BRIEF REPORT

Time Lag of Glucose From Intravascular to Interstitial Compartment in Humans

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The accuracy of continuous interstitial fluid (ISF) 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 performed the first direct measurement of this phenomenon to our knowledge in eight healthy subjects under an overnight fasted condition. Microdialysis catheters were inserted into the abdominal subcutaneous space. After intravenous bolus administrations of glucose tracers, timed samples of plasma and ISF were collected sequentially and analyzed for tracer enrichments. After accounting for catheter dead space and assay noise, the mean time lag of tracer appearance in the interstitial space was 5.3–6.2 min. 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 min. Physiological delay between blood glucose and ISF glucose, therefore, should 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. *Diabetes* 62:4083–4087, 2013

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 (CGMs) that measure interstitial fluid (ISF) glucose concentrations through subcutaneously placed glucose-sensing probes. A recent critique of AEP systems cited large glucose transport 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 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 min (3–11). If the intrinsic physiological delay between blood and interstitial glucose transport is as high as some have hypothesized, it might be challenging to develop CGM systems with sufficient accuracy to permit CLC. However, if this delay is smaller (e.g., <10 min), CGM accuracy might be sufficient to permit the development of safe and effective CLC algorithms. In addition, a modest intrinsic physiological delay might permit the development of specialized algorithms to compensate for the physiological time lag purported to occur across compartmental barriers (5,8).

To our knowledge, there have been no previous studies regarding direct measurement of the transport of glucose from the vascular compartment to the ISF. Smith et al. (12) used a fluorescein tracer to measure the kinetics of blood-to-interstitial transport, but the results were obtained with the use of fluorescein dye as a surrogate for glucose rather than fluorescent or tracer glucose. We used a systematic approach to understand the physiology of glucose transport, combining glucose isotope dilution methodology with a microdialysis technique in overnight fasted healthy adults. After sequential administration of 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 indicates the time delay of glucose transport between intravascular and interstitial compartments because 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 Clinic Institutional Review Board. After obtaining informed consent, screening tests were performed at the Mayo Center for Translational Science Activities, Clinical Research Unit (CRU), to ensure that subjects were healthy, not pregnant, and met enrollment criteria. Subjects with a history of diabetes, glucose intolerance, or family history of diabetes in first-degree members were excluded. Subjects taking any medications except stable thyroid and hormone replacement therapy were excluded.

Subjects were admitted to the CRU at ~1600 h, consumed a standard 10 kcal/kg evening meal at ~1700 h, and remained NPO except water for the remainder of the study. At ~0600 h the next morning, the heated hand vein method was used 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.
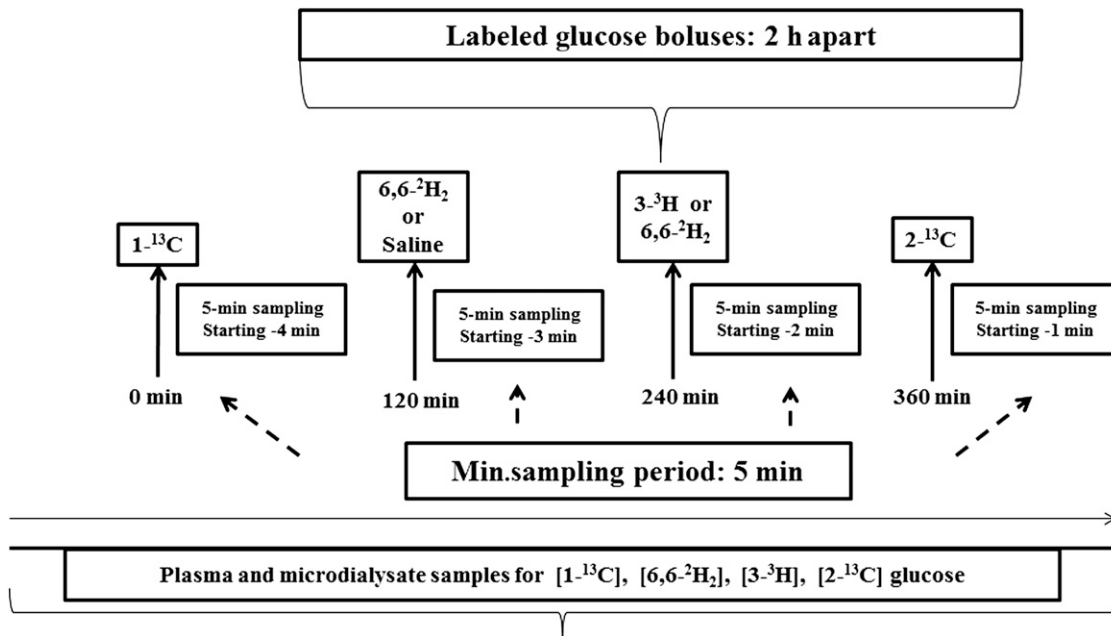
The experimental design is shown in Fig. 1. Four microdialysis catheters (CMA 63, 20-kDa molecular mass; CMA Microdialysis, North Chelmsford, MA) were inserted, under local anesthesia and aseptic precautions, into subcutaneous abdominal fat, two on each side of the anterior abdomen, and were infused with CMA perfusion fluid through a 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 volumes for analyses at the end of each collection point. After insertion, the catheters were allowed to stabilize and reach steady state for at least 1 h before tracer administration. At ~0800 h (0 min), an intravenous bolus of [1-^{13}C] glucose was administered over 10 s. Starting 4 min before the [1-^{13}C] glucose bolus, microdialysate samples were collected every 5 min for the next 30 min and periodically thereafter until 0957 h (117 min). Subsequent tracer glucose boluses and sequential timing of microdialysate and blood sample collections are depicted in Fig. 1. Doses of the stable isotopes ([1-^{13}C], [6,6-^{2}H$_2$], [2-^{13}C] glucose) were estimated to achieve plasma enrichment of ~4%, and [3-^{3}H] glucose (100 μCi) was used as the fourth tracer. Subjects 1–4 were infused with all four tracers, and subjects 5–8 were infused with only stable tracers, eliminating the [3-^{3}H] glucose and replacing it with saline in a different order.

Experimental Design

FIG. 1. Experimental design showing the sequence of tracer glucose boluses and sampling intervals. Subjects 1–4 received [1-^{13}C], [6,6-^{2}H$_2$], [3-^{3}H], and [2-^{13}C] glucose, whereas subjects 5–8 received [1-^{13}C] glucose, saline, [6,6-^{2}H$_2$] glucose, and [2-^{13}C] glucose.

Analytical techniques. Samples were placed on ice, divided into aliquots, and stored at −20°C until assayed. Plasma glucose concentration was measured with a YSI 2300 analyzer (YSI, Inc., Yellow Springs, OH) (14). [3-^{3}H] glucose counts were analyzed as previously described (15). Microdialysis and plasma samples for stable tracer enrichment were analyzed by gas chromatography–mass spectrometry. Selected ion monitoring was used to monitor fragments with a mass/charge ratio of 160 and 161 for [1-^{13}C] glucose and [2-^{13}C] glucose and 319 and 321 for [6,6-^{2}H$_2$] glucose (16).

Statistical analysis. The analysis of the tracer concentrations occurred in two steps. First, the timings of sample collection were reindexed to represent time from infusion to appearance at the microdialysis catheter on the basis of infusion setting and tubing volume to account for the time to cover the catheter dead space. It was determined that a 6.2-min transit time correction factor had to be applied. The second 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, which was defined as enrichment molar ratios (MRs) >0.3%. This corresponds to three times the upper limit of the mass spectroscopy assay noise (MR ±0.1%). In 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 95% CI upper limit of the 75th percentile of the failure distribution (i.e., the estimated time for which 75% of the 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 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 were not used because of interference from the [1-^{13}C] glucose given at 0800 h. The glucose derivative uses a fragment with a mass/charge ratio of 160 and 161 for the determination of [^{13}C] glucose enrichment that contains both the C1 and the C2 carbons of glucose. [1-^{13}C] glucose tracer enrichments were still above the baseline value at the time the [2-^{13}C] glucose tracer was administered, indicating that the [1-^{13}C] glucose was not totally cleared in 360 min. Therefore, we did not use the [2-^{13}C] glucose data. Statistical analyses were conducted with SAS version 9.3 software (SAS Institute, Inc., Cary, NC).

RESULTS

Table 1 shows the demographic characteristics of the study subjects.

Plasma glucose concentrations. Plasma glucose concentrations were normal before the start of the study (5.15 ± 0.45 mmol/L) and remained unchanged for the duration of the study (Fig. 2).

Microdialysate tracer glucose MR. Figure 3A illustrates the subject-specific profiles for [1-^{13}C] glucose and [6,6-^{2}H$_2$] glucose over the entire sampling period, and Fig. 3B shows the subject-specific profiles for the same tracers between 0–10 min. Fig. 3B also illustrates the same data with more resolution over the first 10 min after isotope infusion. 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 Fig. 3A and B, the time from isotope bolus infusion to appearance in the ISF beyond MR >0.3% appeared to be 4–6 min for [6,6-^{2}H$_2$] glucose and slightly longer for [1-^{13}C] glucose. From the Kaplan-Meier survival curve, the mean (SE) time to appearance for [6,6-^{2}H$_2$] glucose and [1-^{13}C] glucose were 5.4 (0.6) and 6.2 (1.2) min, respectively. The 95% CI upper

TABLE 1
Subject characteristics

Characteristic	Value
Age (years)	40 ± 19
Sex (male:female)	2:6
Weight (kg)	73.7 ± 14.9
BMI (kg/m^2)	25.4 ± 3.1
Waist-to-hip ratio	0.83 ± 0.06
Fasting plasma glucose (mg/dL)	80.5 ± 9.7
Total body fat (%)	36.6 ± 13.3
Fat-free mass (kg)	46.5 ± 15.0

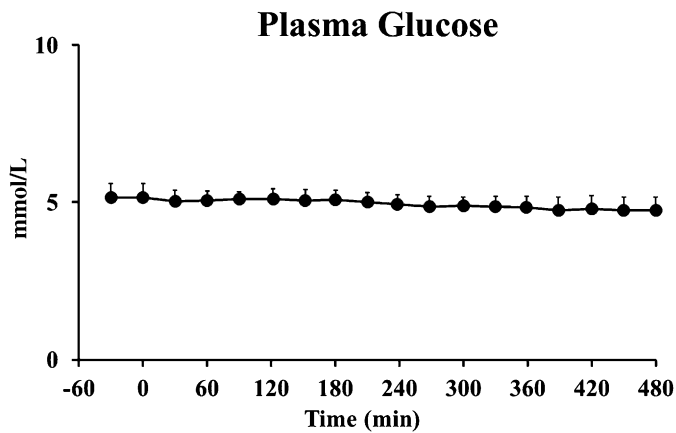Data are mean ± SD unless otherwise indicated.

Plasma Glucose

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

limits for the 75th percentiles of the time-to-appearance distributions were 6.8 (5.8–6.8) and 9.8 (4.8–9.8) min, respectively. Note that the upper limits of the CIs equal the point estimates because all subjects had detectable values by 6.8 and 9.8 min for [6,6-^{2}H$_2$] glucose and [1-^{13}C] glucose, respectively. Thus, 9.8 min is a conservative estimate of the maximum overall time to appearance in the ISF.

Plasma tracer glucose MR. Figure 4A illustrates the subject-specific profiles for [1-^{13}C] glucose and [6,6-^{2}H$_2$] glucose over the entire sampling period, and Fig. 4B shows the subject-specific profiles for the same tracers between 0–10 min. BMI and 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 were −0.34 ($P = 0.41$), −0.59 ($P = 0.12$), and −0.70 ($P = 0.054$), respectively. 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

With the application of glucose isotope infusion and frequent sequential sampling of ISF through microdialysis
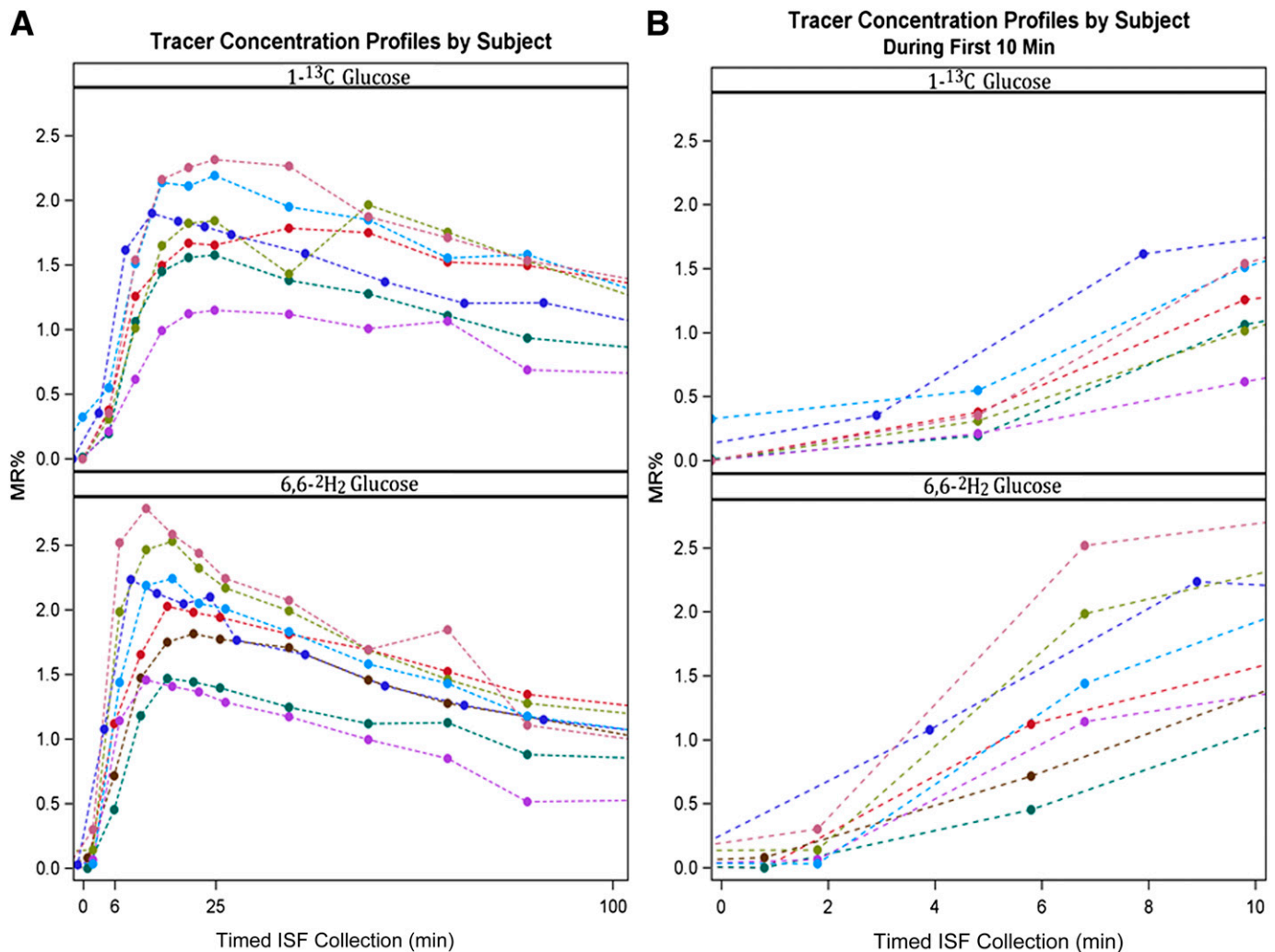
FIG. 3. A: Temporal profiles of microdialysate tracer glucose MRs obtained in each subject over the sampling period of 120 min after the tracer bolus dose at time 0. B: Temporal profiles of microdialysate tracer glucose MRs obtained in each subject over the first 10 min after the tracer bolus dose at time 0. The timed ISF collection included a correction of 6.2 min to allow for transit from the catheter to the collection vial.
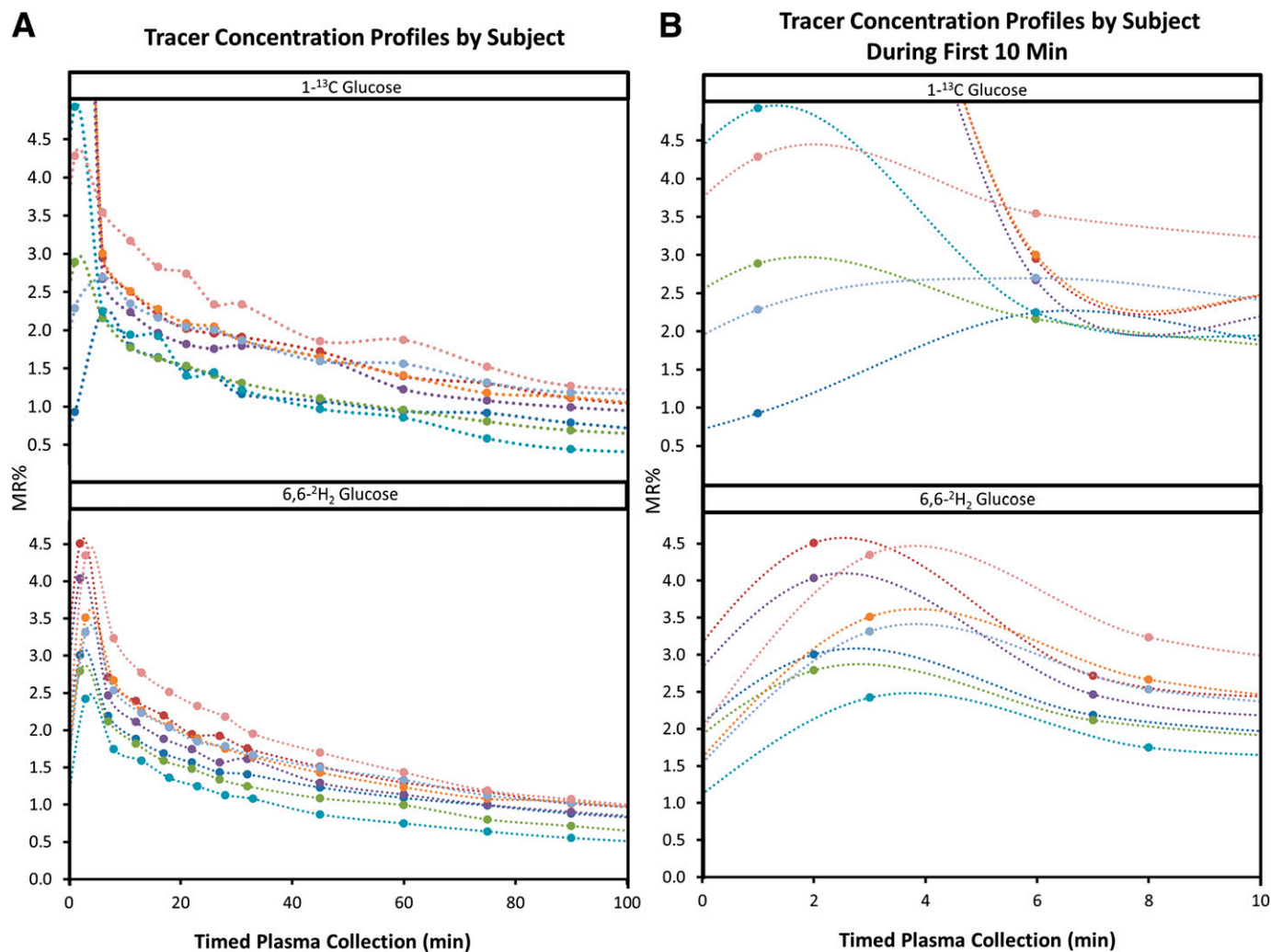
A Tracer Concentration Profiles by Subject

B Tracer Concentration Profiles by Subject During First 10 Min

FIG. 4. *A*: Temporal profiles of plasma tracer glucose MRs obtained in each subject over the sampling period of 120 min after the tracer bolus dose at time 0. *B*: Temporal profiles of plasma tracer glucose MRs obtained in each subject over the first 10 min after the tracer bolus dose at time 0.

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 and 6 min 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 min and used a less stringent detectability cutoff. However, because 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 because the intravenous bolus would traverse the right side of the 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 will address these questions. Additionally, more detailed multicompartmental modeling of the present data are currently being performed. It is important to underscore that the purpose of the present article is to report the time lag of glucose to appear in the ISF from the vascular compartment.

The present study has some 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, for the remaining four subjects, the [3-^{3}H] glucose bolus was replaced by saline, and the time gap was avoided by injecting [6,6-^{2}H$_2$] glucose at 1200 h instead.

The preliminary finding of an inverse relationship between tracer appearance time and degree of central obesity was unexpected yet 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.

Differences in the estimated mean time to appearance for the two isotopes were likely a 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, because of an inability to measure [3-^{3}H] glucose and issues around 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. Of note, by 9.8 min after the bolus, all subjects were observed to have had detectable isotope in the ISF; as seen in Fig. 3, the isotopes may have been detected earlier with a different sampling schedule. Regardless of this difference, the isotopes appeared faster than in prior reports (3–11).

In conclusion, 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, permitting the development of CGM systems and AEP devices with sufficient accuracy and timeliness for improved management of glycemic status. Future studies in type 1 diabetes are required under various dynamic conditions, including meals, exercise, and recovery from hypoglycemia, to determine 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-dependent patients with diabetes.

ACKNOWLEDGMENTS

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