ORIGINAL ARTICLE

Periodic Extraction of Interstitial Fluid from the Site of Subcutaneous Insulin Infusion for the Measurement of Glucose: A Novel Single-Port Technique for the Treatment of Type 1 Diabetes Patients

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Abstract

Background: Treatment of type 1 diabetes patients could be simplified if the site of subcutaneous insulin infusion could also be used for the measurement of glucose. This study aimed to assess the agreement between blood glucose concentrations and glucose levels in the interstitial fluid (ISF) that is extracted from the insulin infusion site during periodic short-term interruptions of continuous subcutaneous insulin infusion (CSII).

Subjects and Methods: A perforated cannula (24 gauge) was inserted into subcutaneous adipose tissue of C-peptide-negative type 1 diabetes subjects ($n=13$) and used alternately to infuse rapid-acting insulin (100 U/mL) and to extract ISF glucose during a fasting period and after ingestion of a standard oral glucose load (75 g).

Results: Although periodically interrupted for extracting glucose (every hour for approximately 10 min), insulin infusion with the cannula was adequate to achieve euglycemia during fasting and to restore euglycemia after glucose ingestion. Furthermore, the ISF-derived estimates of plasma glucose levels agreed well with plasma glucose concentrations. Correlation coefficient and median absolute relative difference values were found to be 0.95 and 8.0%, respectively. Error grid analysis showed 99.0% of all ISF glucose values within clinically acceptable Zones A and B (83.5% Zone A, 15.5% Zone B).

Conclusions: Results show that ISF glucose concentrations measured at the insulin infusion site during periodic short-term interruptions of CSII closely reflect blood glucose levels, thus suggesting that glucose monitoring and insulin delivery may be performed alternately at the same tissue site. A single-port device of this type could be used to simplify and improve glucose management in diabetes.

Introduction

OVER THE PAST 20 YEARS, various glucose monitoring methods have been developed that use the interstitial fluid (ISF) of cutaneous and subcutaneous tissue for the measurement of glucose.¹ These methods include needle-type electrochemical sensors,^{2,3} ultrafiltration,⁴ microdialysis,^{5,6} open-flow microperfusion (MP),^{7,8} and transdermal extraction using reverse-iontophoresis,⁹ sonophoresis,¹⁰ and hypodermic needles.¹¹ Currently, several ISF-based glucose monitoring devices are commercially available and clinically used as an adjunctive device to complement conventional capillary glucose meters in treating diabetes patients.^{3,6} However, to find application as a replacement for the conventional capillary glucose meters, the accuracy, reliability, and comfort of the current ISF-based glucose monitoring

devices may need to be increased, and it is important that the integration with insulin delivery technologies, like insulin pens and pumps, may need to be improved.^{1,12}

In pursuit of a more painless and convenient glucose monitoring method, we recently ascertained whether the site of subcutaneous insulin delivery can also be used for the measurement of glucose.^{13,14} Using the euglycemic clamp technique together with MP or microdialysis catheters for coupling insulin delivery with glucose sampling at the same tissue site, we first examined the kinetics of insulin action on the glucose concentration at the site of subcutaneous insulin delivery.¹³ We observed that within 60 min after exposing adipose tissue of healthy humans to a standard 100 U/mL insulin preparation, insulin's effect on the tissue glucose concentration saturates, and a stable tissue-to-plasma glucose concentration gradient is attained. Having obtained evidence

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that stable tissue-to-plasma glucose concentration gradients are attained at the site of continuous subcutaneous insulin infusion (CSII), we subsequently performed a study in diabetes patients¹⁴ in whom an MP catheter was used to carry out CSII and glucose sampling *simultaneously* at the same adipose tissue site during an overnight fast and after ingestion of a standard oral glucose load (oral glucose tolerance test [OGTT]). In this study we found that glucose levels measured at the insulin delivery site during uninterrupted CSII can be used to reliably estimate blood glucose concentrations.

In our recent study examining the kinetics of insulin action on the tissue glucose concentration at the insulin delivery site,¹³ we also observed that, after cessation of the insulin delivery, insulin's effect on the tissue-to-plasma glucose concentration gradient persisted for 30–60 min. Thus, we reasoned that, when insulin infusion into adipose tissue is interrupted for short times, the glucose levels observed during these interruptions at the infusion site could also be used to reliably estimate plasma glucose concentrations. Therefore, the purpose of the present study was to test the feasibility of estimating plasma glucose concentrations from the ISF glucose levels measured at the insulin infusion site during short-term interruptions of CSII. For this reason, a perforated cannula (24-gauge MP catheter) was inserted into subcutaneous adipose tissue of subjects with type 1 diabetes and used to carry out *sequential* insulin infusion and ISF glucose extraction during a fasting and an OGTT period.

Subjects and Methods

Study subjects

Subjects were men and women required to be in the age group of 18–64 years and diagnosed with type 1 diabetes. They had to have a glycated hemoglobin (HbA_{1c}) level of

<10% and be treated with CSII or multiple daily insulin injections (MDI). Subjects were excluded if they had evidence of clinically overt diabetes complications, had C-peptide levels in blood plasma >22 pmol/L, and used medications (other than insulin) that are known to influence carbohydrate metabolism. Each subject signed a written consent form prior to any study-related procedures. The study was approved by the ethics committee of the Medical University of Graz.

Study design

In the morning after an overnight fast, study subjects were admitted to the clinical research center. Subjects treated with MDI omitted the injection of long-acting insulin on the morning of the study, and subjects with CSII treatment disconnected their insulin pumps upon arrival at the clinical research center.

At approximately 7:00 a.m., an intravenous catheter was inserted into a hand vein for blood sampling. The forearm with the catheter was then placed in a thermoregulated box (55°C) to ensure arterialization of the venous blood. After intravenous catheter insertion, a 24-gauge MP catheter was placed into the periumbilical subcutaneous adipose tissue. Subsequently, a wearable peristaltic pump (MPP101 pump; Joanneum Research, Graz, Austria) was attached to the catheter inflow tubing (Fig. 1). At approximately 7:30 a.m., an infusion of rapid-acting insulin (100 U/mL, insulin aspart; Novo Nordisk, Bagsvaerd, Denmark) using the MP catheter was begun. During a subsequent baseline period lasting 3–5 h, the insulin infusion rate was adjusted periodically so as to achieve and/or maintain plasma glucose levels below 8.33 mmol/L (150 mg/dL). During the last 2 h of the baseline period, two ISF samples were taken at –90 and –30 min using the MP catheter.

At time 0, the subject ingested 75 g of glucose dissolved in 300 mL of water (Glucoral; Unipack, Wiener Neustadt,

FIG. 1. Schematic experimental setup for sequential insulin delivery and glucose sampling. A perforated microperfusion (MP) catheter was inserted into subcutaneous adipose tissue of subjects with diabetes ($n=13$), and the inflow tubing of the catheter was connected to a reservoir filled with rapid-acting insulin (100 U/mL, insulin aspart). By attaching a wearable peristaltic pump to the inflow tube and operating the pump bidirectionally, the MP catheter was then alternately used for insulin delivery and glucose sampling. In the insulin delivery mode (inflow tube end in position 1), the insulin delivery rate was adjusted by adjusting the speed of the peristaltic pump. For extracting tissue glucose (every hour for 10 min), the flow direction was reversed, and interstitial fluid was transported through the perforations of the catheter into the inflow tubing (approximately 100 mm into the 500-mm length of inflow tubing). The extracted interstitial fluid in the inflow tubing was then pumped into the sampling vial (inflow tube end in position 2).

Austria). Twenty minutes before glucose ingestion, an insulin bolus was administered over a period of 15 min via the MP catheter. The size of the insulin bolus administered was calculated using medical records on each subject's insulin-to-carbohydrate ratio. After administration of the insulin bolus, the basal insulin delivery via the MP catheter was continued and periodically adjusted to re-establish normal plasma glucose by approximately 5 h after glucose ingestion.

During the 6-h OGTT phase, ISF samples were drawn every 60 min. For drawing an ISF sample, the peristaltic pump attached to the catheter inflow tubing was stopped and then operated in the reverse direction for 8 min at a flow rate of approximately $0.6 \mu\text{L}/\text{min}$. During the 8-min sampling phase, ISF was continuously drawn from the tissue through the macroscopic catheter perforations into the inflow tubing (Fig. 1).

At the end of the sampling phase, the pump was stopped, and the inflow tubing was disconnected from the catheter inlet and connected to a capped sampling vial (microvial; CMA Microdialysis AB, Stockholm, Sweden) using a 30-gauge syringe needle (disposable injection needle; Transcend, Kiel, Germany). The ISF contained in the inflow tubing was then transferred into the vial by operating the pump in the forward direction for 1 min at a flow rate of approximately $3.0 \mu\text{L}/\text{min}$ (Fig. 1). After the inflow tubing was reconnected to the catheter inlet, insulin infusion was resumed.

Each time before ISF sampling was started, the insulin infusion rate was increased over 1 min, so as to replace the amount of insulin being missed during the subsequent ISF sampling period lasting approximately 10 min. For example, in the case of basal insulin delivery at a rate of $0.6 \text{ U}/\text{h}$, the insulin infusion rate was increased to $6.6 \text{ U}/\text{h}$ for 1 min in order to deliver an additional amount of 0.1 U of insulin (i.e., the amount missed during the sampling period of 10 min). No insulin was infused in sampling situations where basal insulin delivery was 0 and no additional glucose was ingested. The subjects were asked to ingest additional glucose if during the experiment the plasma glucose levels decreased below $3.22 \text{ mmol}/\text{L}$ ($58 \text{ mg}/\text{dL}$).

Blood samples for the measurement of the plasma glucose concentration were drawn every 10–30 min throughout the experiment. Additional blood samples were collected every 30 min in lithium heparin-coated tubes (BD Vacutainer™ Systems, Plymouth, UK) and used for the determination of the plasma electrolyte concentration.

MP catheter

The MP catheters applied were of concentric design with a cylindrical inner and outer tube. The outer tube of the MP catheter (Fig. 1)^{7,8} consisted of a conventional, intravenous cannula (outer diameter, 0.65 mm ; shaft length, 19 mm ; Neoflon™; Becton Dickinson Infusion Therapy AB, Helsingborg, Sweden) in which 27 perforations were formed in the cannula wall using an excimer laser (Laser Zentrum Hannover e.V., Hannover, Germany). The perforations, each having a diameter of 0.3 mm , were distributed over a cannula length of 11 mm . One Tygon® (Saint-Gobain, Paris, France) tube (outer diameter, 0.75 mm ; inner diameter, 0.25 mm ; length, 500 mm ; SCS001 tubing kit; Joanneum Research) was used to connect the catheter inlet with the reservoir containing the insulin solution. A second Tygon tube (length, 50 mm) connected to the catheter outlet was clamped near the catheter outlet

(Fig. 1)⁸ using a tubing clamp (ratchet tubing clamp; Cole-Parmer, Vernon Hills, IL).

Analytical procedures

Plasma glucose concentrations were measured at the bedside using a Beckman glucose analyzer II (Beckman Instruments, Fullerton, CA) with coefficient of variation (CV) of 2%. The glucose analyzer was calibrated with a $150 \text{ mg}/\text{dL}$ glucose standard solution (Beckman Coulter Inc., Fullerton).

Because during ISF sampling the ISF was drawn into the catheter inflow tubing that was also used for insulin infusion, insulin solution residues from previous infusion may have been carried over to the extracted ISF, thereby diluting the ISF sample. To determine the degree of dilution of the extracted ISF by the insulin solution residues, the electrolyte concentration in the insulin solution, blood plasma, and extracted fluid samples was measured using a contactless conductivity detector (TraceDec®; I.S.T., Strasshof, Austria) positioned on a fused silica capillary (inner diameter, $50 \mu\text{m}$; outer diameter, $360 \mu\text{m}$; Agilent Technologies, Santa Clara, CA). Aliquots of the fluid samples (approximately $1 \mu\text{L}$) were drawn from the sample vials into the capillary by using a peristaltic pump (Minipuls® 3; Gilson, Villiers-le-Bel, France) coupled to the capillary. To determine the electrolyte concentration in a sample, the conductivity detector was calibrated against a set of 12 NaCl standards ranging in concentration from 0 to $9 \text{ g}/\text{L}$. The calibration procedure consisted of plotting the standard concentrations versus detector response values and fitting a third-degree polynomial to the obtained data points. The derived regression equation and the measured conductivity values were then used to determine the electrolyte concentration in the plasma, insulin, and extracted ISF samples. The electrolyte concentration was determined with a within-run CV of $<1\%$.

Glucose concentrations in the extracted ISF samples were measured using a glucose test strip device with a sample volume requirement of approximately $0.3 \mu\text{L}$ (FreeStyle® Freedom blood glucose meter; Abbott Diabetes Care, Vienna, Austria). The glucose measurement was performed immediately after the conductivity measurement by transferring the sample fluid contained in the fused silica capillary onto the test strip of the glucose meter using the peristaltic pump coupled to the capillary. Separate in vitro experiments have shown that changes in the ISF sample matrix caused by dilution of the extracted ISF by the insulin solution residues (e.g., decreased electrolyte concentration and presence of insulin solution preservatives) did not affect the precision and accuracy of the ISF glucose measurements with the glucose meter. Furthermore, to determine the accuracy and precision of the glucose meter against the laboratory instrument used for plasma glucose measurements (Beckman glucose analyzer II), plasma samples ($n=61$) were additionally analyzed on the glucose meter, and the results were compared with plasma glucose readings obtained with the laboratory instrument. As the glucose meter is intended for the measurement of glucose in capillary whole blood, the glucose meter results were multiplied by a constant factor of 0.84 to obtain concentrations in plasma.^{15,16} From the converted glucose meter and laboratory instrument results, the correlation coefficient and the mean and median absolute relative differences (ARD) were calculated to be 0.98, 5.2%, and 3.9%, respectively.

The plasma C-peptide concentrations were determined using a two-site enzyme immunoassay (Mercodia C-peptide enzyme-linked immunosorbent assay; Mercodia AB, Uppsala, Sweden) with a lower limit of quantification of 22 pmol/L.

HbA_{1c} was measured by high-performance liquid chromatography (Menarini HA-8160; Menarini Diagnostics, Florence, Italy).

Data analysis

The ISF glucose concentration (G_{isf}) was calculated as the glucose concentration in the extracted ISF sample (G_{ex}) divided by the dilution factor (DF). The DF was determined for each sample as

$$DF = \frac{V_{\text{isf}}}{V_{\text{ins}} + V_{\text{isf}}} = \frac{C_{\text{ex}} - C_{\text{ins}}}{C_{\text{pl}} - C_{\text{ins}}} \quad (1)$$

where V_{isf} is the volume of pure ISF in the sample, V_{ins} is the volume of insulin solution residues in the sample, and C_{ins} , C_{ex} , and C_{pl} are the measured electrolyte concentration in the insulin solution, the extracted ISF sample, and the corresponding plasma sample, respectively. Application of this technique (i.e., ionic reference technique)^{7,8} was possible because the electrolyte concentration in blood plasma is very close to the concentration in the ISF and the electrolyte concentration in the used insulin solution is low compared with that in the blood plasma (insulin aspart, approximately 22.2% of average C_{pl}). By carrying out error propagation analysis, it can be shown that the CV for the determination of G_{isf} ($CV_{G_{\text{isf}}}$) can be calculated as follows:

$$CV_{G_{\text{isf}}} = \sqrt{(CV_{G_{\text{ex}}})^2 + (CV_{DF})^2} \quad (2)$$

where $CV_{G_{\text{ex}}}$ and CV_{DF} are the CVs for the determination of the glucose concentration and DF in the extracted ISF samples, respectively. $CV_{G_{\text{ex}}}$ was found to be approximately 4.3%. Again, using a propagation of errors analysis and assuming a constant electrolyte concentration in the infused insulin solution, it can be shown that the following relationship between CV_{DF} and the CVs of the measured electrolyte concentrations in plasma ($CV_{C_{\text{pl}}}$) and extracted ISF ($CV_{C_{\text{ex}}}$) exists:

$$CV_{DF} = \sqrt{\frac{(CV_{C_{\text{ex}}})^2}{\left(1 - \frac{C_{\text{ins}}}{C_{\text{ex}}}\right)^2} + \frac{(CV_{C_{\text{pl}}})^2}{\left(1 - \frac{C_{\text{ins}}}{C_{\text{pl}}}\right)^2}} \quad (3)$$

By taking an upper limit value of 1% for $CV_{C_{\text{ex}}}$ and $CV_{C_{\text{pl}}}$, and using the C_{ex} , C_{pl} , and C_{ins} values measured in this study, it can be estimated from Eq. 3 that the average CV for the derived DF data was 1.9% (range, 1.7–2.5%). Note that these CV_{DF} values are lower than the $CV_{G_{\text{ex}}}$ value. Estimates of plasma glucose concentration (termed tissue glucose concentrations) were derived from the ISF glucose levels (G_{isf}) by using a prospective one-point calibration procedure¹⁷ that consisted of dividing the ISF glucose values with the ISF-to-plasma glucose ratio calculated from the ISF and corresponding plasma glucose concentrations observed at the beginning of each experiment (at –90 min). Agreement between ISF-derived estimates of plasma glucose levels and directly measured plasma glucose concentrations was as-

sessed by applying error grid analysis and the method of residuals.^{18,19} Correlation analysis was performed using Pearson's product-moment correlation coefficient. The mean and median ARD were calculated by determining the average of the absolute value of the percentage difference between each paired tissue and plasma glucose concentration. Data analysis was performed using an ORIGIN software package (version 8.5; OriginLab Corp., Northampton, MA).

Results

Subject characteristics

The study was conducted in 13 type 1 diabetes patients (two women and 11 men; mean $\pm$ SE age, 38.3 $\pm$ 3.3 years; range, 23–59 years; mean $\pm$ SE body mass index, 25.7 $\pm$ 0.9 kg/m²; body mass index range, 21.1–32.9 kg/m²). Their mean duration of diabetes was 19.0 $\pm$ 2.4 years (range, 8–29 years), and their HbA_{1c} averaged 7.3 $\pm$ 0.2% (range, 5.7–7.8% [normal range, 4.3–5.9%]). Ten patients were treated with CSII and three with MDI. Subjects were all without residual endogenous insulin secretion, as indicated by undetectable C-peptide levels in blood plasma (i.e., < 22 pmol/L).

Insulin infusion and glycemic control

Although periodically interrupted for performing glucose extraction (every hour for approximately 10 min), insulin infusion with the MP catheter was adequate to achieve and maintain near normoglycemia during the fasting period as well as to restore near normal plasma glucose by 5 h after the ingestion of a standard oral glucose load (Fig. 2). Two hours before the glucose load, fasting plasma glucose concentration averaged 7.1 $\pm$ 0.4 mmol/L (mean $\pm$ SE) in the study subjects. At 126 $\pm$ 11 min after glucose ingestion, plasma glucose concentration increased to a peak of 14.3 $\pm$ 0.9 mmol/L. By the end of the study, at 360 min, the plasma glucose concentration had declined to a value (7.2 $\pm$ 0.6 mmol/L) that was similar to the fasting glucose levels. The basal insulin infusion rates used during the fasting and OGTT period averaged 0.91 $\pm$ 0.15 and 0.84 $\pm$ 0.10 U/h, respectively. Furthermore, the amount of insulin given as a bolus before ingestion of the glucose load averaged 8.3 $\pm$ 0.6 U.

Comparison between plasma and tissue glucose levels

ISF-derived estimates of plasma glucose levels (tissue glucose concentrations) agreed well with the measured plasma glucose concentrations during both the fasting and OGTT periods of the experiments (Fig. 2). In total, 103 paired tissue and plasma glucose values were obtained from the experiments in the subjects with diabetes. The correlation coefficient value for all data pairs was found to be 0.95. The median and mean ARD were calculated to be 8.0% and 11.2%, respectively. Furthermore, applying the method of residuals, the residual mean and 2 SD value were found to be 0.9% and 32.3%, respectively (Fig. 3A). In addition, error grid analysis showed that 99.0% of the values were in the clinically acceptable Zones A and B of the error grid, with 83.5% in Zone A and 15.5% in Zone B. There was one value in Zone C (1%) but none in Zones D and E (Fig. 3B). Overall, these results indicate that when insulin infusion into adipose tissue is interrupted for short periods, glucose levels observed at the infusion site during these interruptions may be used to reliably estimate plasma glucose concentrations.

FIG. 2. (Left page and above) Plasma and interstitial fluid-derived glucose concentrations observed during a fasting and an oral glucose tolerance test period in 13 subjects with diabetes. Panels show the time courses of the plasma glucose concentration (solid circles) and the glucose levels in the interstitial fluid that was extracted from the insulin delivery site during periodic short-term interruptions of the insulin infusion (tissue glucose concentration; open circles). Also shown are the time courses of the insulin delivery rate (gray solid bars) applied to control the plasma glucose concentration during experiments. Insulin infusion was interrupted every hour for 10 min to sample the interstitial fluid. The highest differences between plasma and tissue glucose concentrations were observed in subject 4 in the middle of the oral glucose tolerance test. Reasons for these discrepancies may be related to the calibration method applied. Separate linear regression analysis indicated that application of a two-point calibration method would be more appropriate to derive the tissue glucose concentrations for subject 4.

Discussion

The goal of the present investigation was to test the feasibility of estimating plasma glucose concentration from the ISF glucose concentration measured at the insulin infusion site

during short-term interruptions of CSII. To achieve this, we inserted a perforated cannula (MP catheter) into the subcutaneous adipose tissue of subjects with diabetes ($n=13$), attached a wearable peristaltic pump to the catheter tubing, and connected the catheter tubing end to a reservoir filled with

FIG. 3. Assessing agreement between the plasma and interstitial fluid-derived glucose concentrations by use of the method of residuals and error grid analysis. **(A)** Results of applying the method of residuals. Percentage differences in measurements of plasma and tissue glucose concentration (*y*-axis) are plotted against plasma glucose concentration measurements (*x*-axis). The dotted line represents the mean percentage difference between plasma and interstitial fluid-derived glucose values (0.9%), and the dashed lines represent the mean difference plus minus 2 times the SD of the differences (32.3%). **(B)** Error grid analysis results. Of 103 data points, 102 (99.0%) fell within the clinically acceptable Zone A or B of the error grid, with 86 (83.5%) in Zone A and 16 (15.5%) in Zone B. One data point (1.0%) fell within Zone C.

rapid-acting insulin (Fig. 1). By operating the pump bidirectionally, we were then able to alternately use the MP catheter as an insulin delivery and ISF extraction instrument during a fasting and an OGTT period. We found that the insulin infusion was adequate to achieve and maintain euglycemia during the fasting and after glucose ingestion, and, importantly, the glucose concentration measured in the extracted ISF closely reflected glucose levels in blood plasma during both the euglycemic and hyperglycemic periods of the experiments (Fig. 2).

To assess the agreement between the observed ISF-based estimates of plasma glucose levels and directly measured plasma glucose concentrations, we derived several agreement indexes by applying statistical methods, like correlation analysis, the method of residuals, and error grid analysis. The values of the agreement indexes obtained (Fig. 3) were similar to or better than those recently observed with commercial continuous glucose sensing devices that use the ISF of insulin-unexposed adipose tissue for the measurement of glucose.^{6,20–26} For example, recent studies assessing the accuracy of these continuous glucose sensing devices have observed median ARD values (range, 7.7–19.7%) that are similar to^{6,20,22} or higher than^{21,23–26} the median ARD value derived in the present study (8.0%). Furthermore, in a recent study performed in subjects with diabetes,¹⁴ we have assessed the agreement between the plasma glucose concentrations and the ISF glucose levels observed at the insulin delivery site during uninterrupted CSII. The values of the agreement indexes derived in that study¹⁴ were similar to those obtained in the present study. Overall, these results suggest that estimation of plasma glucose concentrations from the glucose levels observed at the insulin delivery site during both CSII and short-term interruptions of CSII is feasible and its quality is similar to or better than that of estimating plasma glucose concentrations from glucose levels measured in insulin-unexposed subcutaneous tissue using the latest commercial continuous glucose

sensing devices.^{6,20–26} Therefore, glucose monitoring and insulin delivery could be carried out both simultaneously or sequentially at the same adipose tissue site using a single tissue catheter (single-port treatment approach), thereby leading to the possibility of simplifying and improving glucose management in diabetes.

During the short-term interruption of CSII (for approximately 10 min every hour), the amount of ISF extracted from the insulin delivery site was about 3 μ L. As commonly used laboratory instruments for the determination of glucose (e.g., Beckman glucose analyzer) require relatively high sample volumes (e.g., >10 μ L), the glucose concentration in the ISF samples was measured by using a conventional glucose test strip device (Abbott Free Style Freedom blood glucose meter) with a sample volume requirement of approximately 0.3 μ L.¹⁵ This glucose meter was previously found to be one of the most accurate glucose test strip devices available²⁷ and was shown to be accurate enough to serve as a replacement for commonly used laboratory instruments (e.g., Beckman glucose analyzer) during glucose clamp studies.²⁸ To estimate the contribution of the applied glucose meter to the overall errors observed in the evaluation of this novel glucose monitoring method, we separately assessed the performance of the glucose meter by comparing venous plasma glucose measurements made using this glucose meter with plasma glucose values obtained by the reference method (Beckman glucose analyzer; see Subjects and Methods). The median ARD and residual mean and 2 SD values derived for the glucose meter were 3.9%, 1.6%, and 13.6%, respectively. These values are similar to those previously obtained for this glucose meter using fingertip capillary blood^{27,29} and venous blood glucose measurements.²⁹ Comparison of the median ARD and 2 SD values derived for the glucose meter with those obtained in the evaluation of this glucose monitoring method (median ARD, 3.9% vs. 8.0%; 2 SD, 13.6% vs. 32.3%) suggests that about half of the overall

errors observed in the method evaluation may have been introduced by the glucose meter alone. Undesired variability associated with the applied ISF sampling procedure (e.g., variations in the extraction and handling of the ISF, uncertainties associated with the determination of the DF), and/or physiological changes in the tissue at the sampling site (e.g., fluctuations in insulin's effect on the local blood flow) may have accounted for the other half of the overall errors observed in this method evaluation.

During the extraction of ISF (Fig. 1), the transfer tube was sometimes not completely filled with ISF. This incomplete filling of the transfer tube caused insulin solution residues also to be transferred to the sample vial, thereby diluting the collected ISF in the vial. In the present study, the degree of dilution due to the insulin residues was determined in each sample by applying the ionic reference technique. This technique is based on the measurement of the electrical conductivity in the ISF, plasma, and insulin infusate (see Subjects and Methods). Using this technique, dilution with insulin residues was observed in 25% of the ISF fluid samples collected. Thus, in the majority of the samples pure ISF was collected. The incomplete filling of the transfer tube with ISF and the subsequent dilution of the collected ISF with insulin residues were mainly caused by the relatively high intrasubject and intersubject variability in the ISF withdrawal rate. The proposed ionic reference technique may be easily integrated into a treatment device, as only the electrical conductivity in the collected ISF needs to be measured for deriving the degree of dilution due to insulin residues. Electrical conductivity in the insulin infusate and plasma may not need to be measured, as it can be safely assumed that the electrolyte concentrations in plasma and insulin infusate are constant and known.^{7,8} Alternatively, the determination of the degree of the dilution due to the insulin residues could also be omitted, if it can be guaranteed that the transfer line is always sufficiently filled with ISF during the sampling phases.

The present study was motivated by our recent observation in healthy humans¹³ that a stable interstitial-to-plasma glucose concentration gradient is attained at the insulin infusion site and that, after cessation of the insulin infusion, insulin's effect on the interstitial-to-plasma glucose concentration gradient persists for 30–60 min. Thus, maintenance of steady-state insulin action conditions for a considerable time after the termination of the insulin infusion may explain why interstitial glucose levels measured during the brief infusion interruptions at the infusion site closely reflect glucose concentrations in blood. There have been other studies evaluating the effect of supraphysiological insulin levels on the glucose concentration in adipose tissue by using either microdialysis-based glucose sensing³⁰ or continuous glucose sensors.³¹ However, whereas we assessed the glucose levels directly at the subcutaneous site of insulin infusion, these studies evaluated the glucose concentrations in tissue regions that were at considerable distances from the insulin administration site (i.e., 5, 9, 10, 20, and 30 mm). The results of these studies indicated that supraphysiological insulin levels affect³¹ or do not affect³⁰ the ISF glucose concentration in adipose tissue. The divergent findings may be partially attributable to technical and procedural differences in the performance of the studies.¹³

The present study demonstrates the feasibility of a novel single-port treatment technique, which is distinct from our

previously described single catheter-based method¹⁴ in that glucose determination and insulin infusion are not performed simultaneously. Instead, insulin infusion and glucose determination are carried out sequentially, and the duration of the periodic interruptions of the insulin infusion is analogous to that used in current insulin pumps.³² Thus, the present study may also provide the basis for the pursuit of a principal concept to the design of a single-port treatment system. This concept involves the integration of a continuous glucose sensor into the lumen of an insulin infusion catheter. By connecting this catheter to a bidirectional insulin pump, the system may then be used alternately to infuse insulin and to transport ISF glucose from the tissue to the glucose sensor in the catheter lumen. To enhance the ISF extraction efficiency of the catheter, the wall of the catheter shaft may also be provided with several perforations (similar to those of the MP catheter in the present study [Fig. 1]). Because insulin pumps usually deliver insulin in discrete pulses,³² with relatively long intervals between pulses (up to several minutes), transport of ISF to the sensor and subsequent ISF glucose sensing could be performed during the intervals between insulin pulses. Furthermore, because in the proposed design concept the sensor resides in the lumen of the infusion catheter, the tissue surrounding the infusion catheter may not come in contact with the sensor during system operation. As a result, the sensor may exhibit reduced biofouling and enhanced analytical performance (see Wisniewski and Reichert³³ and references therein). For the realization of the proposed design concept, however, bidirectional fluid pumping is required. Thus, as current insulin pumps only allow pumping in the forward direction, insulin pumps may need to be modified for bidirectional flow, thereby increasing development and manufacturing cost.

In addition, because of the negative suction pressure applied during ISF sampling, air bubbles may be frequently formed in the ISF that is conveyed in the transfer line. These bubbles did not disturb the subsequent glucose determination with the glucose meter used in the present study. However, if a continuous sensor is applied for the determination of glucose, the formed air bubbles may potentially lead to erroneous sensor signals due to incomplete wetting of the sensor membrane.

Furthermore, the frequency with which the glucose determination can be performed using this approach mainly depends on the withdrawal rate of the ISF, the sample volume requirement of the glucose measuring device used, and the dead space volume of the transfer line. In the present study, as the ISF withdrawal rate was low (approximately 0.5 $\mu\text{L}/\text{min}$) and as the applied sampling cannula and tubing were not optimized regarding the dead space volume, the resulting time needed to collect enough sample volume was approximately 10 min. Because of this relatively long sampling duration, it was not practical to increase the frequency with which the ISF samples were collected to more than one or two samples per hour. However, increases in the measurement frequency could be easily achieved by decreasing the dead space volume of the sampling cannula and the transfer tube and/or by using a continuous glucose sensor that is placed into the sampling cannula lumen close to the cannula tip.

Another limiting factor may be the depletion of ISF in the tissue surrounding a sampling catheter, which has been reported to occur after a few hours of sample site use.³⁴

Fortunately, no sign of ISF depletion at the sampling site was observed in any of our study subjects. The reason for that may be that when ISF is extracted directly from the subcutaneous site of insulin infusion, ISF depletion may not occur as there is a steady fluid influx to the tissue emanating from the insulin infusion. However, in our present study the sampling site was used only for 10–12 h. Therefore, further studies evaluating the approach over longer periods of time (2–3 days) and across the clinically relevant glycemic range (including very low glucose levels) are required.

Finally, in proposing the design concept, the availability of a continuous glucose sensor that is accurate enough to serve as a replacement for conventional glucose test strip devices was assumed. However, if such a sensor is not available in the near future, then the proposed concept could alternatively be realized with a tape-based glucose meter system, like the system recently brought to market by Roche, which contains a continuous tape with 50 glucose test areas.³⁵

In summary, our data show that, when insulin infusion into adipose tissue is interrupted for short periods, glucose concentrations measured at the infusion site during these interruptions closely reflect glucose levels in blood, thus indicating that glucose monitoring and insulin delivery may be carried out sequentially at the same adipose tissue site using a single tissue catheter. A single-port treatment of this type could be used to simplify and improve glucose management in diabetes.

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Author Disclosure Statement

T.R.P. is a co-founder and shareholder of Smart*Med GmbH and a consultant of Novo Nordisk and Hoffmann-La Roche. W.R. and T.R.P. have filed patent applications relating to the methodology described in this article. S.L., S.K., D.T., and M.B. declare no competing financial interests exist. All authors reviewed and edited the manuscript. W.R. conceived the project, designed experiments, planned the statistical analysis, supervised the experiments, and wrote the manuscript. S.L. performed the experiments, conducted data analysis, and helped to draft the manuscript. S.K., D.T., and M.B. contributed in setting up and performing the experiments. T.R.P. provided advice and assistance in performing experiments and statistical analysis. T.R.P. and W.R. are members of the “Artificial Pancreas at Home (AP@home)” Project Consortium.

References

- Vaddiraju S, Burgess DJ, Tomazos I, Jain FC, Papadimitrakopoulos F: Technologies for continuous glucose monitoring: current problems and future promises. *J Diabetes Sci Technol* 2010;4:1540–1562.
- McGarraugh G: The chemistry of commercial continuous glucose monitors. *Diabetes Technol Ther* 2009;11(Suppl 1):S-17–S-24.
- Girardin CM, Huot C, Gonthier M, Delvin E: Continuous glucose monitoring: a review of biochemical perspectives and clinical use in type 1 diabetes. *Clin Biochem* 2009;42:136–142.
- Tiessen RG, Kaptein WA, Venema K, Korf J: Slow ultrafiltration for continuous in vivo sampling: application for glucose and lactate in man. *Anal Chim Acta* 1999;379:327–335.
- Beyer U, Schäfer D, Thomas A, Aulich H, Haueter U, Reihl B, Ehwald R: Recording of subcutaneous glucose dynamics by a viscometric affinity sensor. *Diabetologia* 2001;44:416–423.
- Valgimigli F, Lucarelli F, Scuffi C, Morandi S, Sposato I: Evaluating the clinical accuracy of GlucoMen®Day: a novel microdialysis-based continuous glucose monitor. *J Diabetes Sci Technol* 2010;4:1182–1192.
- Trajanoski Z, Brunner GA, Schaupp L, Ellmerer M, Wach P, Pieber TR, Kotanko P, Skrabal F: Open-flow microperfusion of subcutaneous adipose tissue for on-line continuous ex vivo measurement of glucose concentration. *Diabetes Care* 1997;20:1114–1121.
- Schaupp L, Ellmerer M, Brunner GA, Wutte A, Sendlhofer G, Trajanoski Z, Skrabal F, Pieber TR, Wach P: Direct access to interstitial fluid in adipose tissue in humans by use of open-flow microperfusion. *Am J Physiol* 1999;276:E401–E408.
- Tamada JA, Bohannon NJ, Potts RO: Measurement of glucose in diabetic subjects using noninvasive transdermal extraction. *Nat Med* 1995;1:1198–1201.
- Kost J, Mitragotri S, Gabbay RA, Pishko M, Langer R: Transdermal monitoring of glucose and other analytes using ultrasound. *Nat Med* 2000;6:347–350.
- Collison ME, Stout PJ, Glushko TS, Pokela KN, Mullins-Hirte DJ, Racchini JR, Walter MA, Mecca SP, Rundquist J, Allen JJ, Hilgers ME, Hoegh TB: Analytical characterization of electrochemical biosensor test strips for measurement of glucose in low-volume interstitial fluid samples. *Clin Chem* 1999;45:1665–1673.
- Brauker J: Continuous glucose sensing: future technology developments. *Diabetes Technol Ther* 2009;11(Suppl 1):S-25–S-36.
- Lindpointner S, Korsatko S, Köhler G, Köhler H, Schaller R, Schaupp L, Ellmerer M, Pieber TR, Regittnig W: Glucose levels at the site of subcutaneous insulin administration and their relationship to plasma levels. *Diabetes Care* 2010;33:833–838.
- Lindpointner S, Korsatko S, Köhler G, Köhler H, Kaidar R, Yodfat O, Schaller R, Schaupp L, Ellmerer M, Pieber TR, Regittnig W: Use of the site of subcutaneous insulin administration for the measurement of glucose in patients with type 1 diabetes. *Diabetes Care* 2010;33:595–601.
- Feldman B, McGarraugh G, Heller A: FreeStyle: a small-volume electrochemical glucose sensor for home blood glucose testing. *Diabetes Technol Ther* 2000;2:221–229.
- Fogh-Andersen N, D’Orazio P: Proposal for standardizing direct-reading biosensors for blood glucose. *Clin Chem* 1998;44:655–659.
- Lodwig V, Heinemann L; Glucose Monitoring Study Group: Continuous glucose monitoring with glucose sensors: calibration and assessment criteria. *Diabetes Technol Ther* 2003;5:572–586.

18. Clarke WL, Cox D, Gonder-Frederick LA, Carter W, Pohl SL: Evaluating clinical accuracy of systems for self-monitoring of blood glucose. *Diabetes Care* 1997;20:1114–1121.
19. Bland JM, Altman DG: Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;1:307–310.
20. Weinstein RL, Schwartz SL, Brazg RL, Bugler JR, Peyser TA, McGarraugh GV: Accuracy of the 5-day FreeStyle Navigator Continuous Glucose Monitoring System: comparison with frequent laboratory reference measurements. *Diabetes Care* 2007;30:1125–1130.
21. Wilson DM, Beck RW, Tamborlane WV: The accuracy of the FreeStyle Navigator continuous glucose monitoring system in children with type 1 diabetes. *Diabetes Care* 2007;30:59–64.
22. Mastrototaro J, Shin J, Marcus A, Sulur G; STAR 1 Clinical Trial Investigators: The accuracy and efficacy of real-time continuous glucose monitoring sensor in patients with type 1 diabetes. *Diabetes Technol Ther* 2008;10:385–390.
23. Mazze RS, Strock E, Borgman S, Wesley D, Stout P, Racchini J: Evaluating the accuracy, reliability, and clinical applicability of continuous glucose monitoring (CGM): is CGM ready for real time? *J Diabetes Sci Technol* 2009;11:11–18.
24. Bailey T, Zisser H, Chang A: New features and performance of a next-generation SEVEN-day continuous glucose monitoring system with short lag time. *Diabetes Technol Ther* 2009;11:749–755.
25. Garg S, Jovanovic L: Relationship of fasting and hourly blood glucose levels to HbA1c values: safety, accuracy, and improvements in glucose profiles obtained using a 7-day continuous glucose sensor. *Diabetes Care* 2006;29:2644–2649.
26. Mader JK, Weinhandl H, Köhler G, Plank J, Bock G, Korsatko S, Ratzer M, Ikeoka D, Köhler H, Pieber TR, Ellmerer M: Assessment of different techniques for subcutaneous glucose monitoring in Type 1 diabetic patients during 'real-life' glucose excursions. *Diabet Med* 2010;27:332–338.
27. Freckmann G, Baumstark A, Jendrike N, Zschornack E, Kocher S, Tshiananga J, Heister F, Haug C: System accuracy evaluation of 27 blood glucose monitoring systems according to DIN EN ISO 15197. *Diabetes Technol Ther* 2010;12: 221–231.
28. Cohen O, Shaklai S, Gabis E, Pani MA: FreeStyle Mini blood glucose results are accurate and suitable for use in glycemic clamp protocols. *J Diabetes Sci Technol* 2008;2:890–895.
29. Diabetes Research in Children Network (DirecNet) Study Group: Relative accuracy of the BD Logic and FreeStyle blood glucose meters. *Diabetes Technol Ther* 2007;9:165–168.
30. Hermanides J, Wentholt IM, Hart AA, Hoekstra JB, DeVries JH: No apparent effect of insulin on microdialysis continuous glucose-monitoring measurements. *Diabetes Care* 2008;31: 1120–1122.
31. Rodriguez LT, Friedman KA, Coffman SS, Heller A: Effect of the sensor site-insulin injection site distance on the dynamics of local glycemia in the minipig model. *Diabetes Technol Ther* 2011;13:489–493.
32. Kreagen EW, Chisholm DJ: Pharmacokinetics of insulin—implications for continuous subcutaneous insulin infusion therapy. *Clin Pharmacokinet* 1985;10:303–314.
33. Wisniewski N, Reichert M: Methods for reducing biosensor membrane biofouling. *Colloids Surf B Biointerfaces* 2000;18:197–219.
34. Haar, H-P, Meacham GBK, List H: Direct monitoring of interstitial fluid composition. European Patent Application EP1479344, 2004. <http://worldwide.espacenet.com/> (accessed August 28, 2012).
35. Kaufmann N, Gambke B, Kraft M, Pfrang I, Ramstetter E, Rebernik J, Klaus Rinck K, Thilges J, Wieme-Selle J: Accu-chek® mobile: system evaluation. 2009. www.accu-chek.ch/documents/AC-Mobile-Sytem_Evaluation_e.pdf (accessed August 28, 2012).

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