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On line continuous monitoring of subcutaneous tissue glucose in men by combining portable glucosensor with microdialysis

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Summary. For the normalisation of blood glucose levels in diabetic patients by feedback controlled insulin delivery, a self-manageable and reliable method for continuous glucose estimation is still not available. By combining a commercially available needle type dialysis probe (molecular cutoff 20,000 Da) with a sensitive glucose sensor, we obtained a device for continuous glucose measurement in dialysate. This device was tested in healthy volunteers during a 75-g oral glucose tolerance test and in Type 2 (non-insulin-dependent) diabetic patients. Venous glucose and subcutaneous sensor signal were followed for 300 min (ten healthy subjects), 21 h (three healthy subjects) or 9 h (seven Type 2 diabetic patients). The recovery of the microdialysis was interindividually different, but after calibration, glucose levels in the dialysate and subcutaneous glucose sensor signal correlated well ($r = 0.84$–0.95). Under the assumption of a physiologic and technical delay between intravenous and subcutaneous glucose, correlation coefficient between intravenous glucose and subcutaneous sensor signal ranged from 0.60 to 0.93. We conclude that changes in blood glucose could be monitored in the subcutaneous tissue by the microdialysis technique in a continuous on line manner.

Key words: Continuous glucose sensing, enzyme electrode, glucose oxidase, subcutaneous glucose concentration, microdialysis, oral glucose load.

Although automated continuous blood glucose control by an artificial endocrine pancreas was developed several years ago [1, 2], a reliable method for continuous *self-monitoring* of glucose is still not available. On the other hand, it is not possible for patients to continuously monitor their own blood glucose. It is not advisable for patients to puncture their own veins due to the problems of thrombosis and infection. Hence, if an invasive method is used, the subcutaneous (s.c.) tissue could be the target for glucose estimation. As we reported previously [3], using an enzymatic glucose sensor and the wick technique, the s.c. glucose concentration is equal to the plasma glucose.

There are still considerable problems with implantable needle type enzyme electrodes with a drift of sensor signal and long-term stability [4]. As an alternative, the microdialysis technique might provide dialysates of the extracellular tissue fluid, as shown more than 20 years ago, when amino acids and neurotransmitters were analysed in brain dialysates [5, 6]. In these studies the dialysates were collected in fractions, and the analytes were measured intermittently [7]. This technique has been used for the estimation of various substrates including glucose in extracellular space of different regions [8, 9].

By combining the outlet of a dialysis probe with a glucose sensor, we obtained a device for continuous measurement of glucose content in tissue dialysate.

The technical description of this device was published previously [10]. The present paper refers to the application of this device in healthy volunteers and diabetic patients. Our study had two aims: the first was to check our continuous glucose sensing system s.c. in vivo in both healthy volunteers and diabetic patients. The second was to examine the microdial sis technique under a standardized glucose challenge (healthy volunteers) or under the conditions of disturbed glucose metabolism (Type 2 diabetic patients).

Subjects, materials and methods

Healthy volunteers

Thirteen healthy volunteers (four female, nine male) participated in our study. The age of the subjects was 22.6 ± 5.0 years (mean ± SD, range 19–35 years), the BMI was 22.8 ± 2.7 kg/m^2 (range 19.2–29.3 kg/m^2). In ten subjects, the duration of the experiment was 300 min. In the remaining three subjects, the experiment was extended to 21 h.

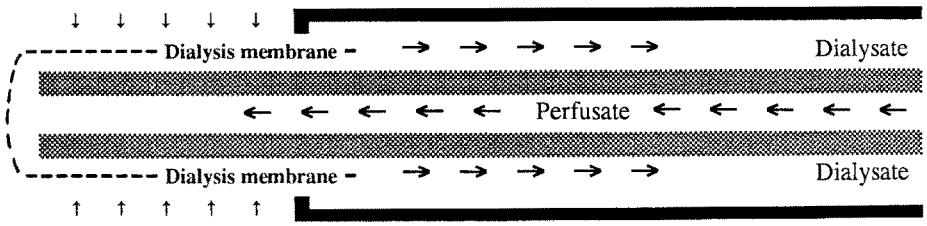

Fig. 1. Scheme of the microdialysis technique. The flow of the fluids is indicated by the bold arrows. A steel shaft (black area) is glued to a dialysis membrane (dotted line). In an inner cannula (dotted area) the perfusate (phosphate buffered saline) is transported to the tip of the probe. The probe is placed under the skin, and substances (e.g. glucose) having a concentration gradient compared with the perfusate, diffuse through the dialysis membrane, as indicated by thin arrows

Diabetic patients

Seven male Type 2 diabetic patients treated by diet, aged 63.7 ± 5.4 years (range 56–68 years) participated in the study. The BMI of the patients was 29.9 ± 1.7 kg/m^2 (range 27.2–31.2 kg/m^2). HbA_{1c} values varied between 5.2 % and 8.5 % (mean 6.3 % ± 1.2 SD; normal range: 4.6–6.0 %). The duration of the experiment was 9 h.

All subjects gave their informed consent, and the study was approved by the local ethical committee.

Microdialysis procedure

A commercially available dialysis probe was used with a molecular cutoff of 20,000 Da (CMA/Microdialysis AB, Stockholm, Sweden; polycarbonate/polyether copolymer, outer diameter 500 µm; membrane length 16 mm). It was perfused by means of a roller pump at a flow rate of 4 µl/min (for experiments of 300 min duration in healthy subjects) or 7 µl/min (for long-term experiments in healthy subjects and experiments in diabetic patients). The perfusion rate was changed, because after 10–12 h at the lower flow rate, a considerable drift of the glucose sensor signal was seen. Polyvinylchloride pump tubing was obtained from Labokron (Sinsheim, FRG). Phosphate buffered saline (0.9 %) was used as perfusion fluid. The principle of the microdialysis method is illustrated in Figure 1.

The dialysis probe was inserted under the paraumbilical skin by the following procedure: the surface of the skin was punctured two times (in and out) by a 20 gauge i.v. cannula (Viggo-Spectramed, Helsingborg, Sweden). The steel mandrin was removed, and the dialysis probe was retrogradely inserted into the plastic cannula. The plastic cannula was then removed, thereby drawing the probe under the surface of the skin. No local anaesthesia was used. At the same time, an indwelling catheter was placed in an antecubital vein.

Glucose sensing

The outlet of the dialysis probe was connected to a flow chamber. A glucose oxidase (GOD) membrane (Ames Division, Miles Lab., Elkhart, Ind., USA) was attached to the flow chamber and covered by a platinum/silver electrode, which was polarized at 700 mV. Glucose diffuses through the membrane and is converted by the GOD to gluconolactone and hydrogen peroxide. Hydrogen peroxide is oxidized at the potential of 700 mV leading to a current proportional to the glucose concentration in the flow chamber. The resulting current was amplified, digitalized and the average value of each minute was recorded.

Blood glucose was continuously measured by our Glucosensor Unitec Ulm [11] using a double lumen catheter and additionally every 30 min by a reference method (23A Glucose Analyzer; Yellow Springs Instruments, Yellow Springs, Ohio, USA).

Calibration

Before each in vivo experiment, the whole system (microdialysis probe, tubing and flow chamber) was perfused using sterile phosphate buffered saline. The resulting background current of the sensor was recorded and the sensor was calibrated to zero. After a 30 min run-in period following the implantation of the microdialysis probe, blood was drawn and the intravenous (i.v.) glucosensor and s.c. glucosensor were calibrated to the actual blood glucose.

Study protocol

Healthy volunteers. At time zero, 30 min after the calibration, the volunteers were given a 75-g oral glucose load and sensor signals were recorded at 1-min intervals for the following 270 min.

Additionally, the dialysate, when having passed the flow chamber, was collected in 30-min fractions and the glucose concentration was estimated by a colorimetric method (glucose oxidase-peroxidase method [12] with slight variations of the volume in order to adapt to the lower glucose concentrations appearing in the dialysate). In the remaining three subjects, the microdialysis probe was inserted at 16.00 hours the day before the oral glucose load. The perfusion procedure and continuous glucose sensing including calibration was performed in the same way as in the other ten subjects, but was extended to 21 h. The subjects had a normal evening meal and fasted between 22.00 hours and 08.00 hours the following morning. A 75-g oral glucose tolerance test was then performed and s.c. glucose sensor signals were recorded at 1-min intervals for the following 300 min. Blood was drawn from an indwelling catheter at 2-h intervals during the night and at 30-min intervals during the glucose tolerance test and subjected to glucose estimation. The same scheme was used in collecting dialysate fractions.

Diabetic patients. At 08.00 hours, the diabetic patients were admitted to the Institute after an overnight fast. The procedure of s.c. and i.v. glucose sensing was performed as in the healthy volunteers. At 09.00 hours and 13.00 hours, the patients were given an 800 kcal meal containing 50 % carbohydrates, 30 % protein and 20 % fat. The values of the i.v. and s.c. glucose sensors were followed until 18.00 hours.

Statistical analysis

To estimate the lag-time between changes in i.v. and s.c. glucose content, the delay due to the dead-space of the tubing was subtracted and linear regression was applied to i.v. glucose sensor values and s.c. glucose sensor values under the assumption of different lag times. Pearson's correlation coefficient r was used as a measure for the goodness of fit. The assumed lag-time leading to the highest correlation coefficient was considered as a measure of the real delay between changes in i.v. and s.c. glucose sensor signal.

Results

Healthy volunteers

Figure 2 shows an example of i.v. and s.c. sensor signal and reference. Intravenous glucose measured by the Glucosensor Unitec Ulm and the reference method were comparable. The correlation coefficient under the assumption of a 6-min delay between i.v. and s.c. glucose sensor signal was 0.85, as shown in Figure 3. The results of

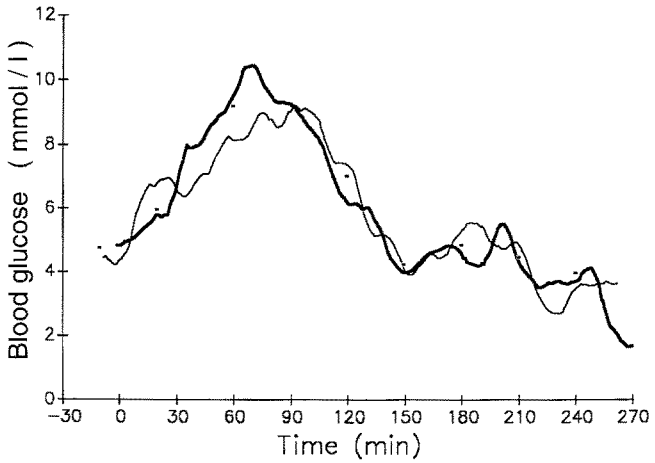

Fig. 2. Measurement of blood glucose (mmol/l; continuously, bold line; reference, mmol/l ■) and s.c. glucose (calibrated in vivo, thin line) in a healthy volunteer. At time zero a 75-g oral glucose tolerance test was started

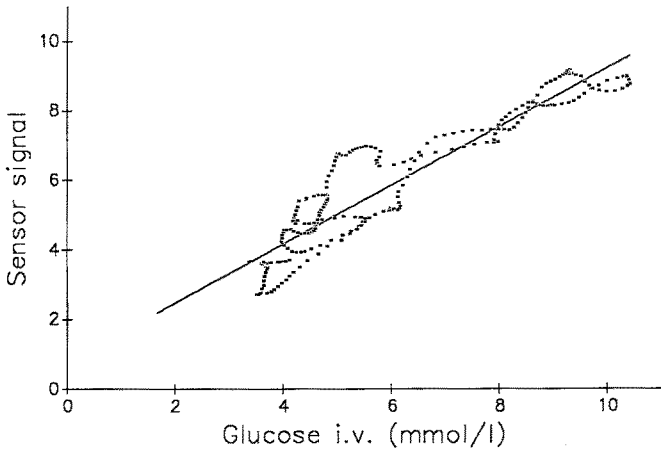

Fig. 3. Correlation between venous blood glucose (mmol/l) and s.c. sensor signal (mmol/l, calibrated in vivo) over 270 min under an assumed 6-min delay between venous blood glucose and s.c. glucose. The values of the i.v. glucose sensor (horizontal axis) are plotted against the values, which were recorded 6 min later by the s.c. glucose sensor. $r = 0.93$; $y = 0.85 \times + 13.8$; $n = 270$

the linear regression between i.v. and s.c. glucose sensor signals and the corresponding lag-times for each subject are given in Table 1. There was no correlation between BMI and the lag-time between i.v. and s.c. glucose sensor signal. In the subject shown in Figure 4, s.c. glucose sensing was performed for 21 h after insertion of the microdialysis probe. The glucose tolerance test was performed in the morning of the second day (08.00 hours). There is a clear parallel between changes in blood glucose and the s.c. glucose sensor signal. Moreover, dialysate glucose parallels the course of blood glucose as well as the s.c. sensor signal over the whole period.

The subcutaneous glucose sensor signal was closely related to the dialysate glucose concentration estimated by the reference method (Fig. 5). The ratio between dialysate glucose and blood glucose, which we refer to as the recovery of the microdialysis, was individually different, but for each volunteer, correlation coefficients between dialysate glucose and s.c. glucose sensor signal ranged between 0.85 and 0.94. Figure 6 shows the relationship between blood glucose and dialysate glucose in ten healthy subjects, where the perfusion rate was 4 µl/min. As indicated by the standard variation, the recovery of the microdialysis was variable (mean 9.4 ± 5.36%, range 4.7–14.1%) and different between volunteers. The recovery remained constant (average of the recoveries at the 30-min intervals over the period of the glucose load: 9.7 ± 0.89%). There was a negative correlation between BMI and recovery: recovery = $-1.2 \times$ BMI + 37, $r = -0.5823$ ($n = 10$, perfusion rate 4 µl/min). This correlation was close to statistical significance ($0.05 < p < 0.1$).

Diabetic patients

The recovery of the microdialysis in the Type 2 diabetic patients varied interindividually between 4.88 and 11.45% (mean ± SD: 6.70 ± 2.26%), but remained constant over the experimental period. The correlation coefficient between BMI and recovery was $r = -0.43$, which is not statistically significant ($p > 0.1$). An individual example of s.c. glucose sensing in comparison to blood glucose is given in Figure 7; the averaged values of sensor signal (± SD) and blood glucose (± SD) are shown in Figure 8. The s.c. glucose sensor signal closely followed the course of blood glucose after the meals.

Discussion

The fact that the glucose concentration in the dialysate correlated well with the glucose sensor signal demonstrates that, over the period of the glucose tolerance test, only a very slight drift of the glucose sensor signal occurred. This was an improvement when compared with

Table 1. Parameters of linear regression between i.v. and s.c. glucose sensor signals over 270 min following oral glucose tolerance test and corresponding lag-time

Subject no.	Slope m	Intercept b	Correlation coefficient r	Lag time (min)
1	1.02	−2.41	0.91	15
2	0.60	0.71	0.67	0
3	0.86	1.67	0.85	13
4	0.55	3.06	0.76	0
5	0.63	2.26	0.89	3
6	0.85	0.72	0.93	6
7	0.91	−2.23	0.93	5
8	0.95	0.48	0.70	0
9	1.07	−0.58	0.81	4
10	0.73	3.67	0.60	0
11[a]	0.67	−1.43	0.58	18
12[a]	0.79	0.92	0.71	8
13[a]	1.16	−1.67	0.78	8

The relation between i.v. and s.c. glucose sensor is assumed to have the form:
s.c. (t) = m × i.v. (t − lag-time) + b
where s.c., s.c. glucose sensor signal; i.v., i.v. glucose sensor signal; t, time t; m, slope; b, intercept
[a] Glucose sensing was performed for 21 h

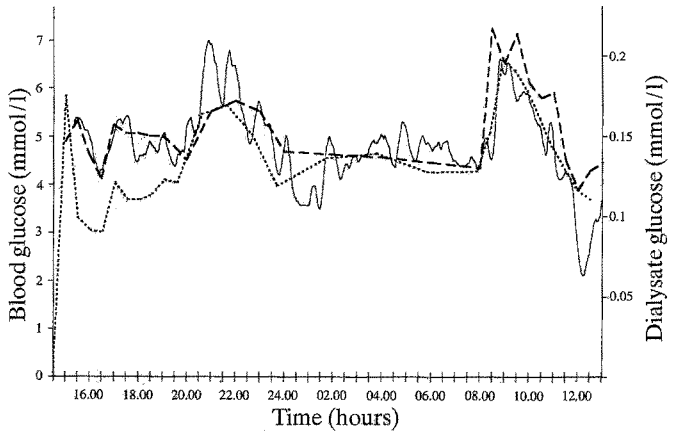

Fig. 4. Measurement of blood glucose (mmol/l; ■) and s.c. glucose (mmol/l, calibrated in vivo, thin line) in a healthy volunteer for 21 h after implantation of the microdialysis probe. At time zero a 75-g oral glucose tolerance test was started

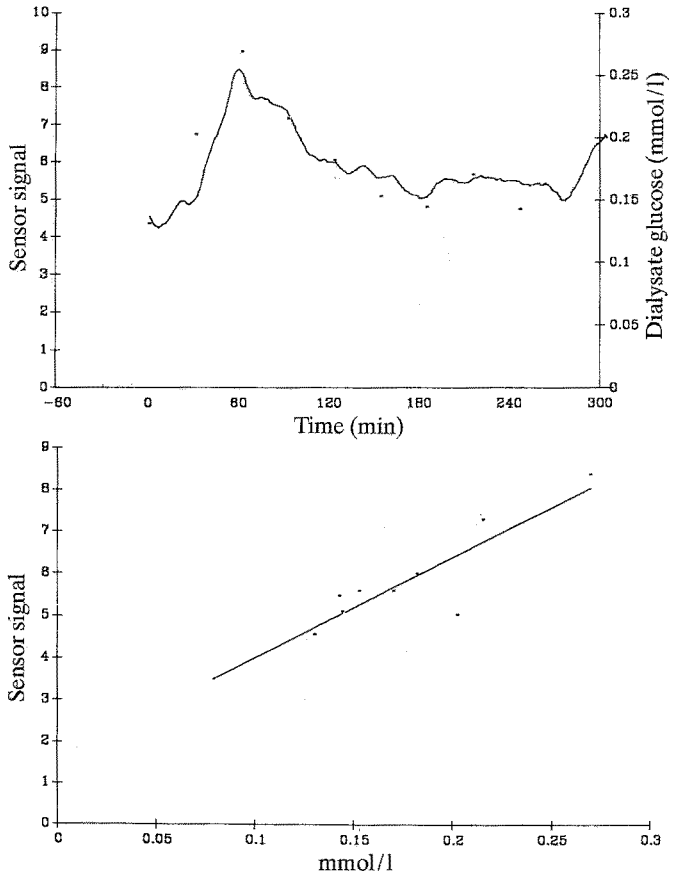

Fig. 5. Upper panel: relationship between s.c. dialysate glucose (mmol/l; ■) and s.c. sensor signal (mmol/l calibrated in vivo, continuous line). At time zero a 75-g oral glucose tolerance test was started. Lower panel: correlation between s.c. dialysate glucose content and s.c. glucose sensor signal in the same volunteer. $r = 0.87$; $y = 23.9 \times + 1.65$; $n = 9$

the implantable enzyme electrodes, which showed a considerable drift immediately after implantation [13, 14].

The pattern of the s.c. glucose concentrations obtained by the combination of the microdialysis technique and the continuous glucose sensing system closely reflected the profile of blood glucose concentrations in the healthy vol-

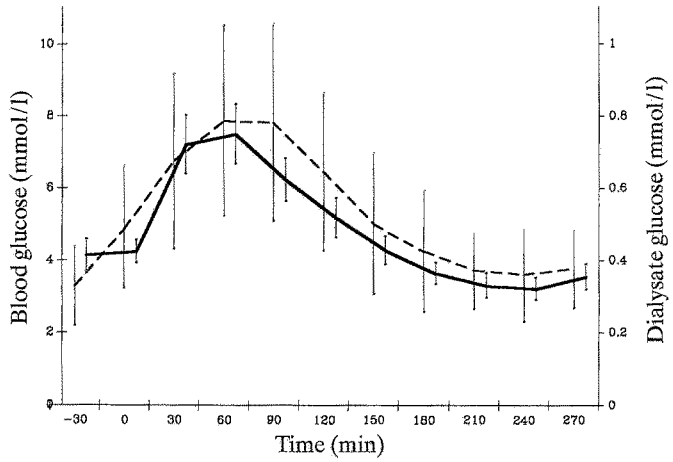

Fig. 6. Relationship between blood glucose (mmol/l, left ordinate; continuous line; ± SD-■) and s.c. dialysate glucose (mmol/l, right ordinate; broken line, ± SD-○) in ten healthy volunteers. At time zero a 75-g oral glucose tolerance test was started

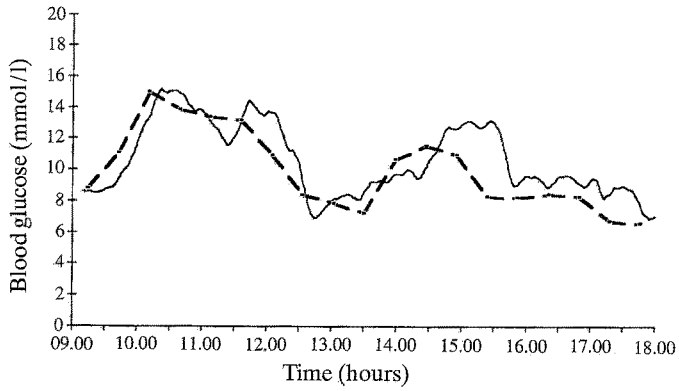

Fig. 7. Measurement of blood glucose (mmol/l; broken line) and s.c. glucose (mmol/l, calibrated in vivo, thin line) in a Type 2 (non-insulin-dependent) diabetic patient. At 09.00 hours and 13.00 hours a 800 kcal meal was given

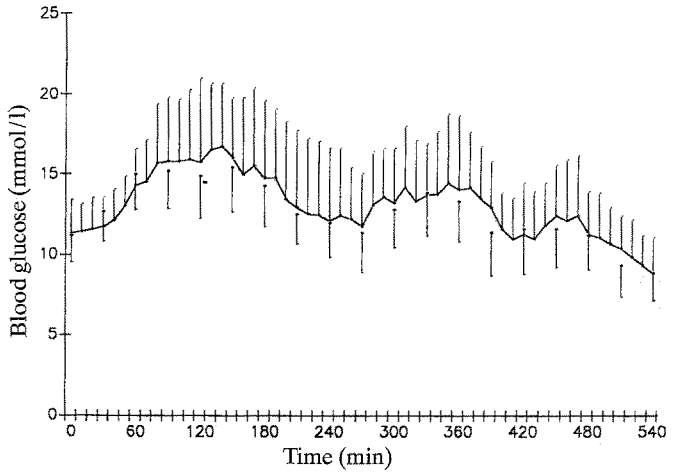

Fig. 8. Blood glucose concentrations at 30-min intervals (mmol/l, closed symbols; − SD) and s.c. glucose concentrations in 10-min intervals (mmol/l, calibrated in vivo, open symbols; + SD) in seven Type 2 (non-insulin-dependent) diabetic patients treated by diet. At time 0 min (09.00 hours) and at time 240 min (13.00 hours) an 800 kcal meal was given

unteers as well as in the diabetic patients. A delay of 0–18 min resulted between i.v. and s.c. glucose sensor signal. This value is comparable to most other studies on s.c. glucose measurement using microdialysis [15, 16] or enzyme electrodes [17]. The pattern of the s.c. glucose concentrations obtained by our device generally paralleled the course of the blood glucose concentrations up to 21 h, demonstrating both stability of the amperometric glucose sensor and constant permeability of the microdialysis probe.

The relative recovery of the microdialysis (ratio between dialysate glucose and corresponding blood glucose) is known to be dependent upon the perfusion rate [10]. When comparing subjects undergoing microdialysis at the same flow rate, however, the recovery appeared to be interindividually different. After calibration, the s.c. glucose sensor signal and the i.v. glucosensor had a linear relation during the experiment. That also applies in the diabetic patients and in the three subjects when the glucose tolerance test was performed over a considerable length of time (16 h) after the insertion of the microdialysis probe. This shows that the relative recovery of the microdialysis in each subject did not change substantially during the period of glucose sensing.

In some subjects, the correlation coefficients between s.c. and i.v. glucose sensor signal were comparatively low (0.58–0.60). In view of the fact that at least 270 single values were correlated, this correlation is highly significant.

The interindividual variability of the recovery can be explained several ways: individual tissue reactions which are known to occur after the insertion of microdialysis probes in the brain of rats [18], e.g. oedema, haemorrhage, infiltration, changes in local blood flow, precipitation of fibrin on the dialysis membrane, could influence the permeability of the dialysis membrane and thereby the resistance to glucose diffusion through the membrane. On the other hand, the resistance of the tissue itself to glucose diffusion also needs to be considered. In a mathematical model of microdialysis [19], the latter is the decisive parameter in influencing the recovery of the analyte (in this case: glucose). This resistance of the tissue is dependent, besides analyte-related diffusion characteristics, upon extracellular volume fraction and the metabolism of the analyte. The latter two points may partly contribute to the borderline-statistically significant correlation between BMI and the recovery of the microdialysis in the ten healthy volunteers undergoing microdialysis at a flow rate of 4 µl/min. This point needs further investigation in a patient population selected with regard to BMI.

Due to the interindividual changes of the recovery, individual calibration is necessary. We used a one-point calibration in vivo after a zero-point calibration in vitro. This calibration should ideally be done under steady-state conditions. This was the case in our fasted healthy volunteers as well as in the Type 2 diabetic patients, whose fasting blood glucose did not change substantially over the period of calibration. In an animal study [20], a two-point in vivo calibration was performed: one calibration point was the fasting blood glucose, and the second calibration point was the peak after an intraperitoneal glucose load. As this procedure would be difficult to perform under the circumstances of daily life, for which a device for continuous glucose monitoring in a diabetic patient must function, we used the easier one-point calibration.

Our technique has the advantage of continuously monitoring glucose in the s.c. tissue, compared to previous studies [16, 21], where dialysate fractions were collected intermittantly and glucose was estimated by a laboratory method. Since a rupture of the dialysis membrane, due to a sudden movement of the subject cannot be excluded, the technique of Aalders et al. [15], requiring continuous perfusion of the microdialysis tube with GOD, has more risks than ours.

In conclusion, the physiologic changes in blood glucose could be followed in the s.c. tissue by the microdialysis technique in a continuous on line fashion over the range of 3.3–10 mmol/l (healthy volunteers) or 8.0–16.0 mmol/l, respectively (Type 2 diabetic patients). Further efforts will aim at miniaturization of the system in order to improve the functionality of the sensor.

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