{2}] glucose for the participants over the entire sampling period for each isotope and the right panel the subject-specific profiles for the same tracers between 0-10 min.

BMI, waist and hip circumferences showed preliminary evidence of an inverse relationship with time to appearance (i.e., the larger the measure of central obesity, the faster the isotope’s peak concentration was observed). The Spearman correlations for these three measures respectively, were -0.34 (p=0.41), -0.59 (p=0.12) and -0.70 (p=0.054). The correlation with weight (-0.31, p=0.45) and waist to hip ratio (-0.31, p=0.47) demonstrated similar patterns of association. These findings, however, are exploratory and require further evaluation.

**Discussion:**

Applying glucose isotope infusion and frequent sequential sampling of ISF using microdialysis catheters we have demonstrated that the mean time to appearance of tracer glucose in the abdominal subcutaneous ISF after an intravenous bolus is between 5-6 minutes in the resting
overnight fasted state. To eliminate mass spectroscopic assay noise, we were stringent in selecting a detectability cutoff that was threefold that of the sensitivity of the assay. The time to appearance of tracer glucose in the ISF may have been even shorter had we sampled ISF more frequently during the first 10 minutes and utilized a less stringent detectability cutoff. However, since the sensitivity of mass spectroscopy to detect tracer glucose concentrations is considerably higher than unlabeled glucose measured by conventional means, we believe that our approach using frequent sampling of tracer glucose, provides accurate estimates of the physiological time lag of glucose transport from the vascular to the ISF compartment.

To provide the ‘cleanest’ initial approach to estimate tracer glucose kinetics, we decided on the intravenous (as opposed to intra-arterial) route for tracer glucose bolus. This is because the intravenous bolus would traverse the right heart and the pulmonary circulation before appearing in the systemic circulation, similar to the route taken by ingested glucose, albeit bypassing the liver at first pass.

The time lag between plasma and ISF glucose appears to differ depending on whether plasma glucose values are rising or falling (9-11) or the type of CGM instrument and sensor algorithm used (17; 18). Future studies are being planned to address these questions. Additionally, more detailed multi-compartmental modeling of the current data is being performed and will be reported in a separate publication. It is important to underscore though that the purpose of this paper is to report the time-lag of glucose to appear in the ISF from the vascular compartment.
Like all studies, this study also has limitations. After reviewing [3-^3^H] glucose data from the first four subjects, we observed that the disintegrations per minute in the microdialysate were below detectable limits of the scintillation counter for several ISF samples, thus providing unreliable data. Hence [3-^3^H] glucose bolus was replaced for remaining 4 subjects by saline and time-gap avoided by injecting [6,6-^2^H_2] glucose at 12 noon instead.

The preliminary finding of a suggestion of an inverse relationship between tracer appearance time and degree of central obesity was unexpected, intriguing and requires further systematic examination. Local metabolism of glucose tracers in the ISF was not accounted for during the study. However, it is unlikely that in the overnight fasted conditions, low physiological insulin concentrations would have resulted in meaningful tracer uptake into the subcutaneous adipose tissues. Studies examining ISF glucose transport during the postprandial state will need to account for local glucose uptake.

There were differences in the estimated mean time to appearance for the two isotopes likely due to direct result of the isotope-specific collection schedules. The intention of using four glucose tracers with staggered and sequential infusion and sample collection times (of both plasma and microdialysate) was to enable minute by minute estimation of tracer glucose appearance in the microdialysate at least in the period immediately after the tracer bolus doses. However, due to analytical reasons of inability to measure [3-^3^H] glucose, and issues about the reliability of [2-^{13}C] glucose assays as mentioned earlier, we chose to analyze the data obtained from [1-^{13}C] glucose and [6,6-^2^H_2] glucose for our purpose. However, it is noteworthy that by 9.8 minutes following bolus dose, all participants were observed to have had detectable isotope in the ISF;
Figure 3 suggests the isotopes may have been detected earlier with a different sampling schedule. Regardless of this difference the isotopes appear faster than prior reports for glucose.

To conclude, we have demonstrated in overnight fasted healthy humans that the physiological time lag of glucose transport between the vascular and ISF compartments is considerably shorter than many have hypothesized. This should permit the development of CGM systems and AEP devices with sufficient accuracy and timeliness to permit improved management of patient glycemic status. Future studies in type 1 diabetes are required under various dynamic conditions, including meals, exercise and recovery from hypoglycemia when glucose transport to and from the ISF could be altered. The preliminary results presented here hold promise for optimization of current and future generation sensor algorithms, sensor augmented pumps and AEP systems to manage insulin requiring patients with diabetes.
**Author Contributions:**

S.D., I.E., and J.C.A assisted in the conduct of the study and data handling; Y.C.K. manuscript review/editing; M.S. and R.E.C. data analyses and manuscript review/editing; T.P. contributed to the idea of using glucose tracers to investigate this issue and assisted in manuscript review/editing; C.C. study design, manuscript review/editing; A.B. and R.B. study design, data analyses and manuscript writing/reviewing/editing.

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Dr. Rita Basu is the guarantor of this work, had full access to all the data, and takes full responsibility for the integrity of data and the accuracy of data analysis. There are no conflicts of interest to declare for any of the authors.

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References:

Table 1: Subject Characteristics

<table>
<thead>
<tr>
<th>Subject Characteristics</th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>40 ± 19</td>
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<tr>
<td>Gender (M:F)</td>
<td>2 : 6</td>
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<tr>
<td>Weight (kg)</td>
<td>73.7 ± 14.9</td>
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<tr>
<td>Body Mass Index (kg/m$^2$)</td>
<td>25.4 ± 3.1</td>
</tr>
<tr>
<td>Waist Hip Ratio</td>
<td>0.83 ± 0.06</td>
</tr>
<tr>
<td>Fasting Plasma Glucose (mg/dL)</td>
<td>80.5 ± 9.7</td>
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<tr>
<td>Total Body Fat (%)</td>
<td>36.6 ± 13.3</td>
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<tr>
<td>Fat Free Mass (kg)</td>
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Figure Legends:

Figure 1: Experimental design showing sequence of tracer glucose boluses and sampling intervals. Subjects 1-4 received 1-\textsuperscript{13}C, 6,6-\textsuperscript{2}H\textsubscript{2}, 3-\textsuperscript{3}H, 2-\textsuperscript{13}C glucose while 5-8 received 1-\textsuperscript{13}C, saline, 6,6-\textsuperscript{2}H\textsubscript{2}, and 2-\textsuperscript{13}C glucose.

Figure 2: Plasma glucose concentrations observed during the study. Data are mean ± SD

Figure 3: Temporal profiles of microdialysate tracer glucose molar ratios obtained in each participant over the sampling period of 120 min after the tracer bolus dose at time 0 (left panel). Temporal profiles of microdialysate tracer glucose molar ratios obtained in each participant over the first 10 min after the tracer bolus dose at time 0 (right panel). The timed interstitial fluid collection includes a correction of 6.2 minutes to allow for transit from the catheter to the collection vial.

Figure 4: Temporal profiles of plasma tracer glucose molar ratios obtained in each participant over the sampling period of 120 min after the tracer bolus dose at time 0 (left panel). Temporal profiles of plasma tracer glucose molar ratios obtained in each participant over the first 10 min after the tracer bolus dose at time 0 (right panel).
Figure 1

Experimental Design

Labeled Glucose boluses: 2 hours apart

1-\(^{13}\)C

6,6-\(^2\)H\(_2\) or Saline

5 min sampling Starting 4 min

0 min

120 min

5 min sampling Starting 3 min

2-\(^{13}\)C

3-\(^3\)H or 6,6-\(^2\)H\(_2\)

5 min sampling Starting 2 min

240 min

5 min sampling Starting 1 min

360 min

Min sampling period: 5 min

Plasma and microdialysate samples for [1-\(^{13}\)C], [6,6-\(^2\)H\(_2\)], [3-\(^3\)H], [2-\(^{13}\)C] glucose

254x190mm (96 x 96 DPI)
Figure 2

Plasma Glucose

254x190mm (96 x 96 DPI)
Figure 3

Tracer Concentration Profiles by Subject

$^{13}$C Glucose

$^{6,6}$H$_2$ Glucose

Timed Interstitial Fluid Collection (mins)

Tracer Concentration Profiles by Subject During First 10 Minutes

$^{13}$C Glucose

$^{6,6}$H$_2$ Glucose

Timed Interstitial Fluid Collection (mins)