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Rapid communication

Microdialysis measurement of the absolute glucose concentration in subcutaneous adipose tissue allowing glucose monitoring in diabetic patients

J. Bolinder[1], U. Ungerstedt[2] and P. Arner[1]

Department of [1] Medicine and the Research Center at Huddinge Hospital, and [2] Department of Pharmacology, Karolinska Institute, Stockholm, Sweden

Summary. The possibility of continuously monitoring the absolute glucose concentration in subcutaneous adipose tissue, using microdialysis of the extracellular water space, was investigated in six Type 1 (insulin-dependent) diabetic patients. By using a large microdialysis probe (30 × 0.62 mm), and by perfusing with a low flow rate (0.5 µl/min), complete recovery of glucose was attained in vitro. In the patients the dialysis probe was implanted subcutaneously, perfused by a wearable microinfusion pump, and dialysate samples were collected in 60-min fractions over 10 h. The absolute glucose concentration in the tissue dialysate was the same or almost the same as the blood glucose concentration (range 87–101 % of the blood glucose value). The changes in blood glucose were closely paralleled by the variations in adipose tissue glucose ($r = 0.93, p < 0.01$), and the recovery of glucose in the microdialysate remained constant during the 10-h study period. In conclusion, it is possible, using microdialysis, to directly determine the absolute glucose concentration in subcutaneous adipose tissue. Hence, this technique may be used for continuous glucose monitoring in diabetic patients.

Key words: Microdialysis, glucose monitoring, subcutaneous adipose tissue.

In practice, the various forms of intensive insulin therapy and, ultimately, the introduction of closed-loop insulin delivery systems, in the treatment of Type 1 (insulin-dependent) diabetes mellitus depend on the development of reliable techniques for continuous glucose monitoring. Because of the potential risks associated with long-term vascular access, research in the field of intracorporal glucose monitoring has focused on the possibility of using the extracellular space of subcutaneous adipose tissue as a site for glucose sensing, since the glucose concentration in this compartment is almost identical with that in venous blood [1]. By using various types of implantable enzymatic glucose sensors it has been shown that the variations in subcutaneous tissue glucose levels closely parallel the changes in blood glucose concentrations [2]. However, problems relating to in situ calibration of the sensor signal and maintenance of sensor sensitivity have largely prevented the clinical introduction of electrochemical glucose sensors.

Recently, the microdialysis technique has been used as an alternative for monitoring glucose (and other metabolites) in adipose tissue [3]. The general principle underlying this technique is to mimic the function of a capillary blood vessel by slowly perfusing a dialysis tube implanted in the tissue [4]. The metabolite concentrations in the outgoing dialysate are then determined and reflect the levels in the interstitial fluid because of the diffusion of substances across the semipermeable membrane.

Until now a limitation of the microdialysis technique has been that the absolute metabolite concentration in the outgoing tissue dialysate is lower than that in the adipose tissue interstitial compartment because of incomplete recovery. Consequentially, an initial calibration of the microdialysis device must be made when microdialysis is being used for continuous glucose monitoring. In the present study this problem has been solved by improving the diffusion capacity of the microdialysis probe. To evaluate the feasibility of this microdialysis method for clinical use, the glucose concentrations in the adipose tissue dialysate were compared to conventionally measured blood glucose levels in Type 1 diabetic patients.

Subjects and methods

Subjects

Six Type 1 diabetic patients (five male and one famale), of normal weight, aged 19–49 years, participated in the study. The duration of diabetes ranged between 2–12 years. All patients were on intensive conventional insulin therapy (i. e. preprandial injections of short-acting insulin and a bedtime injection of intermediate-acting insulin). The study was approved by the ethics committee at Huddinge Hos-

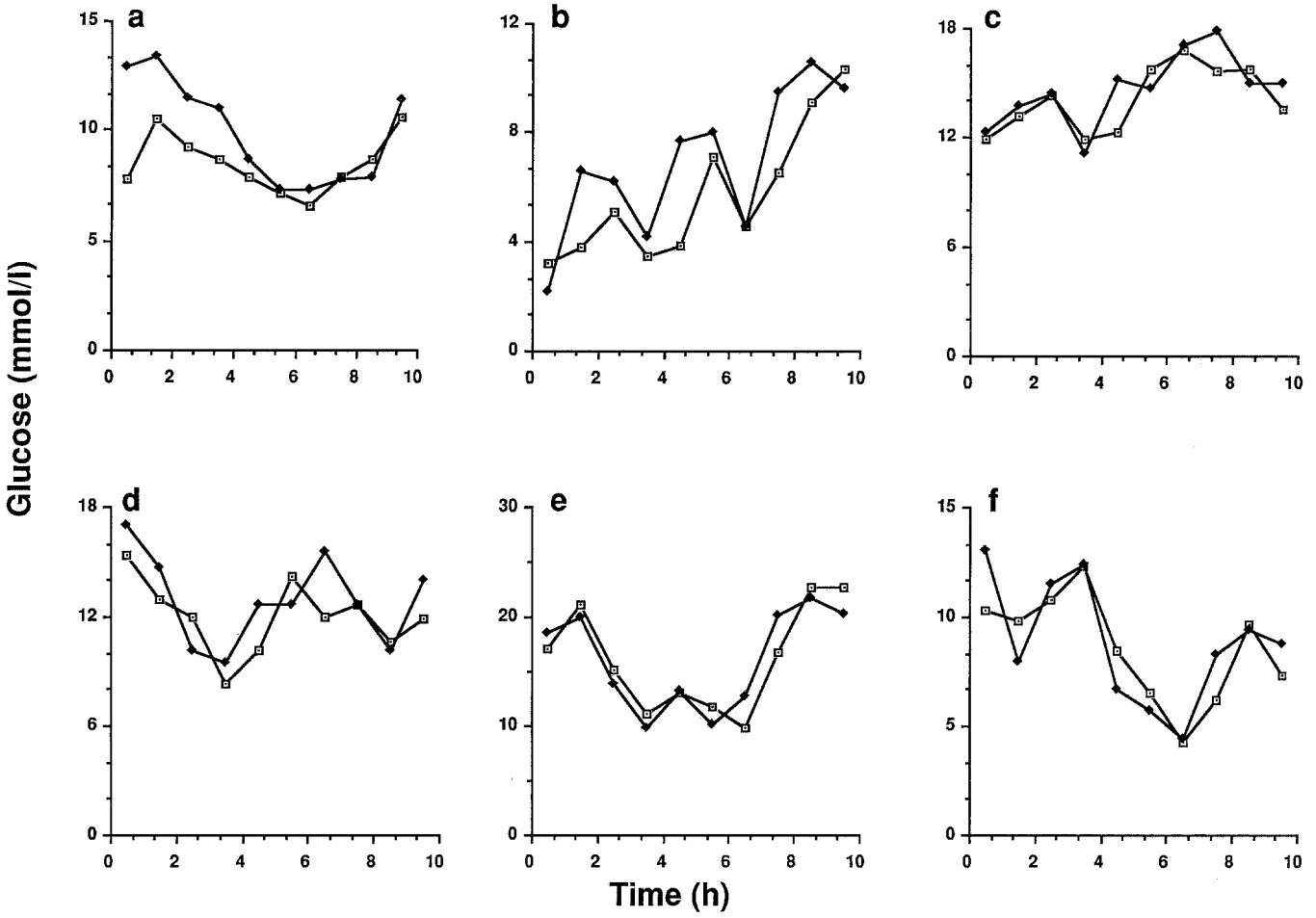

Fig. 1 a–f. Ten-hour profiles of glucose in the adipose tissue dialysate (□) and in blood (■) in six Type 1 (insulin-dependent) diabetic patients. A microdialysis probe was implanted subcutaneously and was perfused at 0.5 µl/min with Ringer's solution via a wearable microinfusion pump. During 10 h 60-min fractions of the tissue dialysate were collected for the analysis of glucose. A venous blood sample was drawn in the middle of each 60-min period for the determination of blood glucose. The ratio between dialysate glucose vs blood glucose × 100 (i.e. the relative recovery of glucose) was calculated for each 60-min period during the 10 h experiments. The mean ± SEM recovery values for patients **a–f** were 88 ± 4, 87 ± 8, 97 ± 3, 94 ± 4, 101 ± 4 and 100 ± 6%, respectively

pital. The patients were given a detailed description of the study and informed consent was obtained.

Microdialysis device

The principle of the microdialysis probe (CMA Research AB, Stockholm, Sweden) has been described in detail previously [5]. Briefly, a tubular dialysis membrane (30 × 0,62 mm, 20,000 mol. wt. cut-off), was glued to the end of a double-lumen catheter. The inlet of the catheter was continuously perfused by a wearable microinfusion pump (Minimed 504; Minimed Technologies, Sylmar, Calif., USA). The perfusion solvent then enters the probe through the space between the outer inlet lumen of the catheter and the dialysis membrane. Thereafter it leaves the probe through the inner outgoing lumen from which it is collected.

In vitro experiments

To characterize the recovery of glucose in vitro, two dialysis probes were placed in glass beakers containing various concentrations of glucose (1, 5, and 10 mmol/l), and were perfused with Ringer's solution (contents in 1,000 ml water: 147 mmol sodium, 4 mmol potassium, 2.3 mmol calcium, 156 mmol chloride, osmolality: 290 mosm/kg). Following a 30-min equilibration period, dialysate fractions of 15 µl each were collected in triplicate. The dialysate and media glucose concentration were determined by a glucose-oxidase method [6]. The influence of the perfusion flow rate on the glucose recovery was evaluated by varying the pump speed (0.5, 0.8, 1.0, 1.2 µl/min).

In vivo experiments

The patients reported to the laboratory at 07.30 hours in a non-fasting state. A microdialysis probe was inserted percutaneously, under sterile conditions, into the subcutaneous adipose tissue in the periumbilical region with a steel cannula guide for which anaesthesia was not necessary. The inlet tubing of the microdialysis catheter was connected to the wearable microinjection pump and was perfused with Ringer's solution. After a 15–30 min equilibration period, 60-min fractions of the outgoing tissue dialysate were collected in polyethylene test tubes over 10 h for the analysis of glucose [6]. Simultaneously, in the middle of each 60-min period, a venous blood sample was drawn from an indwelling polyethylene catheter placed in a cubital vein to determine blood glucose [6]. The dialysate and blood samples were obtained in the hospital when the patients were

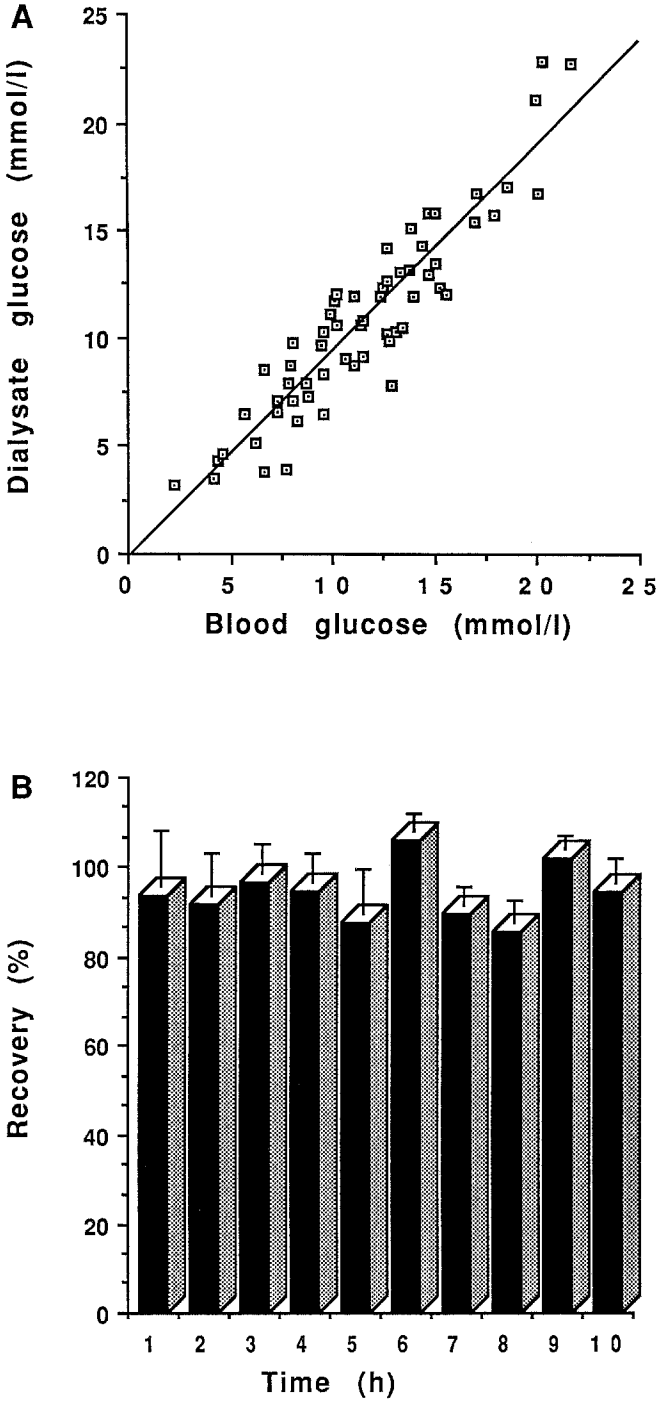

Fig. 2 A, B. Relationship between the blood glucose concentration and the dialysate glucose level (**A**), and the in vivo glucose recovery during 10 h (**B**). Pooled values for the six Type 1 (insulin-dependent) diabetic patients were calculated. Linear regression analysis by the method of least squares was performed in (**A**). $y = 0.957x - 0.210$, $r = 0.93$. The mean ± SEM recovery values are given in (**B**)

following their usual pattern of physical activities, food intake and insulin injections.

Statistical analysis

The reported values are the mean ± SEM. Linear regression analysis was performed by the least-squares method.

Results

The relative recovery of glucose in vitro (dialysate glucose concentration divided by medium glucose concentration × 100) increased progressively at decreasing perfusion flow rates. At the two lowest perfusion velocities, the recovery of glucose in the dialysate was complete at all medium concentrations of glucose tested; the recovery at 1, 5, and 10 mmol/l of glucose being 102.5 ± 0.5, 100.3 ± 0.4, and 104.8 ± 1.8%, respectively, at 0.5 µl/min, and 101.4 ± 0.6, 101.9 ± 0.4, and 99.1 ± 1.1%, respectively, at 0.8 µl/min. At perfusion flow rates of 1.0 and 1.2 µl/min, the corresponding relative recovery values in vitro averaged about 98% and 92%, respectively.

The 10-h profiles of glucose in the adipose tissue dialysate, and the corresponding blood glucose concentrations, in the six Type 1 patients are depicted in Figure 1. In these experiments the microdialysis probe was perfused at a perfusion flow rate of 0.5 µl/min. In all patients the changes in blood glucose levels were closely paralleled by the variations in glucose in the adipose tissue dialysate. The absolute glucose concentrations in the dialysate were identical, or close to identical, with the simultaneously measured blood glucose concentrations; ranging from 87% to 101% of the blood glucose level.

When the data from all patients were calculated together, a close correlation ($r = 0.93$, $p < 0.01$) was found between the glucose concentrations in venous blood and in the tissue dialysate (Fig. 2 A). The deviations of the data points from the regression line were small in both the low and the high ranges of glucose concentrations. The recovery of glucose remained constant at approximately 95–100% throughout the 10-h study period (Fig. 2 B).

Discussion

The results of this study have demonstrated for the first time that it is possible, using microdialysis, to directly determine the absolute glucose concentration in subcutaneous adipose tissue, and that this technique may thus be used clinically for continuous glucose monitoring in diabetic patients. While in previous investigations using microdialysis of subcutaneous fat, the variations in glucose levels in the tissue dialysate closely paralleled the changes in blood glucose concentrations, the dialysate glucose concentration was much lower (1–30%) than the true glucose concentration in the tissue [7–10]. In the present study the recovery of glucose was improved, first, by using a large-sized tubular dialysis membrane (tube length 30 mm) and, secondly, by reducing the perfusion flow rate. By doing so, complete recovery of glucose was achieved in vitro when the perfusion velocity was less than or equal to 0.8 µl/min. More important, when this device was applied in vivo in diabetic patients, using the 0.5 µl/min perfusion flow rate, total, or near-total, recovery of glucose was attained. This was observed over a wide range of blood glucose concentrations, varying from about 2.5 to 25 mmol/l. Moreover, the close similarity of the glucose concentration in the tissue dialysate to the blood glucose level remained unchanged, despite rapid

fluctuations in the circulating glucose level. In this respect it should be noted that the glucose concentration in the dialysate represents an integrated measure of the glucose level in the tissue (and in blood) during the sampling period. It has been shown, using microdialysis, that changes in the blood glucose level are found in the adipose tissue extracellular space within a time-lag of between 2–8 mm [8]. Hence, if the sampling period (presently 60 min) were shortened, it would be possible to detect the ongoing changes in the blood glucose concentration in even more detail. However, if the purpose were to monitor the diurnal glycaemic profile in a diabetic patient, sufficient resolution would probably be obtained with the sampling frequency used in this study.

The present findings may also apply to the microdialysis method in general. Since microdialysis allows continuous sampling of the extracellular space, the technique has been increasingly used for kinetic investigations of various tissues in experimental animals and in humans [3, 4]. However, as discussed earlier for glucose, the main problem with such experiments is that the recovery of the compounds measured in the tissue dialysate is incomplete. Consequently, the true concentration of the substances in the tissue interstitial compartment is not known. Owing to differences in the diffusion properties in water and tissue, the recovery of compounds in vivo is lower than that in vitro [4]. Therefore, the absolute metabolite concentration in the tissue cannot be determined indirectly by extrapolating from in vitro recovery values. However, it is possible to use a calibration technique to estimate the true metabolite concentration in the extracellular water space with microdialysis. With this method, the microdialysis probe is perfused with increasing concentrations of the substance of interest, and the outgoing dialysate concentration, that is in equilibrium with the ingoing perfusate level, is calculated; this concentration reflects the absolute tissue concentration [7]. Unfortunately, the in vivo calibration method is not suitable for routine use since it is much too time-consuming and requires long-term steady-state conditions. Furthermore, perfusion of the tissue with supraphysiological concentrations of the compound during the calibration procedure may increase the interstitial level of the substance, and thereby artificially influence the results of a subsequent kinetic experiment. On the other hand, by using the presently-used dialysis probe and perfusion flow rate, this problem may be overcome, since complete recovery of most low molecular weight substances should be attained. It should also be noted that there is a non-linear relationship between the perfusion velocity and the relative recovery of compounds in the tissue dialysate [4]. Hence, at low recovery rates small variations in the perfusion flow rate may artificially lead to major but incorrect changes in the contents of compounds in the dialysate. Conversely, when the recovery is total or near-total, as in this study, changes in the composition of the dialysate more accurately reflect the true kinetic behaviour of the substances in the tissue interstitial compartment.

In summary, the results of this study have shown that direct measurements of the absolute glucose concentration in subcutaneous adipose tissue can be carried out using the microdialysis technique. Hence, this method, owing to its simplicity and limited invasiveness, may offer unique prospects for continuous long-term glucose monitoring in diabetic patients.

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Dr. J. Bolinder
Department of Medicine, M54
Huddinge Hospital
S-141 86 Huddinge
Sweden