special communication

Direct access to interstitial fluid in adipose tissue in humans by use of open-flow microperfusion

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[1]*Department of Biophysics, Institute of Biomedical Engineering, Graz University of Technology, A-8010 Graz;* [2]*Department of Internal Medicine, Diabetes and Metabolism, Karl Franzens University Graz, A-8036 Graz; and* [3]*Department of Internal Medicine, Krankenhaus der Barmherzigen Brüder, Teaching Hospital, Karl Franzens University Graz, A-8020 Graz, Austria*

Schaupp, L., M. Ellmerer, G. A. Brunner, A. Wutte, G. Sendlhofer, Z. Trajanoski, F. Skrabal, T. R. Pieber, and P. Wach. Direct access to interstitial fluid in adipose tissue in humans by use of open-flow microperfusion. *Am. J. Physiol.* 276 (*Endocrinol. Metab.* 39): E401–E408, 1999.—To gain direct access to the interstitial fluid (ISF), a new technique called open-flow microperfusion has been evaluated. This method is based on a double-lumen catheter with macroscopic (0.3–0.5 mm diameter) perforations that is inserted into the subcutaneous adipose tissue and constantly perfused. Thus partial equilibration between the ISF and the perfusion fluid occurs. The glucose concentration of the ISF was determined by established (zero flow rate, no net flux, and recirculation procedures) and new (ionic reference and suction technique) calibration methods by use of open-flow microperfusion. The data show that *1*) the glucose concentration in the ISF is significantly lower than the corresponding arterialized venous plasma values during basal steady-state conditions (adipose tissue 3.2 ± 0.10 mM, plasma 5.27 ± 0.12 mM) as well as during hyperglycemic clamp experiments (adipose tissue 7.3 ± 0.13 mM, plasma 9.91 ± 0.16 mM), and *2*) it is possible to determine the recovery continuously by using the ion concentration of the ISF as an internal standard (ionic reference).

glucose; ionic reference; mass transfer resistance; hyperglycemia

ACCESS TO THE INTERSTITIAL FLUID in the intercellular space allows the measurement of relevant metabolic substrates, such as glucose (16, 19), lactate (20, 22), or glycerol (9, 17), and therefore offers new insights into the substrate exchanges. Various techniques have been proposed to gain access to the interstitial space: capillary ultrafiltration (14), the wick technique (6), the transcutaneous sampling technique (1), or transdermal extraction (28). Microdialysis, the most promising and most widespread approach to monitor the interstitial fluid (ISF), has been established as one of the major research tools to investigate tissue metabolism. The basic principle of microdialysis consists of sampling ISF through a dialysis membrane, which allows diffusion between the ISF and the dialysate. Microdialysis does not obtain pure samples of ISF; the concentrations of the substances in the dialysate only reach partial equilibration (recovery). Furthermore, the membrane itself limits the diffusion of large molecules (cutoff of membrane).

To allow calculation of the absolute in vivo concentration of a specific substance from the concentration in the collected sample, several calibration techniques have been proposed to determine the recovery in microdialysis experiments (13). Nevertheless, most calibration techniques are time consuming, need a calibration against blood values, or require long-term steady-state conditions. Recently, Rosdahl el al. (22) reported about microdialysis at very low perfusion flow rates. The advantage of this approach is the complete equilibrium with the ISF, and therefore no recalculation from a prior calibration or standard is necessary. The disadvantage of this low perfusion flow is the small amount of fluid collected per time, which results in a poor time resolution. It is apparent that these techniques may be of great value; nevertheless, the system's inherent membrane remains a limiting factor for the free exchange of molecules.

An alternative method to gain access to the ISF water space was proposed by Skrabal et al. (26) and recently also by Trajanoski et al. (29) by use of open-flow microperfusion. Open-flow microperfusion is based on a double-lumen catheter with macroscopic perforations, circumventing the use of a membrane; thus direct access to the ISF is possible. Furthermore, to calculate the recovery, a novel approach using electrical conductivity as internal standard was proposed that simplifies the calibration procedure considerably.

The overall aims of this study were to gain direct access to the ISF by using open-flow microperfusion without an intervening membrane and to validate a calibration technique (ionic reference) with ions as an endogenous marker for the estimation of the recovery. Therefore, the glucose concentration in the subcutaneous adipose tissue during basal steady-state conditions was determined with established calibration tech-

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niques known from microdialysis. Furthermore, pure ISF was sampled using a new suction technique at very low flow rates. Finally, the ISF glucose concentration was monitored during a standardized change of glycemia by use of open-flow microperfusion combined with the ionic reference technique. For this purpose, we investigated open-flow microperfusion in healthy volunteers during basal steady-state as well as hyperglycemic clamp conditions.

METHODS

Subjects

The study group consisted of 25 healthy volunteers [age 25.6 ± 3.4 (SD) yr, body mass index 23.4 ± 2.0 kg/m^2] who were not under medication. After explanation of the aims, purpose, and potential risks of the study, all subjects gave written informed consent to the study protocol, which was approved by the local ethical committee.

Sampling System

The technique of open-flow microperfusion has been described in detail previously (29). A schematic representation of the sampling system is given in Fig. 1. The double-lumen catheter was prepared from a conventional intravenous cannula (18 gauge × 50 mm, 1.2 mm OD; Angiocath, Becton-Dickinson, Sandy, UT) by perforating 120 holes (0.5 mm diameter) with an Excimer Laser. The catheter was inserted into the adipose tissue of the anterior abdominal wall by a steel mandrin, which was subsequently removed and replaced by the inner cannula of the double-lumen catheter (PTFE tubing, 0.76 OD, 0.3 mm ID; Cole-Parmer Instrument, Barrington, IL). The inner cannula was connected to a plastic bag containing perfusate [either isotonic, ion-free mannitol in aqueous solution (275 mM, 288 mosmol/l) or Ringer solution (in mM: 2.3 Ca^{2+}, 4 K^+, 155.6 Cl^-, and 147 Na^+), 309 mosmol/l; Leopold, Graz, Austria]. The perfusion fluid entered the catheter through the inner lumen and passed to the tip of the probe. Thereafter, it streamed in the annular space between the inner cannula and the outer perforated catheter, back where partial equilibration between the ISF and the perfusate occurred. The outer lumen was connected to a peristaltic pump (Minipuls 3, Gilson, France), which transported the diluted ISF effluents through the tubing system to a collecting vial on ice. The vials were sealed with a film to prevent evaporation during sampling. The flow rate was set by the peristaltic pump. To verify appropriate average flow rate, each vial was weighed before and after sampling.

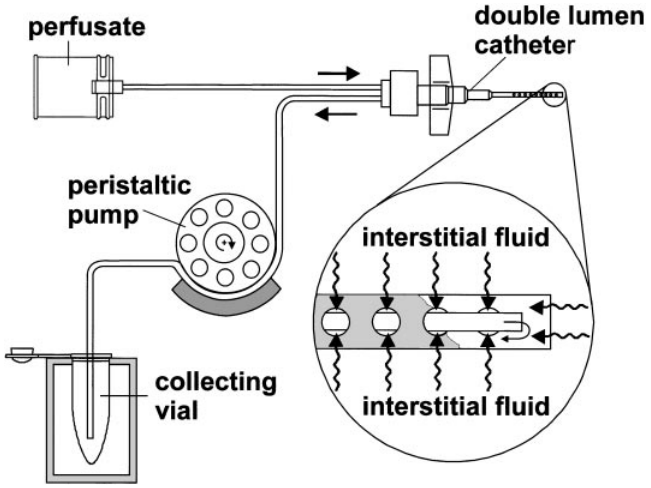

Fig. 1. Schematic representation of sampling system. Double-lumen catheter is inserted into adipose tissue and perfused. Partial equilibration between perfusate and interstitial fluid occurs through macroscopic perforations of the outer catheter. Fluid is transported by a peristaltic pump through the tubing system to a collecting vial.

Protocol 1: Basal Steady-State Experiments

After a 12-h overnight fast, an intravenous cannula was placed into a dorsal hand vein to obtain blood samples. The hand was kept in a thermoregulated (55°C) box to sample arterialized venous blood (18). The volunteers rested in a supine position throughout the experiment. According to the protocol, one or two double-lumen catheters were inserted into the abdominal subcutaneous adipose tissue. Local skin anesthesia (Novanaest purum 2%, Gebro Broschek, Vienna, Austria) was used before the adipose tissue was cannulated. Perfusion was started immediately, but sampling of the perfusate began 60 min after the insertion of the catheter (equilibration period) to allow the initial trauma to subside. ISF and plasma samples were stored at −70°C instantly after sampling until they were analyzed. Some of the samples were measured twice, before and after freezing, to assure that there was no loss of glucose due to freezing and thawing.

Zero flow rate. The method of zero flow rate is based on the fact that the recovery of a substance depends on the perfusion flow rate, such that, at zero flow rate, the interstitial and perfusion fluid are in complete equilibrium. By sampling at different flow rates it is possible to calculate the absolute concentration c_0 of the ISF by extrapolating to flow rate of zero [zero-flow-rate protocol (11)] by nonlinear regression according to the formula (4)

$$c_{out} = c_0 \cdot \left[1 - e^{\overline{R \cdot Q}^{-1}}\right] \qquad (1)$$

where c_{out} is the concentration of the sample at the given flow rate Q (in µl/min) and the overall mass transfer resistance R (min/mm^3).

To verify stable equilibration of the perfusate with the ISF, open-flow microperfusion using mannitol as perfusion fluid was carried out in five volunteers over a period of 420 min with a fixed flow rate (one set of experiments). Five different flow rates (0.25, 0.5, 1, 2, and 4 µl/min) were applied (five sets of experiments, $n = 25$).

The ISF effluent samples were collected according to the flow rate in 30-min (4 and 2 µl/min) or 60-min (1, 0.5, and 0.25 µl/min) intervals. In the middle of each interval, a blood sample was drawn.

Recirculation. To achieve complete equilibrium between the ISF and the perfusate, it was proposed to recirculate the sample within the tubing-catheter system several times (13, 27). Because of the large internal volume of the double-lumen catheter (18 gauge × 54 mm) described before, a smaller catheter (24 gauge × 19 mm, diameter 0.6 mm, Neoflown, Viggo, Helsingborg, Sweden) with 36 holes (0.3 mm diameter) (29) was used for this experiment. The catheter was inserted into the subcutaneous adipose tissue of five volunteers without local anesthesia, and after a rinse of the system at a flow rate of 2 µl/min for 60 min and discard of the sample, the ends of the tubing system were connected to each other. The perfusate (275 mM mannitol) was recirculated ~11 times during 420 min at a flow rate of 1 µl/min (total volume of the tubing system and catheter was 40 µl). At the end of the experiment, the recirculated perfusate was collected in a vial as described before.

Suction technique. A catheter of the same size (24 gauge × 19 mm) as that used in the recirculation protocol was inserted into the subcutaneous adipose tissue of five volunteers without local anesthesia. After a 60-min equilibration period, the catheter and tubing system were emptied and the sample was discarded. The inner lumen was clamped, and the flow rate of the peristaltic pump was set to 0.1 μl/min. During the experiment, the samples were stored in the tubing system (to avoid evaporation) and diluted with sterile water thereafter. The dilution was controlled by weighing the vials before and after dilution.

No-net-flux protocol. The principle of the no-net-flux procedure is the measurement of glucose concentration in the samples by allowing the calculation of the absolute ISF concentration when the catheter is perfused with different concentrations of glucose. When the net exchange across the perforations is zero, the perfusate and effluent glucose concentrations are equal (15).

Two double-lumen catheters (18 gauge × 54 mm) were placed into the subcutaneous adipose tissue. One catheter was perfused with isotonic ion-free mannitol and the second catheter with isotonic Ringer solution. Different glucose concentrations of the perfusate (0, 1, 2, 3.5, and 5.3 mM) were achieved by adding glucose to the perfusion fluid. At each stage, the effluent was collected for 30 min with 50-min equilibration periods between each change in perfusate to allow reequilibration with the new perfusate concentration. To exclude an order effect, the sequence in which the different perfusate glucose concentrations were used was randomized. Linear regression analysis was applied to calculate the glucose concentration at which no net flux occurred through the perforations.

Protocol 2: Hyperglycemic Clamp Experiment

Five volunteers of *protocol 1* participated in these hyperglycemic clamp experiments. After a 12-h overnight fast, an intravenous cannula was inserted into a vein in the dorsum of the hand for manually drawing blood samples. The hand was kept in a thermoregulated (55°C) box for sampling arterialized venous blood (18). Another catheter was placed in the contralateral cubital vein for variable glucose infusion. A double-lumen catheter (18 gauge × 54 mm) was inserted into the abdominal subcutaneous adipose tissue and perfused with mannitol at a flow rate of 2 μl/min. After an equilibration period of 60 min, sample collection was started (30-min fractions during the entire experiment). After 90 min of basal sampling, the plasma glucose concentration was clamped to 10 mM by variable glucose infusion over a 180-min period.

Ionic Reference Technique

This technique is based on the simultaneous measurement of glucose and ions in the samples. With the assumption of constant and known ionic concentrations in the ISF, which are very close to plasma values except for minor differences resulting from the Gibbs-Donnan effect (see Ref. 8), the "ionic recovery" can be estimated as the ratio of ion concentrations in the sample to the ion concentrations of plasma by use of an ion-free perfusate. On the basis of the assumption that the recovery of glucose and ions is the same, the glucose concentration of the ISF can be calculated as the ratio of glucose concentration in the sample to ionic recovery. Within this study, the sodium concentration was used as ionic reference because of its high and tightly regulated concentration in the ISF (8).

Analytic Methods

Sodium and potassium concentrations in the samples and in plasma were measured using a flame photometer (IL 943, Instrumentation Laboratory, Milano, Italy). The glucose concentration in plasma and ISF samples was measured enzymatically with glucose hexokinase programmed on a Cobas Integra analyzer (Hoffman-La Roche, Basel, Switzerland). In addition, to allow adjustments of the glucose infusion rate, plasma glucose during the clamp experiments was measured in duplicate by use of a Beckman Glucose Analyzer 2 (Beckman Instruments, Fullerton, CA).

Statistical Methods

Unless otherwise indicated, results in the text, figures, and table are presented as means ± SE. The coefficient of variation (CV) was calculated as the standard deviation (SD) divided by the mean. Significance of difference was tested with Student's *t*-test for paired or unpaired observations. $P < 0.05$ was considered significant. The nonlinear fit was based on the Levenberg-Marquard algorithm, with the reciprocal variance used as a weight of each point. Linear and nonlinear regression analysis and statistical calculations were performed with Origin (Microcal Software, Northampton, MA) on a personal computer.

RESULTS

Zero Flow Rate and Recirculation

Results of the various flow rates and recirculation experiments are shown in Fig. 2. The depicted values of the ISF effluents were obtained during steady-state conditions (≥90 min before and after no significant change). The values of the recirculation experiments correspond to flow rate "zero." Complete equilibration was confirmed by nearly identical ion concentrations of plasma and the obtained ISF samples.

Applying nonlinear regression to the data with *Eq. 1* resulted in the adipose ISF concentrations of 4.67 ± 0.40, 142.95 ± 4.64, and 3.32 ± 0.39 mM and the overall mass transfer resistances of 1.13 ± 0.17, 1.23 ± 0.25, and 1.26 ± 0.25 min/mm^3 for potassium, sodium, and glucose, respectively. The corresponding plasma concentrations were 4.33 ± 0.35, 139.35 ± 0.53, and 5.27 ± 0.12 mM ($n = 5$). The estimated adipose ISF glucose concentration was 63% of the corresponding plasma glucose ($P < 0.01$).

Suction Technique

The concentrations of the ISF obtained with the suction technique were 4.58 ± 0.88, 140.65 ± 28.17, and 3.19 ± 0.51 mM for potassium, sodium, and glucose, respectively. The corresponding plasma values were 4.28 ± 0.10, 139.89 ± 0.50, and 5.14 ± 0.15 mM ($n = 5$). Again, ISF glucose was 63% of the simultaneous plasma glucose ($P < 0.01$; Fig. 3).

No-Net-Flux Protocol

The estimated adipose ISF glucose concentration derived from the intercept of the regression line on the *x*-axis was 2.99 ± 0.28 and 3.56 ± 0.25 mM for mannitol and Ringer solution, respectively ($r = 0.99$, $P < 0.001$; Fig. 4), which was significantly lower than the corre-

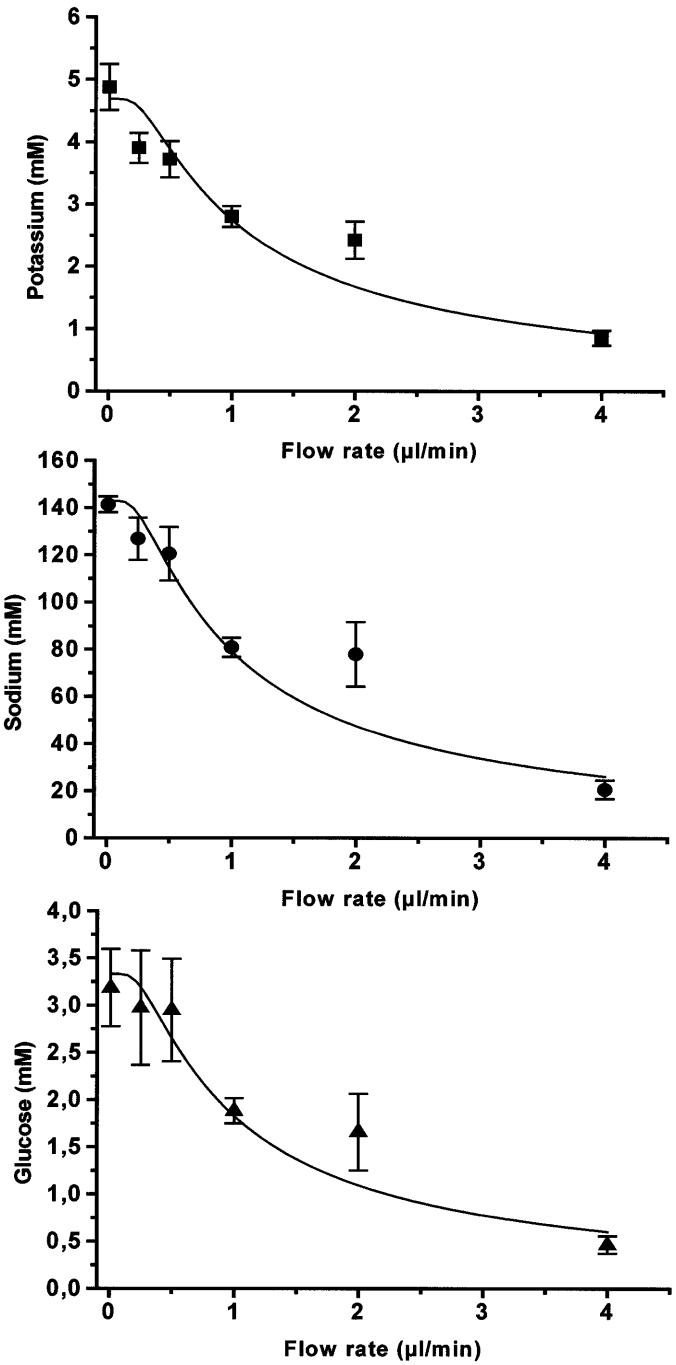

Fig. 2. Mean values of potassium, sodium, and glucose concentrations at various flow rates during steady-state experiments. Data of recirculation experiments correspond to flow rate zero. Solid line represents nonlinear fit of data according to *Eq. 1*. Values are means ± SE ($n = 5$).

sponding plasma glucose concentrations (by 56 and 67%, respectively, $P < 0.01$). The estimated recovery determined from the slope of the regression lines was 0.39 ± 0.04 and 0.37 ± 0.06 for mannitol and Ringer solution, respectively.

Ionic Reference Technique

Estimations of the subcutaneous glucose concentrations during steady state by means of the ionic reference technique are shown in Fig. 3. The mean estimated ISF glucose concentration was 3.2 ± 0.10 mM, which was 62% of the corresponding arterialized venous plasma concentration ($P < 0.01$).

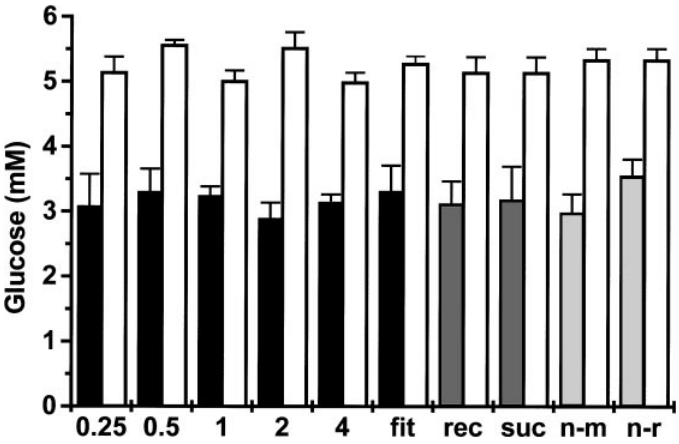

Fig. 3. Estimated interstitial fluid (ISF, solid or dark gray and light gray solid bars) and corresponding plasma glucose concentrations (open bars) of *protocol 1*. Estimations were performed using the ionic reference technique ($glucose_{sample}/sodium_{sample} \times sodium_{plasma}$). Nos. 0.25, 0.5, 1, 2, 4, flow rates (μl/min) of basal steady-state experiments; fit, estimated glucose concentrations of nonlinear fit of *protocol* 1; rec and suc, recirculation and suction technique experiments, respectively ($n = 5$); n-m and n-r, estimated glucose concentrations of no-net-flux experiments in which catheter was perfused with mannitol or Ringer solution, respectively ($n = 6$). Values are means ± SE.

Hyperglycemic Clamp Experiment

In Fig. 5, the mean time courses of the hyperglycemic clamp experiments are given for sodium, glucose, and the ISF glucose concentration estimated with the ionic reference technique. The absolute difference between

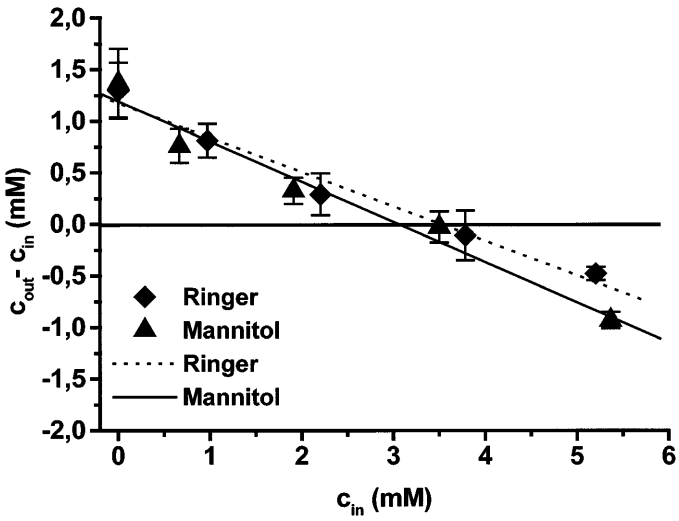

Fig. 4. Mean values and linear regression of no-net-flux experiments. Two catheters were placed in the sc adipose tissue and perfused with mannitol and Ringer solution containing different glucose concentrations (c_{in}). Net increase or decrease of glucose concentration in recollected perfusate (c_{out}) was determined. Intercept of regression line with *x*-axis indicates estimated glucose concentration, whereas slope of regression line represents overall recovery. Values are means ± SE ($n = 6$).

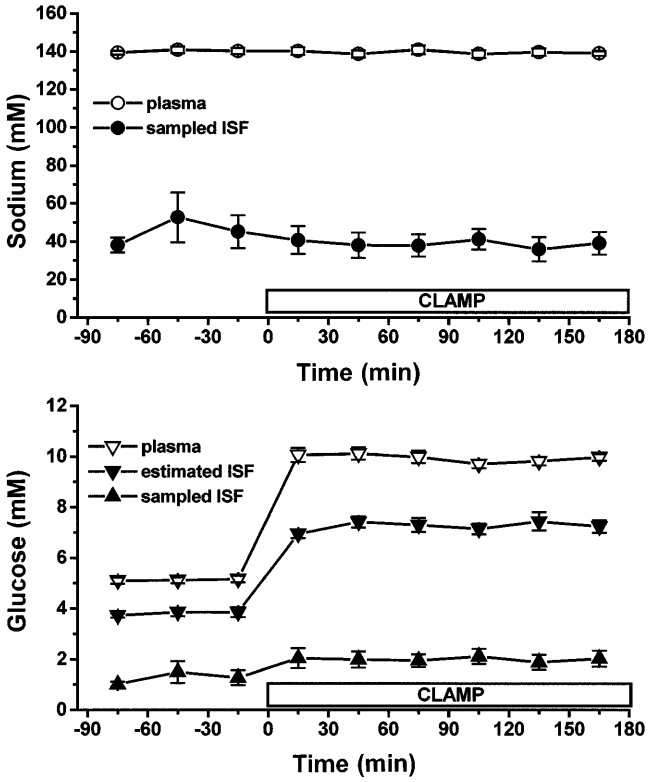

Fig. 5. Mean values of sodium, glucose (plasma and sampled), and estimated ISF glucose concentration (sodium as internal reference) during hyperglycemic clamp experiments. After 90 min of basal sampling, plasma glucose concentration was clamped to 10 mM over a 180-min period. Values are means ± SE (n = 5).

plasma glucose and the estimated tissue glucose concentration was 1.27 ± 0.04 mM during basal conditions and 2.61 ± 0.07 mM during hyperglycemia. This indicates that the ISF glucose concentration was significantly ($P < 0.01$) lower than the corresponding plasma values. In Table 1, the CV values for plasma, estimated, and sampled glucose concentrations during the basal condition and during hyperglycemia are shown. Note the marked reduction of SE of the estimated ISF glucose concentration by use of the ionic reference technique.

Table 1. *CV for plasma, measured ISF effluent, and estimated ISF glucose concentrations of protocol 2 during basal conditions and hyperglycemia*

	Basal, %	Hyperglycemia, %
Plasma glucose	5.4 ± 0.17	4.6 ± 0.51
Measured ISF effluent glucose	43.2 ± 9.30	35.2 ± 1.86
Estimated ISF glucose	8.0 ± 1.36	7.7 ± 0.76

Estimation is based on the ionic reference technique. Coefficient of variation (CV) was calculated as the ratio of the SD and the mean for each point of time. These values were averaged for basal and hyperglycemic periods and expressed as means ± SE in %. Note diminished CV of estimated interstitial fluid (ISF) glucose concentration; variations due to changes or differences of (relative) recovery of glucose are mirrored by (relative) recovery of ions. Estimation of glucose concentration by use of the ionic reference technique reduces or cancels these variations and/or differences.

DISCUSSION

Open-flow microperfusion was used to gain direct access to the ISF and to estimate the glucose concentration by using different calibration techniques: well-established methods such as zero flow rate, recirculation, and the no-net-flux protocol, known from microdialysis, as well as a new suction technique to obtain undiluted ISF. These procedures were used to validate a new calibration method called the ionic reference technique. The results of these techniques are very similar and indicate that the ionic reference technique can be used to determine the recovery for glucose. Furthermore, the interstitial glucose concentration is significantly lower than the glucose concentration in the arterialized venous plasma.

It is a well-known fact that, shortly after insertion of the probe into the subcutaneous adipose tissue, the collected samples are not in a steady state with the surrounding tissue because of disruption of local blood vessels, cells, and capillaries. Schmidt et al. (24) reported that within 6 h the ISF glucose concentration decreased to a stable value. To enable equilibration of the system, a modified zero flow rate protocol (11) was used in the current investigation: instead of varying the flow rate within one experiment, the flow rate was kept unchanged to assure stable conditions. On different days different flow rates were applied. Furthermore, to avoid extrapolation of a nonlinear function, the values of the recirculation experiments were included as representatives of flow rate zero, thus enabling a fit of the function.

Lönnroth et al. (15) proposed the no-net-flux protocol to estimate the glucose concentration of the adipose ISF. This method was applied with open-flow microperfusion with mannitol and Ringer solution as perfusion fluids. Although there was a slight difference between the estimated ISF glucose concentration for mannitol and that for Ringer solution (probably due to the more physiological composition of Ringer solution), there was no significant difference compared with the values obtained with the other protocols.

In addition, the adipose ISF glucose concentration was determined with the open-flow suction technique. With this technique, pure ISF was sampled. Therefore, no assumptions had to be made regarding recovery. The results of the glucose concentrations were also significantly lower than the corresponding plasma values.

In addition to the estimation of the concentrations in the ISF, the curve fit of *protocol 1* provided values for the overall mass transfer resistances, which were nearly identical for potassium, sodium, and glucose (1.13 ± 0.17, 1.23 ± 0.25, and 1.26 ± 0.25 min/mm^3). Similar mass transfer resistances are a prerequisite for calculating the glucose concentration with the ionic reference technique. Identical mass transfer resistances indicate identical recoveries at a given flow rate. The good agreement of the estimated subcutaneous glucose concentration by use of the ionic reference technique with the glucose concentrations obtained with the other calibration procedures (Fig. 3) confirms the assumption

of identical recoveries for glucose and ions. Therefore, simultaneous measurements of ions and glucose in the recollected perfusate allow not only the estimation of the recovery but also the estimation of the glucose concentration of the ISF.

Within *protocol 2*, we investigated the influence of hyperglycemia on the glucose concentration of the ISF by use of the ionic reference technique. Although the absolute difference between plasma and estimated adipose ISF glucose concentration increased during hyperglycemic conditions (basal 1.27 mM, hyperglycemic 2.61 mM), the ratio of ISF to arterialized venous plasma glucose concentration was nearly the same (75.1 vs. 73.7%, not significant). This again indicates significantly lower ($P < 0.01$) tissue glucose concentrations than the corresponding plasma values, irrespective of the glycemic condition. Furthermore, the CV of the glucose concentration in the sampled perfusate was ~5 times the CV of the estimated glucose concentration of the ISF during basal as well as hyperglycemic conditions (Table 1). This confirms the need and potential of the ionic reference technique for accurate and continuous calculation of the recovery of the ISF (Fig. 5).

Originally, the mass transfer resistance concept was developed to provide a quantitative basis for microdialysis (4, 11). The overall mass transfer resistance is given by the sum of the resistances for the perfusate, the membrane, and the external medium (e.g., tissue). Bungay et al. (4) showed that, in microdialysis for low-molecular-weight species, the tissue was generally more important than the probe membrane in determining the sample-to-tissue concentration relationship. Because there is no membrane with open-flow microperfusion and the resistance within the perfusate is sufficiently small (4), the rate-limiting medium for the overall transport from the tissue to the catheter is the tissue itself.

One of the underlying assumptions of the mass transfer resistance concept (4) is the concentration gradient as the driving force (diffusion). It may be asked whether this assumption is also true for open-flow microperfusion, because the catheter's operation mode is either the pull or push-pull (recirculation) mode, whereas microdialysis uses the push mode. It may possibly be argued that the driving force with open-flow microperfusion may be convection instead of diffusion. The no-net-flux protocol, in which glucose was added to the perfusate at varying concentrations, provided high linear correlation over the entire concentration range and not only at glucose concentrations that were lower in the perfusate than in the surrounding tissue. Glucose was certainly transported out of the catheter to the tissue by diffusion. On the other hand, the similar mass transfer resistances for solutes with different diffusion coefficients are in contrast to a model that assumes diffusion as the only driving force. Probably the transport mechanism from the tissue to the catheter is a combination of convection and diffusion (11). To elucidate the mechanisms of exchange of fluids by use of open-flow microperfusion, simultaneous measurement of substrates whose diffusion coefficients are very different from each other (e.g., glucose and albumin) should give further insight into the principles underlying the molecular exchange mechanisms. Because the albumin concentration in the ISF is not known exactly, further investigations are necessary to understand the transport mechanisms involved with open-flow microperfusion.

It may be argued that rinsing the catheter with glucose-free perfusion fluid may lead to a local depletion of glucose in the tissue around the catheter and hence to glucose concentrations that are lower than in the undisturbed tissue (15). The ISF glucose concentrations estimated with the no-net-flux method, which involves the addition of glucose to the perfusate at different concentrations, were very similar to the results of the other protocols. This makes a local depletion artifact very unlikely.

In addition, the results from the recirculation experiments, which are not expected to deplete the tissue glucose, were also indistinguishable from the values obtained with the other protocols. During 420 min, 3.2 mmol/l of glucose were withdrawn into a volume of 40 µl (volume of the catheter and the tubing system), which corresponds to an average removal of 0.3 nmol/min. The distance of the drained tissue is unknown, but ~1 mm around the probe seems realistic (4). This corresponds approximately to 50 mg of drained tissue. Therefore, the total amount of harvested glucose is ~6 $\mu mol \cdot kg^{-1} \cdot min^{-1}$. Rosdahl et al. (22) reported complete equilibrium for glucose (5.29 mmol/l) at 0.16 µl/min, which corresponds to ~8.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, which is very similar to the amount withdrawn with open-flow microperfusion. Simonsen et al. (25) reported glucose uptake levels between 5 and 17 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ during basal steady-state conditions, which increased to 60 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ during an oral glucose load, values that are far beyond the amount withdrawn with open-flow microperfusion.

With increasing flow rates, the absolute recovery (rate of removal of a substrate per unit time) also increases, which implies the potential of local depletion around the probe. Apparently, the relative change of the recovery for ions and glucose is the same, which means that a local change of the glucose concentration due to the sampling process is mirrored by a local change of the ions (Fig. 2). Otherwise, the estimated glucose concentration by use of the ionic reference technique would depend on the flow rate, which is entirely not the case (Fig. 3). This enables sampling at higher flow rates yet the calculation of the true concentration (*protocol 2*). The higher flow rates enable larger sample volumes, which result in higher time resolution.

With the pull mode, which is mainly used in the current investigations, the perfusate is able to enter the catheter without control of an intervening pump that might dilute the ISF. Only the push-pull mode (recirculation) enables the exact control of the fluid shifts. Because the results for glucose concentrations were similar for both methods, it can be assumed that either the same amount of fluid is delivered and

withdrawn, or as in case of net fluid shifts, the use of ionic reference corrects for this possible phenomenon. This is based on the fact that glucose and ionic concentrations are diluted by the same amount.

Because of the macroscopic perforations when open-flow microperfusion is used, there is no limiting membrane that prevents large molecules from entering the catheter, a situation that is theoretically also true for enzymes. It may be argued that these enzymes could degrade the glucose in the outgoing perfusate. To the best of our knowledge, there is no evidence of glucose-degrading enzymes in the ISF, and contamination by intracellular enzymes can be excluded because relevant cell rupture due to the catheter would increase the potassium concentration, which was not observed in any experiment. Furthermore, glucose degradation would have been possible only on the way from the catheter to the collecting vial because, as mentioned before, the collecting vials were kept on ice, and it is very unlikely that enzymes are still active at this temperature.

Another possibility for loss of glucose is by freezing and thawing (5). Measurements of the glucose concentration in the same samples before and after freezing and thawing resulted in identical glucose concentrations. This indicates that freezing and thawing do not have any influence on the glucose concentration of the samples.

The glucose concentration in the ISF is still the subject of discussion: concentrations that were almost identical to plasma (2, 6, 10, 12, 15, 19, 29) but also values that were considerably lower (50–70%) (16, 17, 20, 24, 27) have been reported so far. It should be acknowledged that ISF glucose was compared with different blood specimens (whole blood or plasma; venous, arterialized venous, or arterial samples), which can explain some of the differences of the various studies up to ~10% (18).

Because different approaches were used, a direct comparison of the results is difficult. But even if the same method, e.g., microdialysis, was applied to determine the subcutaneous glucose concentration, different values were obtained (2, 12, 15–17, 19, 24, 27). One possible explanation for this finding is the indirect access to the ISF through the membrane. Water molecules and small ions are more likely to pass through the membrane than larger molecules and are less likely to experience hydrophobic or electrostatic interactions with the membrane. In addition, protein molecules may adhere to the membrane in an unpredictable manner during the experiment. The above mentioned effects may alter not only the composition of the collected fluid, depending on the size or the charge of the molecules, but also its properties, such as osmolarity or viscosity, and hence the concentration of the various substrates (14, 23).

It may be argued that the sampled fluid obtained by open-flow microperfusion is not pure ISF but a mixture of blood, ISF, and intracellular fluid. Because of the macroscopic perforations with open-flow microperfusion, local bleeding can be detected by reddish color from erythrocytes in the sampled fluid. Furthermore, contamination of the samples with intracellular fluid would increase the potassium concentration [within the cell ~140 mM (8)], which was not the case as depicted in Fig. 2. Thus it can be assumed that the samples represent entirely ISF diluted with the perfusion fluid.

To conclude, open-flow microperfusion was used to gain direct access to the ISF and to estimate the glucose concentration. The results are in agreement with those in earlier publications, indicating that glucose concentration in the adipose tissue is significantly lower than in plasma (16, 17, 20, 24, 27). This was confirmed in the present study by five different calibration techniques during basal and hyperglycemic conditions. This finding is also in keeping with physiological expectation, namely glucose uptake by adipose tissue. The measurement of arteriovenous differences (25) confirms significant glucose uptake, suggesting that the adipose tissue may play a significant role in glucose utilization. In addition, it could be shown that the mass transfer resistances for ions and glucose are similar, which makes continuous estimation of the recovery with the ionic reference technique possible. Thus time-consuming calibration procedures or the exposure to labeled markers can be avoided. This finding is essential for continuous monitoring and, therefore, for the implementation of a glucose-monitoring device by open-flow microperfusion.

Finally, open-flow microperfusion enables direct access to the ISF without an intervening membrane. This not only allows the measurement of small molecules (ions, glucose) but also implies the potential of sampling macromolecules, which would open a new field in clinical experimental research.

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REFERENCES

1. **Boer, J., de, H. Plijter-Groendijk, and J. Korf.** Microdialysis probe for transcutaneous monitoring of ethanol and glucose in humans. *J. Appl. Physiol.* 75: 2825–2830, 1993.
2. **Bolinder, J., E. Hagström, U. Ungerstedt, and P. Arner.** Microdialysis of subcutaneous adipose tissue in vivo for continuous glucose monitoring in man. *Scand. J. Clin. Lab. Invest.* 49: 465–474, 1989.
3. **Bolinder, J., U. Ungerstedt, and P. Arner.** Microdialysis measurement of the absolute glucose concentration in subcutaneous adipose tissue allowing glucose monitoring in diabetic patients. *Diabetologia* 35: 1177–1180, 1992.
4. **Bungay, P. M., P. F. Morrison, and R. L. Dedrick.** Steady-state theory for quantitative microdialysis of solutes and water in vivo and in vitro. *Life Sci.* 46: 105–119, 1990.
5. **Clark, M. L., S. M. Humphreys, and K. N. Frayn.** Stability of plasma glucose during storage. *Ann. Clin. Biochem.* 27: 373–377, 1990.

6. **Fischer, U., R. Ertle, P. Abel, K. Rebrin, E. Brunstein, V. D. Hahn, and E. J. Freyse.** Assessment of subcutaneous glucose concentration: validation of the wick technique as a reference for implanted electrochemical sensors in normal and diabetic dogs. *Diabetologia* 30: 940–945, 1987.
7. **Fuchi, T., H. Rosdahl, R. C. Hickner, U. Ungerstedt, and J. Henricksson.** Microdialysis of rat skeletal muscle and adipose tissue: dynamics of the interstitial glucose pool. *Acta Physiol. Scand*. 151: 249–260, 1994.
8. **Guyton, A. C.** Capillary dynamics, and exchange of fluid between the blood and interstitial fluid. In: *Textbook of Medical Physiology* (6th ed.). Philadelphia, PA: Saunders, 1981, p. 358–369.
9. **Hagström-Toft, E., S. Enoksson, E. Moberg, J. Bolinder, and P. Arner.** Absolute concentrations of glycerol and lactate in human skeletal muscle, adipose tissue, and blood. *Am. J. Physiol.* 273 (*Endocrinol. Metab.* 36): E584–E592, 1997.
10. **Hashiguchi, Y., M. Sakakida, K. Nishida, T. Uemura, K. Kajiwara, and M. Shichiri.** Development of a miniaturized glucose monitoring system by combining a needle-type glucose sensor with microdialysis sampling method. Long-term subcutaneous tissue glucose monitoring in ambulatory diabetic patients. *Diabetes Care* 17: 387–396, 1994.
11. **Jacobson, I., M. Sandberg, and A. Hamberger.** Mass transfer in brain dialysis devices—a new method for the estimation of extracellular amino acids concentration. *J. Neurosci. Methods* 15: 263–268, 1985.
12. **Jansson, P.-A., J. Fowelin, U. Smith, and P. Lönnroth.** Characterization by microdialysis of intercellular glucose level in subcutaneous tissue in humans. *Am. J. Physiol.* 255 (*Endocrinol. Metab.* 18): E218–E220, 1988.
13. **Kehr, J.** A survey on quantitative microdialysis: theoretical models and practical implications. *J. Neurosci. Methods* 48: 251–261, 1993.
14. **Linhares, M. C., and P. T. Kissinger.** Capillary ultrafiltration: in vivo sampling probes for small molecules. *Anal. Chem.* 64: 2831–2835, 1992.
15. **Lönnroth, P., P. A. Jansson, and U. Smith.** A microdialysis method allowing characterization of intercellular water space in humans. *Am. J. Physiol.* 253 (*Endocrinol. Metab.* 16): E228–E231, 1987.
16. **Maggs, D. G., R. Jacob, F. Rife, S. Caprio, W. V. Tamborlane, and R. S. Sherwin.** Counterregulation in peripheral tissues: effect of systemic hypoglycemia on levels of substrates and catecholamines in human skeletal muscle and adipose tissue. *Diabetes* 46: 70–76, 1997.
17. **Maggs, D. G., R. Jacob, F. Rife, R. Lange, P. Leone, M. J. During, W. V. Tamborlane, and R. S. Sherwin.** Interstitial fluid concentrations of glycerol, glucose, and amino acids in human quadricep muscle and adipose tissue. Evidence for significant lipolysis in skeletal muscle. *J. Clin. Invest.* 96: 370–377, 1995.
18. **McGuire, E. A. H., J. H. Helderman, J. D. Tobin, R. Andres, and M. Berman.** Effects of arterial versus venous sampling on analysis of glucose kinetics in man. *J. Appl. Physiol.* 41: 565–573, 1976.
19. **Moberg, E., E. Hagström-Toft, P. Arner, and J. Bolinder.** Protracted glucose fall in subcutaneous adipose tissue and skeletal muscle compared with blood during insulin-induced hypoglycaemia. *Diabetologia* 40: 1320–1326, 1997.
20. **Müller, M., A. Holmang, O. K. Andersson, H. G. Eichler, and P. Lönnroth.** Measurement of interstitial muscle glucose and lactate concentrations during an oral glucose tolerance test. *Am. J. Physiol.* 271 (*Endocrinol. Metab.* 34): E1003–E1007, 1996.
21. **Müller, M., R. Schmid, M. Nieszpaur, A. Fassolt, P. Lönnroth, P. Fasching, and H. G. Eichler.** Key metabolite kinetics in human skeletal muscle during ischaemia and reperfusion: measurement by microdialysis. *Eur. J. Clin. Invest.* 25: 601–607, 1995.
22. **Rosdahl, H., K. Hamrin, U. Ungerstedt, and J. Henriksson.** Metabolite levels in human skeletal muscle and adipose tissue studied with microdialysis at low perfusion flow. *Am. J. Physiol.* 274 (*Endocrinol. Metab.* 37): E936–E945, 1998.
23. **Rosdahl, H., U. Ungerstedt, and J. Henriksson.** Microdialysis in human skeletal muscle and adipose tissue at low flow rates is possible if dextran-70 is added to prevent loss of perfusion fluid. *Acta Physiol. Scand.* 159: 261–262, 1997.
24. **Schmidt, F. J., W. J. Sluiter, and A. J. Schoonen.** Glucose concentration in subcutaneous extracellular space. *Diabetes Care* 16: 695–700, 1993.
25. **Simonsen, L., J. Bülow, and J. Madsen.** Adipose tissue metabolism in humans determined by vein catheterization and microdialysis techniques. *Am. J. Physiol.* 266 (*Endocrinol. Metab.* 29): E357–E365, 1994.
26. **Skrabal, F., Z. Trajanoski, H. Kontschieder, P. Kotanko, and P. Wach.** Portable system for on-line continuous ex vivo monitoring of subcutaneous tissue glucose using open-tissue perfusion. *Med. Biol. Eng. Comput.* 33: 116–118, 1995.
27. **Sternberg, F., C. Meyerhoff, F. J. Mennel, F. Bischof, and E. F. Pfeiffer.** Subcutaneous glucose concentration in humans. Real estimation and continuous monitoring. *Diabetes Care* 18: 1266–1269, 1995.
28. **Tamada, J. A., N. J. Bohannon, and R. O. Potts.** Measurement of glucose in diabetic subjects using noninvasive transdermal extraction. *Nat. Med.* 1: 1198–1201, 1995.
29. **Trajanoski, Z., G. A. Brunner, L. Schaupp, M. Ellmerer, P. Wach, T. R. Pieber, P. Kotanko, and F. Skrabal.** Open-flow microperfusion of subcutaneous adipose tissue for on-line continuous ex vivo measurement of glucose concentration. *Diabetes Care* 20: 1114–1121, 1997.