Glucose Levels at the Site of Subcutaneous Insulin Administration and Their Relationship to Plasma Levels

Stefan Lindpointner, MD[1]
Stefan Korsatko, MD[1]
Gerd Köhler, MD[1]
Hans Köhler, MSC[2]
Roland Schaller, MSC[2]
Lukas Schaupp, PHD[1,2]
Martin Ellmerer, PHD[1]
Thomas R. Pieber, MD[1,2]
Werner Regittnig, PHD[1]

OBJECTIVE — To examine insulin's effect on the tissue glucose concentration at the site of subcutaneous insulin administration.

RESEARCH DESIGN AND METHODS — A CMA-60 microdialysis (MD) catheter and a 24-gauge microperfusion (MP) catheter were inserted into the subcutaneous adipose tissue of fasting, healthy subjects ($n = 5$). Both catheters were perfused with regular human insulin (100 units/ml) over a 6-h period and used for glucose sampling and simultaneous administration of insulin at sequential rates of 0.33, 0.66, and 1.00 units/h (each rate was used for 2 h). Before and after the insulin delivery period, both catheters were perfused with an insulin-free solution (5% mannitol) for 2 h and used for glucose sampling only. Blood plasma glucose was clamped at euglycemic levels during insulin delivery.

RESULTS — Start of insulin delivery with MD and MP catheters resulted in a decline of the tissue glucose concentration and the tissue-to-plasma glucose ratio (TPR) for ~60 min ($P < 0.05$). However, during the rest of the 6-h period of variable insulin delivery, tissue glucose concentration paralleled the plasma glucose concentration, and the TPR for MD and MP catheters remained unchanged at 83.2 ± 3.1 and 77.1 ± 4.8%, respectively. After subsequent switch to insulin-free perfusate, tissue glucose concentration and TPR increased slowly and reattained preinsulin delivery levels by the end of the experiments.

CONCLUSIONS — The results show the attainment of a stable TPR value at the site of insulin administration, thus indicating that insulin delivery and glucose sensing may be performed simultaneously at the same adipose tissue site.

Diabetes Care 33:833–838, 2010

When insulin is secreted from the pancreas, the resulting rise in the blood insulin level acts to lower the blood glucose concentration by both suppressing glucose production in the liver and enhancing glucose uptake in insulin-sensitive tissues (mainly muscle and adipose tissue) (1). Studies (2,3) investigating insulin's effect on glucose uptake in isolated muscle and fat cells have shown that the cellular response to increasing insulin concentration is a continuous increase in glucose uptake until a maximal response is reached. Further increases in the prevailing insulin concentrations beyond this threshold level does not further increase glucose uptake. Similar saturation-type concentration-response characteristics were found for insulin-stimulated arteriovenous glucose difference, blood flow, and glucose uptake (4,5) in the forearm and leg as well as for insulin-stimulated whole-body glucose uptake (1,4,5). In these in vivo studies, the maximal response to insulin in healthy humans was observed to occur at a blood plasma concentration of 300–1,000 μU/ml (1,4,5). Most notably, this maximally effective insulin level is ~100,000 times lower than the insulin concentration in preparations currently used in the replacement therapy of diabetic patients (100 units/ml) (6). Thus, given the observed saturation-type relationship between insulin concentration and insulin's effect on arteriovenous glucose difference, blood flow, and glucose uptake in human peripheral tissues, we reasoned that a similar saturation-type concentration-response characteristic for the insulin-stimulated difference between arterial blood glucose levels and interstitial fluid (ISF) glucose concentrations might exist in these tissues (online appendix Fig. 1 [available at http://care.diabetesjournals.org/cgi/content/full/dc09-1531/DC1]). An interstitial infusion of insulin into adipose tissue at rates used in the replacement therapy of diabetic patients might therefore evoke insulin concentrations at the infusion site that are much higher than the maximally effective insulin level of this tissue and, in turn, might ensure an essentially maximal and stable blood-to-ISF glucose concentration gradient in the tissue at this infusion site. If this were the case, then despite the presence of variable insulin delivery rates, reliable estimation of blood glucose concentrations from glucose concentrations measured in the ISF at the insulin delivery site would be possible.

The present study was undertaken to investigate this possibility in the subcutaneous adipose tissue of healthy subjects. Microperfusion and microdialysis techniques were utilized to perform glucose sampling and simultaneous insulin delivery at the same adipose tissue site. Using these techniques, ISF glucose samples from the site of insulin delivery were obtained in the presence of variable insulin delivery rates. The observed ISF glucose levels were then compared with plasma glucose concentrations as well as to glucose levels in ISF samples from insulin-unexposed adipose tissue.

From the [1]Department of Internal Medicine, Division of Endocrinology and Nuclear Medicine, Medical University of Graz, Graz, Austria; and the [2]Institute of Medical Technologies and Health Management, Joanneum Research Forschungsgesellschaft, Graz, Austria.
Corresponding author: Werner Regittnig, werner.regittnig@healthsite.at.
Received 16 August 2009 and accepted 14 January 2010. Published ahead of print at http://care.diabetesjournals.org on 22 January 2010. DOI: 10.2337/dc09-1531. Clinical trial reg. no. NCT00812591, clinicaltrials.gov.

RESEARCH DESIGN AND METHODS

— The study was conducted in five healthy male adult volunteers. The age of the subjects was 29.8 ± 4.0 years (range 19–44) and the BMI was 23.0 ± 0.9 kg/m^2 (20.7–26.0). Written informed consent was obtained after the purpose, nature, and potential risks of the study were explained to the subjects. The study was approved by the ethics committee of the Medical University of Graz.

In the morning after an overnight fast, study subjects were admitted to the clinical research center. At ~7:30 A.M., an intravenous catheter was placed into an arm vein for glucose infusion. Another catheter was inserted into a vein of the contralateral hand for blood sampling. The forearm with the sampling catheter was then placed in a thermoregulated box (55°C) to ensure arterialization of the venous samples. Subsequently, a 24-gauge microperfusion (MP) catheter (7,8) and a microdialysis (MD) catheter (CMA60, modified as described below; CMA/Microdialysis, Solna, Sweden) were inserted into the periumbilical subcutaneous adipose tissue, and peristaltic pumps (Minipuls 3; Gilson, Villiers-le-Bel, France) were attached to the inflow and outflow tubing of each catheter (Fig. 1). Both catheters were then sequentially perfused with an insulin-free solution (5% mannitol; Fresenius Kabi, Graz, Austria) for 2 h, a standard human insulin preparation (100 units/ml Actrapid; Novo Nordisk, Bagsvaerd, Denmark) for 6 h, and, again, an insulin-free solution (5% mannitol) for 2 h. During the 6-h insulin perfusion period, the insulin delivered to the tissue by the MP catheter was at a rate of ~0.33 units/h over the first 2 h, ~0.66 units/h over the subsequent 2 h, and ~1.00 units/h over the final 2 h. Similar insulin delivery rates, but in reverse chronological order, were administered via the MD catheters. As the outflow rates of the two catheters were maintained at a constant value (~0.45 μl/min) throughout the experiment, the insulin delivery rate of each catheter was adjusted by simply adjusting the inflow rate of the catheter (dual-pump operation mode) (9) (Fig. 1). During the experiment, catheter effluents were continuously collected in 30-min fractions in vials (Microvial; CMA/Microdialysis) kept on ice and covered to avoid fluid evaporation. In parallel, glucose concentrations in blood plasma were measured frequently (i.e., every 5–30 min), and, to prevent a fall in the blood glucose concentration during and after the subcutaneous insulin delivery period, glucose (0.1 g/ml; Fresenius Kabi) was intravenously infused at a variable rate.

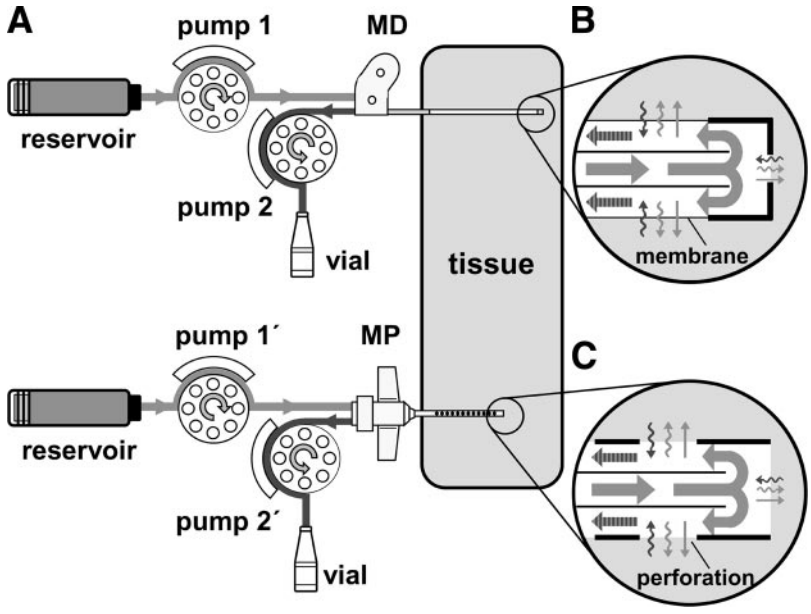

Figure 1—*Schematic of the experimental set-up for the assessment of insulin's effect on the glucose concentration at the tissue site of insulin delivery. A: A microperfusion (MP) and a microdialysis (MD) catheter were inserted into subcutaneous adipose tissue of healthy subjects (n = 5). Both catheters were perfused sequentially with an insulin-free solution (mannitol) for 2 h, a standard human insulin preparation (100 units/ml, Actrapid) for 6 h, and, again, an insulin-free solution (mannitol) for 2 h. Perfusion of each catheter was accomplished by applying two peristaltic pumps, with one pump (1, 1′) attached to the inflow tubing and another (2, 2′) to the outflow tubing (dual-pump operation mode [9]). B and C: The perfusate streamed through the inflow tubing and the inner cannula to the tip of the catheter and then entered the space between inner and outer cannula of the catheter* (bold arrows)*. By operating the inflow pump at a higher speed than the outflow pump, a fraction of the perfusate entering the space between inner and outer cannula was forced to flow through the perforations and membrane pores of the MP and MD catheter, respectively, to the surrounding tissue (tissue-directed flows indicated by* thin straight arrows)*. The magnitude of this flow fraction and, thus, the amount of perfusate solutes (e.g., insulin) convectively transported to the tissue was controlled by controlling the difference between the inflow and outflow rates of the catheter. In parallel to this convective tissue-directed solute transport, diffusive bidirectional solute transport took place across the perforations and membrane pores of the MP and MD catheter, respectively* (wavy arrows)*, thereby causing ISF solutes (e.g., glucose) to enter the fluid fraction that flowed in the space between inner and outer cannula of the catheter* (bold striped arrows)*. Along with the permeated ISF solutes, this fluid fraction was pumped continuously through catheter outlet and outflow tubing to the collecting vial. The efficiency by which glucose was transported via diffusion from the ISF of the tissue into the catheter (glucose recovery) was measured by applying the ionic reference technique (7,8).*

Microperfusion, microdialysis, and analytical procedures

MP and MD catheters applied were of concentric design with a cylindrical inner and outer tube (Fig. 1*B* and *C*). The outer tube of the MP catheter (7,8) consisted of a conventional, intravenous 24-G cannula (shaft length: 19 mm, Neoflon; Becton Dickinson, Helsingborg, Sweden) in which 27 perforations (each 0.3 mm in diameter) were formed in the cannula wall using an Excimer laser (LZH, Hannover, Germany). Two 750-mm lengths of Tygon tubing (outer diameter: 2.0 mm, inner diameter: 0.19 mm; Cole-Parmer, Vernon Hills, IL) were used to connect catheter inlet and outlet with perfusate reservoir and sampling vial, respectively. In the case of the MD catheter (CMA60), part of the outer tube was made from a polyamide membrane with a molecular weight cutoff of 20 kDa (membrane length: 30 mm, pore sizes: ~1 μm). To operate MD catheters in the dual pump mode (Fig. 1), the original inflow and outflow tubing of the catheters were replaced by two 750-mm lengths of Tygon tubing. In addition, to increase the insulin delivery efficiency of MD catheters, a fluid communication between the distal inner tube end and the outer surface of the cath-

eter tip was established by forming a perforation into the catheter tip using a 30-G syringe needle (point length: 1.2 mm; Microlance, Becton Dickinson, Drogheda, Ireland). To monitor the outflow rate of the MP and MD catheters and to determine the exact sampled effluent volume, the sample vials were weighed before and after sample collection. The effluent sampling delay time introduced by the dead space volume of the catheter outflow tubing was taken into account when the sample collection was begun.

Plasma glucose concentrations were measured at the bedside using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA) with a coefficient of variation (CV) of 2%. The glucose concentrations in catheter effluents were determined using an automated CMA600 analyzer (CMA/Microdialysis) with a within-run CV of 2%. Separate in vitro experiments have shown that preservatives contained in the regular human insulin solution used to perfuse the catheters (Actrapid) do not compromise the accuracy of the CMA600 glucose analyzer. The conductivities in the plasma, perfusate, and catheter effluent samples were measured using a contactless conductivity detector (TraceDec; I.S.T., Strasshof, Austria). The electrical conductivity was determined with a within-run CV of <1%.

Data analysis

The ISF glucose concentration was calculated (7,8) as the glucose concentration in the catheter effluent sample divided by the glucose recovery of the catheter (*R*). The recovery was determined for each sampling period as $R = (C_{out} - C_{in})/(C_{pl} - C_{in})$, where C_{in}, C_{out}, and C_{pl} are the measured electrical conductivity in the perfusate, the effluent sample, and the corresponding plasma sample, respectively. Application of this technique (ionic reference technique) (7,8) was possible because the electrical conductivity in the used catheter perfusates was either negligible (mannitol) or low compared with that in blood plasma (Actrapid: ~1.7% of average C_{pl}). To facilitate comparison of glucose time courses observed in the tissue ISF with those determined in the blood plasma, we calibrated the MP-derived and MD-derived ISF glucose values against the mean plasma glucose concentration observed during the 2-h baseline period (basal calibration). These glucose values (termed tissue glucose concentrations) and the corresponding plasma glucose levels were then used to calculate the time courses of the tissue-to-plasma glucose ratio (TPR). The differences of mean glucose and TPR results were examined by one-factor repeated-measures ANOVA. If significance was achieved, post hoc comparison of means with Dunnett's test was performed. A *P* value <0.05 was considered to indicate statistical significance. Normality of data were assessed using normal probability plots. Data are presented as means ± SE. Data analysis was performed using MATLAB (MathWorks, Natick, MA) and SPSS (SPSS, Chicago, IL) software packages.

RESULTS

Glucose dynamics at the site of subcutaneous insulin administration

Figure 2*A* shows the time courses of the glucose concentration in arterialized blood plasma and in the tissue surrounding the MP and MD catheters. Commencement of insulin delivery with the MD and MP catheters induced a fall in the tissue glucose concentration for ~60 min ($P < 0.05$). However, during the rest of the 6-h period of variable insulin delivery, the tissue glucose concentration paralleled the glucose concentration observed in plasma, thereby indicating that insulin's effect on the tissue glucose concentration saturated within 60 min after the start of insulin administration. After switching back from insulin to mannitol perfusates, tissue glucose concentration increased slowly and reattained plasma glucose levels at the end of the experiments (Fig. 2*A*).

Figures 2*B* and *C* depict the TPR for the MD and MP catheters as a function of time. As can be seen, during the first ~60 min of insulin delivery, the TPR declined to a level of 83.8 ± 3.6 and 78.5 ± 5.0% for MD and MP catheters, respectively, and then remained at these levels until the end of the 6-h period of variable insulin delivery. During the final 5 h of the insulin delivery period, the TPR levels for MD and MP catheters averaged 83.2 ± 3.1 and 77.1 ± 4.8%, respectively.

Taken together, the temporal pattern of change in tissue and plasma glucose and TPR values suggests that within 60 min after exposing adipose tissue to a standard 100 units/ml insulin preparation, insulin's effect on the tissue glucose concentration saturates and a stable ratio between the tissue and plasma glucose concentration is attained.

Glucose recoveries and insulin infusion rates

At the used outflow rate of ~0.47 μl/min, the transport efficiency (recovery) of glucose for the MP catheter was found to be 25.1 ± 5.4% during the first 2 h of insulin delivery and remained unchanged during the subsequent 2 h and final 2 h of insulin delivery (24.6 ± 4.4 and 24.8 ± 4.9%, respectively; $P = 0.93$, one-factor repeated-measures ANOVA). Similarly, recovery for the MD catheter was 96.8 ± 1.2% during the first 2 h of insulin delivery and remained at this level during the subsequent 2 h (98.0 ± 1.3%) and final 2 h (98.1 ± 1.3%) of insulin delivery ($P = 0.20$, one-factor repeated-measures ANOVA). The lower glucose recovery for MP catheters compared with MD catheters may be mainly attributable to the different size of the exchange area of the applied catheters (MP perforations distributed over an 11-mm shaft length versus MD membrane length of 30 mm). Figure 2*B* and *C* show the average time course of the total insulin delivery rate (i.e., the sum of the convective and diffusive portions of the insulin delivery rates) for the MD and MP catheters (see also online appendix supplementary methods).

Glucose infusion rates

The time course of the intravenous glucose infusion rate is shown in online appendix Fig. 2. As can be seen, the glucose infusion rate needed to maintain euglycemia gradually increased during the second half of the 6-h insulin delivery period and reached a peak value of 1.75 ± 0.31 $mg \cdot kg^{-1} \cdot min^{-1}$ 1 h after termination of insulin delivery. Glucose infusion was required until the end of the experiments.

CONCLUSIONS — The present investigation was undertaken to assess insulin's effect on the tissue glucose concentration at the site of subcutaneous insulin administration. For this reason, we subcutaneously administered insulin at variable rates and simultaneously sampled ISF glucose directly from the administration site. To carry out ISF glucose sampling and simultaneous insulin administration at the same adipose tissue site, we utilized MP and MD catheters together with standard insulin preparations as catheter perfusates and used two peristaltic pumps to operate each catheter (Fig. 1). This technique of catheter operation allowed the withdrawal of ISF at a

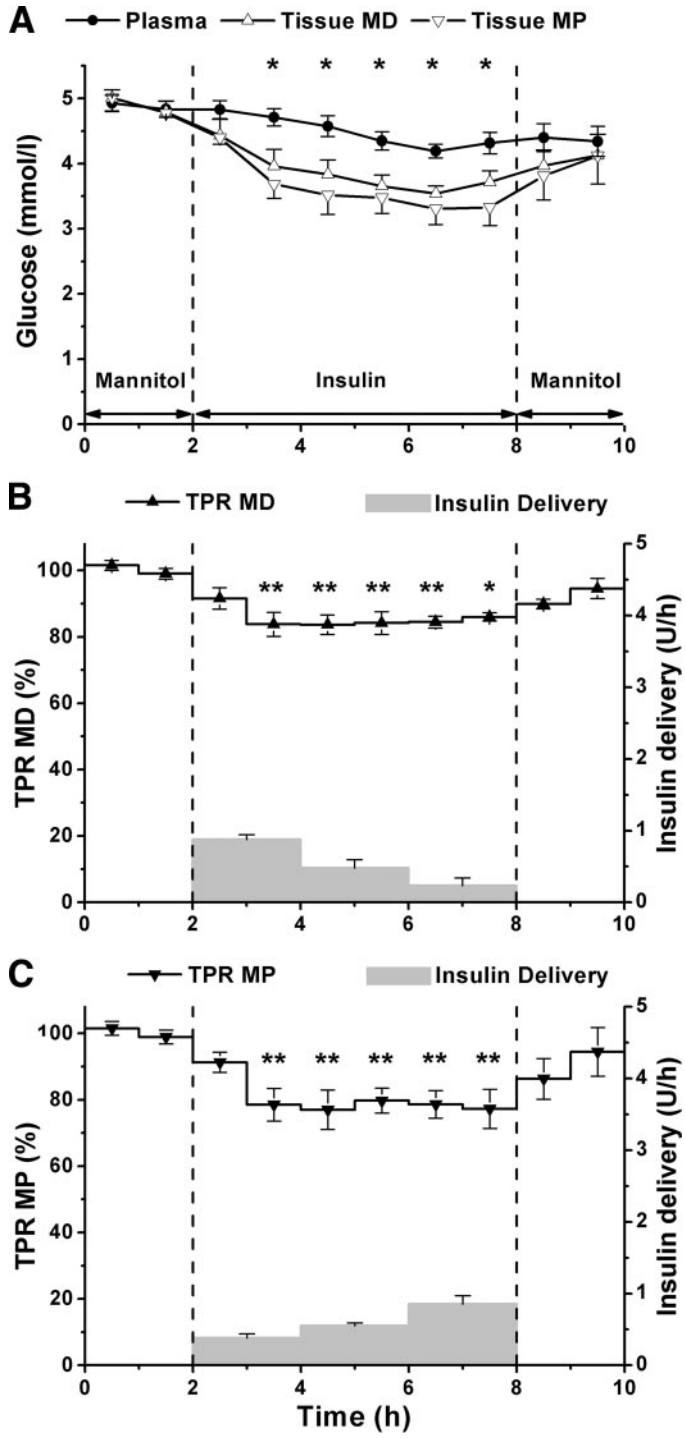

Figure 2—*Glucose dynamics at the subcutaneous tissue site of insulin delivery.* A*: Average time course* (n = *5, means ± SE) of plasma glucose concentration* (●) *as well as tissue glucose concentration determined with MD* (△) *and MP* (▽) *catheters.* *P < *0.05 tissue glucose vs. corresponding plasma glucose values, one-factor repeated-measures ANOVA, and Dunnett's post hoc test.* B *and* C*: Average time course* (n = *5, means ± SE) of the TPR obtained with MD* (▲) *and MP* (▼) *catheters.* *P < *0.05;* **P < *0.01 vs. the first basal TPR value, one-factor repeated-measures ANOVA (Huynh-Feldt corrected), and Dunnett's post hoc test. Panels also show the average time course* (n = *5, means ± SE) of the insulin delivery rates* (bars) *of the MD* (B) *and MP* (C) *catheters.*

constant rate as well as the simultaneous adjustment of the insulin delivery rate during the experiments.

When insulin delivery to the adipose tissue of the healthy humans was started at basal rates similar to those used in the replacement therapy of diabetic patients, there was an initial delay of ~60 min before the effect of insulin on the tissue-to-blood glucose concentration gradient at this delivery site reached its maximum (Fig. 2). This observed delay in insulin action (activation time) may be a reflection of the time required for the convectional and diffusional transport of insulin from the catheter to the surface of the insulin-sensitive fat cells surrounding the catheter as well as for the subsequent recruitment and activation of glucose transport proteins stimulating cell glucose uptake (10). After this activation time, despite changes in the insulin delivery rates, the tissue–to–blood glucose concentration gradient remained stable, thereby supporting our hypothesis that insulin's effect on the tissue glucose concentration at the insulin delivery site is saturated and attains steady-state values. This steady-state condition in insulin action at the insulin delivery site in turn allowed us to reliably estimate blood glucose concentrations from glucose concentrations measured in the ISF of this tissue site (11). After switching back from insulin to mannitol perfusates, the tissue glucose concentration and the tissue–to–blood glucose concentration gradient increased slowly and reattained preinsulin delivery levels by the end of the 2-h mannitol perfusion period (Fig. 2), thereby indicating that insulin's effect on the tissue glucose concentration vanished within 2 h after termination of the insulin delivery. Overall, the observed activation and deactivation kinetics of insulin action on the tissue–to–blood glucose concentration gradient in adipose tissue are compatible with previous findings in humans showing similar activation and deactivation kinetics of insulin's action on the forearm (12), leg (4), and peripheral (13) glucose uptake.

Conclusions in the present study differ from the recent study of Hermanides et al. (14), who reported no influence of insulin on the glucose concentration in the adipose tissue located near the insulin infusion site. The disparity between our results and those of Hermanides et al. may be attributable to the different experimental procedure used to assess the effect of insulin on the tissue glucose at the insulin administration site. Whereas the MP and MD catheters used in the present study functioned simultaneously as insulin delivery and ISF sampling instruments (single-port approaches), Hermanides et al. (14) used a dual-port approach consisting

of a subcutaneously inserted insulin infusion catheter and an MD catheter, which had a membrane length of 25 mm (14,15) and which was inserted in subcutaneous tissue at a closest mean distance of 9 ± 2 mm from the infusion catheter. Thus, in contrast to our study in which ISF glucose was withdrawn from the insulin-exposed tissue layer surrounding the MD and MP catheter, Hermanides et al. (14) performed ISF glucose sampling from tissue regions that were at considerable distances from the insulin administration site. A previous study by Linde and Philip (16), using radiography of the radioactivity distribution of subcutaneously injected ^{125}I-insulin, indicated that when a bolus amount of 0.25 ml of a 40 unit/ml insulin solution (10 units) is administered subcutaneously, the maximal volume of distribution of the administered insulin may have an average cross-sectional area of ~170 mm^2 or an average radius of ~7 mm (when a near-spherical shape is assumed) (17). Furthermore, our recent study in diabetic subjects (11), using MP catheters for insulin delivery and simultaneous glucose sampling, showed that a subcutaneous administration of a bolus amount of ~0.08 ml of a 100 unit/ml insulin solution (~8 units) is causing a dilution of the ISF at the insulin delivery site, thereby decreasing the glucose concentration around the insulin delivery catheter during and shortly after the bolus delivery period. In comparison, in the study of Hermanides et al. (14), a bolus amount of ~0.11 ml of a 100 unit/ml insulin solution (~11 units) was administered. Apparently, no decrease in the effluent glucose during the bolus administrations and, thus, no dilution effect of the insulin solvent were observed. Therefore, a possible reason for not observing a significant effect of insulin and/or insulin solvent on the local tissue glucose concentration in their study may be that, due to the considerable length of the microdialysis membrane used (25 mm), the whole or most parts of the glucose-exchanging membrane, especially the membrane part near the catheter outlet, may not have been positioned in the insulin-exposed tissue layer surrounding the insulin infusion catheter. There have been other studies evaluating the effect of insulin on the glucose concentration in human adipose tissue by using either continuous glucose sensors (18,19) or microdialysis-based glucose sensing (19–21). However, whereas we and Hermanides et al. (14) assessed the effect of supraphysiological insulin levels (i.e., 100 units/ml) on the adipose tissue glucose concentration, these studies evaluated the effect of physiological insulin levels (<150 μU/ml) on this glucose concentration. Furthermore, in contrast to the study of Hermanides et al. (14) and our study, where insulin was locally introduced into the ISF of subcutaneous adipose tissue, these studies invariably increased the insulin levels in the central circulation by intravenous insulin infusions (18–21) and/or by enhancing endogenous insulin secretion using intravenous glucose infusions (18,19). The results of these studies indicated that physiological changes in the insulin levels affect (19,21) or do not affect (18,20) the ISF-to-plasma gradient in human adipose tissue. The divergent findings may be partially attributable to technical and procedural differences in the performance of the studies (18).

Besides the effect of insulin on the fat cell glucose uptake, an additional mechanism potentially influencing the glucose concentration at the site of insulin delivery may be the local dilution of the ISF by the insulin solvent. We reasoned that if an increase in the perfusate flow fraction directed to the tissue (Fig. 1, *thin straight arrows*) is causing a dilution of the ISF surrounding the catheter, then there will be a decrease in the efficiency of exchange of solutes between the ISF and the perfusates of the two catheters. We found that changes in the tissue-directed flow rates did not cause changes in the exchange efficiency (recovery) of the two catheters, thereby suggesting that insulin solvent delivery at basal rates is not diluting the ISF in the tissue surrounding the catheters. This observation is in agreement with the results of our recent study in diabetic subjects (11), in which after changes in the basal delivery rates, no changes in the catheter recovery were observed. The relatively high blood flow per unit adipose tissue weight (20–30 $\mu l \cdot min^{-1} \cdot g^{-1}$) (22) compared with the amount of insulin solution infused during basal delivery (0.1–0.2 μl/min) may be the likely reason for not observing a local dilution of the ISF when basal insulin is delivered.

Our present and recent study (11) 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 directly onto the shaft wall of an insulin infusion catheter. When connected to an insulin pump, the system may then permit simultaneous insulin infusion and glucose sensing. Available information on solute transport in ISF surrounding infusion catheters (which is governed by a combination of convection, diffusion, and tissue clearance) (17,23,24) may be used to guide the exact placement of the glucose sensor onto the shaft of an insulin infusion catheter. For example, when a typical bolus amount of 0.1–0.2 ml (10–20 units) of a standard insulin solution is administered through a conventional infusion catheter (diameter: ~0.5 mm; shaft length: ~10 mm), there may be a significant backflow of infusate from the catheter tip along the catheter shaft (24) toward the skin, so that the initial area of distribution of infused insulin (initial depot volume) (17,23) may correspond to a cylindrical tissue layer surrounding the catheter shaft (24) and having a width of a few millimeters (17). Shortly after bolus administration, the relative rates of diffusion and clearance may then produce first an expansion of the insulin-exposed tissue layer about the catheter shaft to a width of several millimeters (e.g., ~7 mm) (16) and, as soon as clearance prevails, a decrease of the size of this layer, so that 6–10 h after bolus administration, all the insulin is cleared from the tissue site of insulin administration (17,21). Furthermore, in the case of insulin delivery at basal rates (~0.01 ml/h = ~1 unit/h, usually administered as microboluses with 0.025–0.1 units per pump stroke) (25), backflow distances may be shorter and hence the volume of insulin-exposed tissue about the catheter shaft may be smaller than after bolus administrations (24). Therefore, when a basal-bolus insulin regimen is applied, the best placement location of a continuous glucose sensor on the catheter shaft may be close to the catheter tip because at this site the tissue surrounding the sensor may be permanently exposed to insulin. In the case of suspension of the insulin pump treatment, a recalibration of the glucose sensor may be required when the length of pump suspension exceeds the deactivation time of insulin action. The deactivation time, however, may largely depend on the actual insulin depot size at the delivery site, which can be very high after bolus administrations or lower after prolonged basal insulin delivery (two to five times the hourly infusion rates) (25).

In summary, our results show that within 60 min after exposing adipose tis-

sue to a standard 100 units/ml insulin preparation, insulin's effect on the tissue glucose concentration saturates and a stable ratio between the tissue and plasma glucose concentration is attained, thus indicating that insulin delivery and glucose sensing may be performed simultaneously at the same adipose tissue site via a single tissue catheter. This single-port treatment approach may hold great promise to simplify and improve glucose management in diabetes.

Acknowledgments— This work was supported in part by funding from Science Park Graz and the Federal Ministry of Economics and Labor of the Republic of Austria.

T.R.P. is a cofounder and shareholder of Smart*Med and a consultant of Novo Nordisk and sanofi-aventis. M.E. is a consultant of Sensile Medical and BBraun and a cofounder and shareholder of Smart*Med. L.S. and H.K. are cofounders and shareholders of Smart*Med. W.R., L.S., G.K., M.E., and T.R.P. have filed patent applications relating to the methodology described in this report. No other potential conflicts of interest relevant to this article were reported.

Parts of this study were presented in abstract form at the 2nd European Diabetes Technology and Transplantation Meeting, Innsbruck, Austria, 27–29 January 2008.

We are grateful to A. Wutte for her expert assistance in conducting the study, M. Suppan for performing insulin assays, A. Groselj-Strele for her assistance with statistical analysis, and all volunteers for participating in the study.

References

1. Rizza RA, Mandarino LJ, Gerich JE. Dose-response characteristics for effects of insulin on production and utilization of glucose in man. Am J Physiol 1981;240:E630–E639
2. Kono T, Barham FW. The relationship between the insulin-binding capacity of fat cells and the cellular response to insulin: studies with intact and trypsin-treated fat cells. J Biol Chem 1971;246:6210–6216
3. Nesher R, Karl IE, Kipnis DM. Dissociation of effects of insulin and contraction on glucose transport in rat epitrochlearis muscle. Am J Physiol 1985;249:C226–C232
4. Laakso M, Edelman SV, Brechtel G, Baron AD. Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man: a novel mechanism for insulin resistance. J Clin Invest 1990;85:1844–1852
5. Bonadonna RC, Saccomani MP, Seely L, Zych KS, Ferrannini E, Cobelli C, DeFronzo RA. Glucose transport in human skeletal muscle: the in vivo response to insulin. Diabetes 1993;42:191–198
6. Hirsch IB. Insulin analogues. N Engl J Med 2005;352:174–183
7. 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
8. 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
9. Regittnig W, Koehler G, Bodenlenz M, Schaller HC, Koehler H, Ellmerer M, Schaupp L, Pieber TR. Coupling of subcutaneous insulin delivery and subcutaneous glucose sensing by means of a microperfusion or microdialysis probe. Abstract presented at the 5th Annual Diabetes Technology Meeting, San Francisco, CA, 10–12 November 2005
10. Carruthers A. Facilitated diffusion of glucose. Physiol Rev 1990;70:1135–1176
11. Regittnig W, Lindpointner S, Korsatko S, Kohler G, Kaidar R, Yodfat O, Kohler H, Ellmerer M, Pieber TR. Glucose concentrations at the site of subcutaneous insulin delivery in patients with type 1 diabetes. Diabetologia 52(Suppl. 1):S42, 2009
12. Utriainen T, Malmström R, Mäkimattila S, Yki-Järvinen H. Methodological aspects, dose-response characteristics and causes of interindividual variation in insulin stimulation of limb blood flow in normal subjects. Diabetologia 1995;38:555–564
13. Prager R, Wallace P, Olefsky JM. In vivo kinetics of insulin action on peripheral glucose disposal and hepatic glucose output in normal and obese subjects. J Clin Invest 1986;78:472–481
14. 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
15. Maran A, Crepaldi C, Tiengo A, Grassi G, Vitali E, Pagano G, Bistoni S, Calabrese G, Santeusanio F, Leonetti F, Ribaudo M, Di Mario U, Annuzzi G, Genovese S, Riccardi G, Previti M, Cucinotta D, Giorgino F, Bellomo A, Giorgino R, Poscia A, Varalli M. Continuous subcutaneous glucose monitoring in diabetic patients. Diabetes Care 2002;25:347–352
16. Linde B, Philip A. Massage-enhanced insulin absorption: increased distribution or dissociation of insulin? Diabetes Res 1989;11:191–194
17. Trajanoski Z, Wach P, Kotanko P, Ott A, Skrabal F. Pharmacokinetic model for the absorption of subcutaneously injected soluble insulin and monomeric insulin analogues. Biomed Technik 1993;38:224–231
18. Steil GM, Rebrin K, Hariri F, Jinagonda S, Tadros S, Darwin C, Saad MF. Interstitial fluid glucose dynamics during insulin-induced hypoglycaemia. Diabetologia 2005;48:1833–1840
19. Monsod TP, Flanagan DE, Rife F, Saenz R, Caprio S, Sherwin RS, Tamborlane WV. Do sensor glucose levels accurately predict plasma glucose concentrations during hypoglycemia and hyperinsulinemia? Diabetes Care 2002;25:889–893
20. Rosdahl H, Lind L, Millgard J, Lithell H, Ungerstedt U, Henriksson J. Effect of physiological hyperinsulinemia on blood flow and interstitial glucose concentration in human skeletal muscle and adipose tissue studied by microdialysis. Diabetes 1998;47:1296–301
21. Moberg E, Hagström-Toft E, Arner P, Bolinder J. Protracted glucose fall in subcutaneous adipose tissue and skeletal muscle compared with blood during insulin-induced hypoglycaemia. Diabetologia 1997;40:1320–1326
22. Rosell S, Belfrage E. Blood circulation in adipose tissue. Physiol Rev 1979;59:1078–1104
23. Mosekilde E, Jensen KS, Binder C, Pramming S, Thorsteinsson B. Modelling absorption kinetics of subcutaneous injected soluble insulin. J Pharmakokinet Biopharm 1989;17:67–87
24. Morrison PF, Chen MY, Chadwick RS, Lonser RR, Oldfield EH. Focal delivery during direct infusion to brain: role of flow rate, catheter diameter, and tissue mechanics. Am J Physiol 1999;277:R1218–R1229
25. Kreagen EW, Chisholm DJ. Pharmacokinetics of insulin: implications for continuous subcutaneous insulin infusion therapy. Clin Pharmacokinet 1985;10:303–314