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

YASUHIRO HASHIGUCHI, MD
MICHIHARU SAKAKIDA, MD, PHD
KENRO NISHIDA, MD
TAKERO UEMURA, MD
KEN-ICHIRO KAJIWARA, MD, PHD
MOTOAKI SHICHIRI, MD, PHD

OBJECTIVE — To develop a reliable and practical glucose monitoring system by combining a needle-type glucose sensor with a microdialysis sampling technique for long-term subcutaneous tissue glucose measurements.

RESEARCH DESIGN AND METHODS — A microdialysis Cuprophan hollow-fiber probe (inner diameter, 0.20 mm; length, 15 mm) was perfused with isotonic saline solution (120 μl/h) and glucose concentrations in the dialysate were measured by a needle-type glucose sensor extracorporeally. This system was tested both in vitro and in vivo. Subcutaneous tissue glucose concentrations were then monitored continuously in 5 healthy and 8 diabetic volunteers for 7 to 8 days. A hollow-fiber probe was inserted into the abdominal subcutaneous tissue.

RESULTS — This monitoring system achieved excellent results in vitro. Subcutaneous tissue glucose concentrations were measured in a wide range from 1.7 to >27.8 mM glucose, with a time delay of 6.9 ± 1.2 min associated with a rise in glucose and 8.8 ± 1.6 min with a fall in the glucose level (means ± SE). The overall correlation between subcutaneous tissue (Y) and blood (X) glucose concentration was $Y = 1.08X + 0.19$ ($r = 0.99$). The subcutaneous tissue glucose concentration could be monitored precisely for 4 days without any in vivo calibrations and for 7 days by introducing in vivo calibrations.

CONCLUSIONS — Glycemic excursions could be monitored precisely in the subcutaneous tissue by this microdialysis sampling method with a needle-type glucose sensor in ambulatory diabetic patients.

From the Department of Metabolic Medicine, Kumamoto University School of Medicine, Kumamoto, Japan.

Address correspondence and reprint requests to Yasuhiro Hashiguchi, MD, Department of Metabolic Medicine, Kumamoto University School of Medicine, 1-1-1 Honjo, Kumamoto 860, Japan.

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IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; BMI, body mass index; FBG, fasting blood glucose; Y, subcutaneous tissue; X, blood glucose concentration.

In 1982, Shichiri et al. (1) developed a highly sensitive needle-type glucose sensor, suitable for in vivo studies. This was followed by the development of the wearable artificial endocrine pancreas, which when used in ambulatory diabetic patients has achieved physiological glycemic regulation (2). The major obstacle in extending the term of glycemic control, however, is the lack of a stable implantable glucose sensor. Therefore, before long-term clinical applications of this wearable artificial endocrine pancreas can be undertaken, further studies are required to develop an implantable long-lasting glucose sensor.

Microdialysis sampling techniques have been used to measure metabolic events, such as amino acid and neurotransmitter concentrations in brain tissue (3,4) and tissue glucose concentrations (5,6). Because this microdialysis sampling technique might provide stable dialysis conditions for several days, several investigators have been trying to develop a device that will continuously measure glucose concentrations in the tissue dialysate by combining the outlet of a dialysis probe with a glucose sensor (7–9). However, in these experiments the monitoring systems were only applied on a short-term basis. Aalders et al. (10) reported that 9 days after insertion of the probe, the dialysis system was still functioning well, although the dialysis functions varied significantly day by day. However, the Aalders et al. study did not continuously monitor subcutaneous tissue glucose concentration. Previously, Meyerhoff et al. (11) reported that changes in blood glucose could be continuously monitored in the subcutaneous tissue for one day using this microdialysis technique.

As an alternative method for long-term subcutaneous tissue glucose monitoring, we have developed a miniaturized extracorporeal glucose monitoring system that combines a needle-type glucose sensor with a microdialysis hollow-fiber probe. This study deals with the in vitro

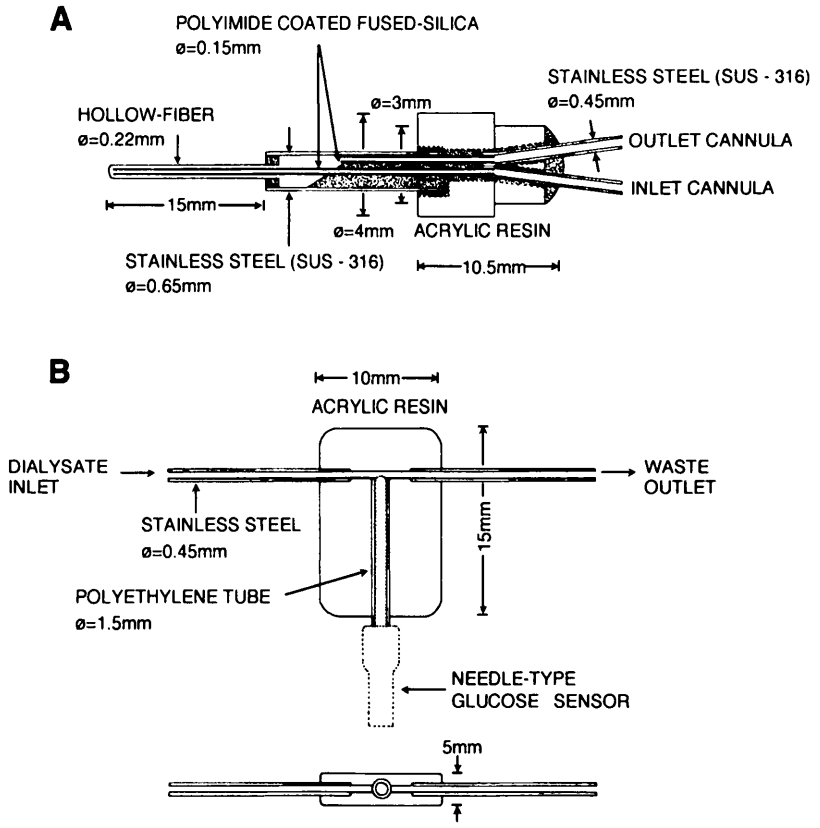

Figure 1—*Schematic diagram of hollow-fiber probe and flow-cell with a needle-type glucose sensor for extracorporeal glucose measurement in the miniaturized subcutaneous tissue glucose monitoring system based on microdialysis sampling method.* A: *Hollow-fiber probe. In an inner cannula, the perfusate is transported to the tip of the probe, diffused through the dialysis membrane, and the dialysate then comes out through an outlet cannula.* B: *Sensor flow-cell.*

and in vivo characteristics of this subcutaneous tissue glucose monitoring system. With a single dialysis hollow-fiber probe, subcutaneous tissue glucose concentrations can be monitored continuously for up to 4 days without any in vivo calibrations and for 7 days by introducing in vivo calibrations in healthy and diabetic volunteers.

RESEARCH DESIGN AND METHODS

Development of the system

The miniaturized extracorporeal monitoring system consists of a microdialysis hollow-fiber probe, a flow-cell with a needle-type glucose sensor for extracorporeal sensing, a precision microroller pump, and a monitoring system. A microdialysis Cuprophan hollow-fiber probe (Fig. 1*A*) was used with a molecular cutoff of 50,000 dalton (regenerated cellulose dialysis hollow-fiber membrane, inner diameter: 0.20 mm; outer diameter: 0.22 mm; length: 15 mm; AM-Neo-2000H, Asahi Medical, Tokyo, Japan). The microdialysis hollow-fiber probe was steadily perfused with isotonic saline solution (0.15 M NaCl in water) using a microroller pump (RA-20GM-CA20–06, Sayama Precision, Sayama, Japan) at a flow rate of 120 μl/h. The perfusate is transported through an inlet cannula (polyimide coated fused-silica tube, outer diameter 0.15 mm) to the tip of the probe, where it diffuses through the dialysis membrane, and the dialysate then comes out through an outlet cannula.

The outlet of the dialysis probe was connected to a flow-cell (clear acrylic resin plate, 15 × 10 × 5 mm) with vinyl chloride tubing (inner diameter: 0.25 mm; outer diameter: 2.0 mm; length; 30 mm) (Fig. 1*B*). The dialysis hollow-fiber probes were fabricated and purchased from Eicom (Kyoto, Japan) (model BDP-I-12–15).

A needle-type glucose sensor with high sensitivity was fixed in the flow-cell. This sensor (diameter, 1.0 mm; length, 20 mm) is a hydrogen-peroxide electrode covered with immobilized glucose oxidase, which generates a current signal depending on the glucose concentrations in the dialysate. Characteristics of the needle-type glucose sensors were as follows (means ± SE, n = 5–10): residual current, 0.3 ± 0.1 nA; output current generated to 5.5 mM glucose, 11.5 ± 0.5 nA. The response time to reach 90% of the steady-state level ($T_{90\%}$) was achieved by switching the sensor from 5.5 to 11.1 mM, 2.1 ± 0.3 min, and from 11.1 to 5.5 mM, 3.8 ± 0.4 min, for baseline drift, 0.9 ± 0.2%/24 h (Table 1).

The lower and upper detection limits of glucose concentrations were 0.6 ± 0.1 and 22.1 ± 0.3 mM, respectively. The linear relationship between output currents of glucose sensor (subcutaneous tissue [Y]: nA) and glucose concentrations in the glucose solution (blood glucose concentration [X]: mM, 0.6 to 22.2 mM) was $Y = 2.09X + 0.02$, $r = 0.99$. The needle-type glucose sensors were individually fabricated in our laboratory. The preparation and the in vitro tests for performance of a needle-type glucose sensor were described in detail elsewhere (1,2,12). A saline solution reservoir (15 ml), waste-bag (15 ml), flow-cell with a needle-type glucose sensor, and microroller pump were packed into a small unit (13.0 × 7.8 × 3.0 cm; weight, 160 g). The output currents generated from a glucose sensor were then connected to the glucose monitoring system.

Table 1—*Characteristics of the extracorporeal subcutaneous tissue glucose monitoring system based on the microdialysis sampling method in vitro*

Test	Performance	Number tested
Characteristics of the needle-type glucose sensor		
Residual current (nA)	0.3 ± 0.1	10
Output current generated to 5.5 mM glucose (nA)	11.5 ± 0.5	10
Lower detection limit of glucose concentration (mM)	0.6 ± 0.1	10
Upper detection limit of glucose concentration (mM)	22.1 ± 0.3	10
Linear relationship between output currents (Y: nA) and glucose concentrations (X: mM, 0.6–22.2 mM)	Y = 2.09X + 0.02 (r = 0.99)	10
$T_{90\%}$ response time to reach steady-state level		
5.5–11.1 mM (min)	2.1 ± 0.3	5
11.1–5.5 mM (min)	3.8 ± 0.4	5
Baseline drift (%/24 h)	0.9 ± 0.2	10
Characteristics of the hollow-fiber probe (cuprophan hollow-fiber; length: 15 mm, perfusion rate: 120 μl/h)		
Recovery of glucose in dialysate in saline solution (%)	35.6 ± 0.3	5
$T_{90\%}$ response time to reach steady-state level		
5.5–11.1 mM (min)	1.6 ± 0.5	10
11.1–5.5 mM (min)	1.7 ± 0.7	10
Characteristics of the glucose monitoring system (hollow-fiber probe; connecting tubing [length: 30 mm], needle-type glucose sensor)		
Lower detection limit of glucose concentration (mM)	1.7 ± 0.1	10
Linear relationship between glucose concentrations in dialysate (Y: mM) and glucose concentrations (X: mM, 1.7–27.8 mM)	Y = 0.36X + 0.01 (r = 0.99)	10
$T_{90\%}$ response time to reach steady-state level		
5.5–11.1 mM (min)	5.6 ± 0.4	5
11.1–5.5 mM (min)	7.4 ± 0.5	5

Data are mean ± SE. Residual current denotes the output current of the sensor in the absence of glucose. Baseline drift is expressed as percentage change of output current in response to 5.5 mM glucose solution during continuous operation. Linear relationships are studied in a regression analysis between output currents or glucose concentrations measured and glucose concentrations in the ranges indicated. $T_{90\%}$ denotes the response time to reach 90% of the final plateau value. $T_{90\%}$ response time to reach steady-state dialysis condition in the hollow-fiber probe is estimated by subtracting response times of glucose sensor and lag-time of connecting tubing (1.9 min) from the overall response time of the monitoring system.

The glucose monitoring system (12.4 × 6.0 × 2.2 cm; weight, 330 g) (SMP-X1, Nikkiso, Tokyo, Japan) consisted of a current-voltage converting amplifier, a 10 bit AD converter (voltage/frequency converter), an 8 bit CPU, an 8 KB PROM for storing measured glucose data, a liquid crystal display mechanism, and a battery supply. In this monitoring system, the output current of the glucose sensor was transformed into glucose concentration according to the following equation: Y = GST × I/(IST—Io). Y is glucose concentration in an unknown sample, GST is glucose concentration in a standard glucose solution (11.1 mM), IST and Io are output currents in dialysates when the probe is placed into a saline solution and a standard glucose solution, and I is output current in the dialysate against an unknown sample. The average glucose value is recorded every minute for a day, and the stored glucose data can be recalled using a data terminal (SDM-X1, Nikkiso). Hypoglycemia beyond the prefixed threshold is notified by the sound of an alarm.

Characteristics of the monitoring system with dialysis hollow-fiber probe in vitro and in vivo

The characteristics of the monitoring system with dialysis hollow-fiber probe (n = 5–10) were assessed by placing the probe in a saline solution with different glucose concentrations (0, 2.8, 5.6, 11.1 16.7, 22.2, and 27.8 mM) in a temperature controlled (37°C) chamber. To assess the lower detection limit of glucose concentration, a saline-glucose solution at concentrations of 0.5, 1.0, 1.5, and 2.0 mM was used. With the hollow-fiber length of 15 mm and the perfusion rate of 120 μl/h, the percentage of glucose recovery in the dialysate in vitro was calculated by dividing glucose concentration in the dialysate by glucose concentration in saline solution. Response time to reach 90% of the final plateau value ($T_{90\%}$) was calculated by switching the probe from 5.5 to 11.1 mM glucose solution and vice versa. To

test the decrease in dialysis function attributable to protein fixation on the surface of the hollow-fiber membrane, glucose concentrations in the dialysate were continuously monitored in glucose solution (11.1 mM) with albumin at concentrations of 10, 30, and 50 g/L.

After evaluating this system in animal experiments (intravenous glucose and insulin infusion tests and 7 days continuous monitoring in normal and mild diabetic rats), the system was then assessed in 5 healthy subjects (28.4 ± 2.3 years of age; body mass index [BMI], 22.6 ± 1.4 kg/m^2, means ± SE) to characterize its in vivo functions. After calibration was made by placing the hollow-fiber probe in a saline solution with and without glucose (11.1 mM) in vitro, a hollow-fiber probe was inserted into abdominal subcutaneous tissue by the following procedure. The surface of the skin was punctured by an indwelling catheter (20 gauge, SR-OT2051C, Terumo, Tokyo, Japan). 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. Then, a hollow-fiber probe was perfused with sterile perfusate. No local anesthesia was used. A hollow-fiber probe was sterilized with ethylene oxide gas. The subcutaneous tissue glucose concentrations were monitored continuously with this microdialysis sampling method. Simultaneously, the whole blood glucose concentrations were also continuously monitored with the aid of a bedside-type glucose monitoring system (model STG-22, Nikkiso) (13,14). In this system, a glucose sensor, which was developed by combining glucose oxidase membrane with a hydrogen-peroxide electrode, was incorporated for continuous blood glucose monitoring. After monitoring the baseline for more than 30 min, each healthy subject was given a 75-g oral glucose load. After their blood glucose returned to the fasting level (usually 1–2 h after the oral glucose tolerance test), insulin at a dose of 0.1 U/kg was injected intravenously.

The in vivo calibration factor was calculated as the quotient of apparent subcutaneous tissue glucose concentration and whole blood glucose concentration in the fasting state. The in vivo calibration procedure in this monitoring system was performed by dividing the sensor output by the in vivo calibration factor, then multiplying a factor of 1.10. Because subcutaneous tissue glucose concentrations were identical to plasma glucose concentrations, but were 10% higher than whole blood glucose concentrations, a factor of 1.10 was multiplied to adjust apparent values estimated by whole blood glucose concentrations to true subcutaneous tissue glucose concentrations. In vivo response times in this monitoring system were defined as the time lag between the time to reach the peak subcutaneous tissue and blood glucose concentrations after the oral glucose load, or the time lag between the time to reach the lowest subcutaneous tissue and blood glucose concentrations after intravenous insulin injection.

Continuous subcutaneous tissue glucose monitoring in healthy and diabetic volunteers

This system of continuous subcutaneous tissue glucose monitoring was then assessed in 5 healthy subjects and 8 diabetic patients. All diabetic patients (3 patients with insulin-dependent diabetes mellitus [IDDM] and 5 patients with non-insulin-dependent diabetes mellitus [NIDDM]; age, 35.2 ± 10.9 years of age; duration of diabetes, 5.7 ± 1.1 years; BMI, 24.0 ± 3.4 kg/m^2) were hospitalized in our department and treated with multiple insulin injection therapy. Their fasting blood glucose (FBG) and HbA_{1C} values ranged between 3.8 and 10.4 mM (7.8 ± 1.9 mM) and between 7.2 and 8.9% (8.3 ± 0.2%), respectively. Their mean blood glucose and mean amplitude of glycemic excursions values ranged between 6.4 and 12.1 mM (9.1 ± 0.3 mM) and between 2.8 and 6.5 mM (4.6 ± 0.2 mM), respectively. The diabetic patients did not have liver disease, kidney dysfunction, or chronic pancreatitis. Patients with severe diabetic microvascular complications were also excluded. Five healthy subjects were studied within a 2-week period after the experiments on oral glucose load and intravenous insulin injection. Informed consent was obtained from each volunteer. During the experimental period, subjects were scheduled to take each meal at 0800, 1200, and 1800 (1,600–2,200 kcal/day for healthy subjects and 1,200–1,800 kcal/day for diabetic patients). The subjects were allowed to walk for 30 min starting at 1 h after each meal.

After calibration with a standard glucose solution in vitro, the sterile dialysis hollow-fiber probe was inserted into the abdominal subcutaneous tissue in the same manner as described previously and subcutaneous tissue glucose concentrations were continuously monitored. During continuous monitoring, the in vivo calibration factors were calculated in the fasting state each morning and compared. Three series of clinical experiments were designed.

1. Subcutaneous tissue glucose monitoring for 7 days without in vivo calibration. Subcutaneous tissue glucose concentrations were monitored continuously for 7 days without in vivo calibration in 3 healthy subjects and 2 diabetic patients (1 IDDM and 1 NIDDM patient).
2. Subcutaneous tissue glucose monitoring for 7 days with in vivo calibration. During subcutaneous tissue glucose monitoring in 2 healthy subjects and 3 diabetic patients (1 IDDM and 2 NIDDM patients), in vivo calibration procedure was introduced every morning after 4 days continuous monitoring.
3. Subcutaneous tissue glucose monitoring for 8 days by replacing the hollow-fiber probe at day 5. To demonstrate the possibility of long-term use, continuous subcutaneous tissue glucose monitoring was performed for 8 days by replacing the probe on day 5 in 2 healthy subjects

and the remaining 3 diabetic patients (1 IDDM and 2 NIDDM patients). Two healthy subjects (subjects 4 and 5) participated in this series of the experiment 2 weeks after the first series of the experiment.

Blood samples were obtained from the antecubital vein before each meal, 1 and 2 h after each meal, and at bedtime. Blood glucose concentrations in whole blood samples were measured by the glucose oxidase method.

Statistical analysis

Results are expressed as means ± SE. Statistical analysis was performed with paired Student's *t* tests. Linear regression analysis was by the method of least squares and the F-distribution test were used for statistical evaluation of the slopes of regression lines. The relationships between variables were estimated with Pearson's correlation coefficients.

RESULTS

Characteristics of the extracorporeal subcutaneous tissue glucose monitoring system based on the microdialysis sampling method in vitro and in vivo

In vitro characteristics. Table 1 shows the in vitro characteristics of the extracorporeal subcutaneous tissue glucose monitoring system based on the microdialysis sampling method. The percentage for recovery of glucose in the dialysate in vitro was constant (35.6 ± 0.3%) over the concentration ranges tested (35.6 ± 0.7, 35.4 ± 0.5, 35.5 ± 0.4, 35.9 ± 0.4, 35.3 ± 0.4, and 35.7 ± 0.6% at glucose concentrations of 2.8, 5.6, 11.1, 16.7, 22.2, and 27.8 mM, respectively). The lower detection limit of glucose concentration with this monitoring system was 1.7 ± 0.1 mM. A linear relationship between glucose concentration in the dialysate (Y) and glucose concentration in the saline-glucose solution (X) was observed in the range between 1.7 and 27.8 mM ($Y = 0.36X + 0.01$, $r = 0.99$). By recording rapid changes of the glucose concentrations by switching the probe from 5.5 to 11.1 mM glucose and vice versa, the times to reach 90% of the maximum or minimum values ($T_{90\%}$) were 5.6 ± 0.4 min and 7.4 ± 0.5 min, respectively.

To test dialysis failure attributable to protein adhesion on the surface of the hollow-fiber membrane, the percentages of glucose recovery were continuously monitored for 7 days with saline-glucose (11.1 mM) solution with or without albumin. During a 7-day experimental period, the percentages of glucose recovery were not significantly different for 4 days monitoring in the solutions with albumin at the concentrations of 10, 30, and 50 g/L (35.6 ± 0.1, 35.6 ± 0.1, and 35.6 ± 0.1% on day 1; 35.5 ± 0.1, 35.2 ± 0.1, and 35.2 ± 0.1% on day 2; 35.3 ± 0.1, 35.5 ± 0.1, and 35.5 ± 0.1% on day 3; and 35.1 ± 0.3, 35.1 ± 0.3, and 34.7 ± 0.2% ($P < 0.01$) on day 4, respectively). Recovery then decreased gradually but significantly with time (32.7 ± 0.6, 32.0 ± 0.4, and 32.0 ± 0.4%, [$P < 0.01$] on day 5) and reached the significant lower values of 31.6 ± 0.9, 29.5 ± 0.6, and 28.4 ± 0.9% ($P < 0.01$) with increasing albumin concentrations, respectively, on day 7.

Continuous subcutaneous tissue glucose monitoring during oral glucose load and intravenous insulin injection in healthy volunteers

The in vivo calibration factor measured at the fasting state conditions before 75-g oral glucose loads and intravenous insulin injection in 5 healthy subjects was 1.07 ± 0.04. As shown in Fig. 2, during 75-g oral glucose loads and intravenous insulin injections, a clear parallel relationship was observed between the changes in blood glucose concentrations and subcutaneous tissue glucose concentrations measured. With this monitoring system, nadir values of hypoglycemic range (1.7–2.4, 1.8 ± 0.6 mM) were detected and notified by the sound of an alarm. Subcutaneous tissue glucose concentrations (Y) measured by the monitoring system were 9.8 ± 0.6% higher than blood glucose concentrations (X) ($Y = 1.08X + 0.12$, $r = 0.99$, $n = 240$ determinations in 5 healthy subjects). Response times to reach the peak values after oral glucose loads and the nadir values after intravenous insulin injections were 6.9 ± 1.2 and 8.8 ± 1.6 min, respectively.

Two daily continuous subcutaneous tissue glucose monitoring in healthy and diabetic volunteers

Changes to in vivo calibration factors and linear regression analyses between apparent subcutaneous tissue glucose concentrations and blood glucose concentrations during continuous monitoring in healthy subjects and diabetic patients are summarized in Tables 2 and 3, respectively. Linear regression analyses in representative cases of diabetic patients in three series of this experiment are also depicted in Fig. 3.

As shown in Table 2, the in vivo calibration factors were not significantly different, although slightly decreased, during 4 days of continuous subcutaneous tissue glucose monitoring in both healthy subjects and diabetic patients. However, after 4 days, during the 7-day monitoring period, the in vivo calibration factors decreased gradually but significantly to levels of 65.7%, found on the first day of application, by day 7 (Table 2). Even after the introduction of the in vivo calibration procedures during days 5, 6, and 7 of monitoring, the in vivo calibration factor, recalibrated with the aid of in vivo calibration procedure, again decreased significantly. However, by replacing the hollow-fiber probe at day 5, the in vivo calibration factors were not significantly changed during 5 to 8 days of continuous monitoring.

In comparison of the mean parameters, regression lines obtained during 5 to 7 days of monitoring were significantly different from those obtained during 1 to 4 days of monitoring, as shown in Table 3 and in Fig. 3*A*. However, by introducing the in vivo calibration technique every morning after 4 days

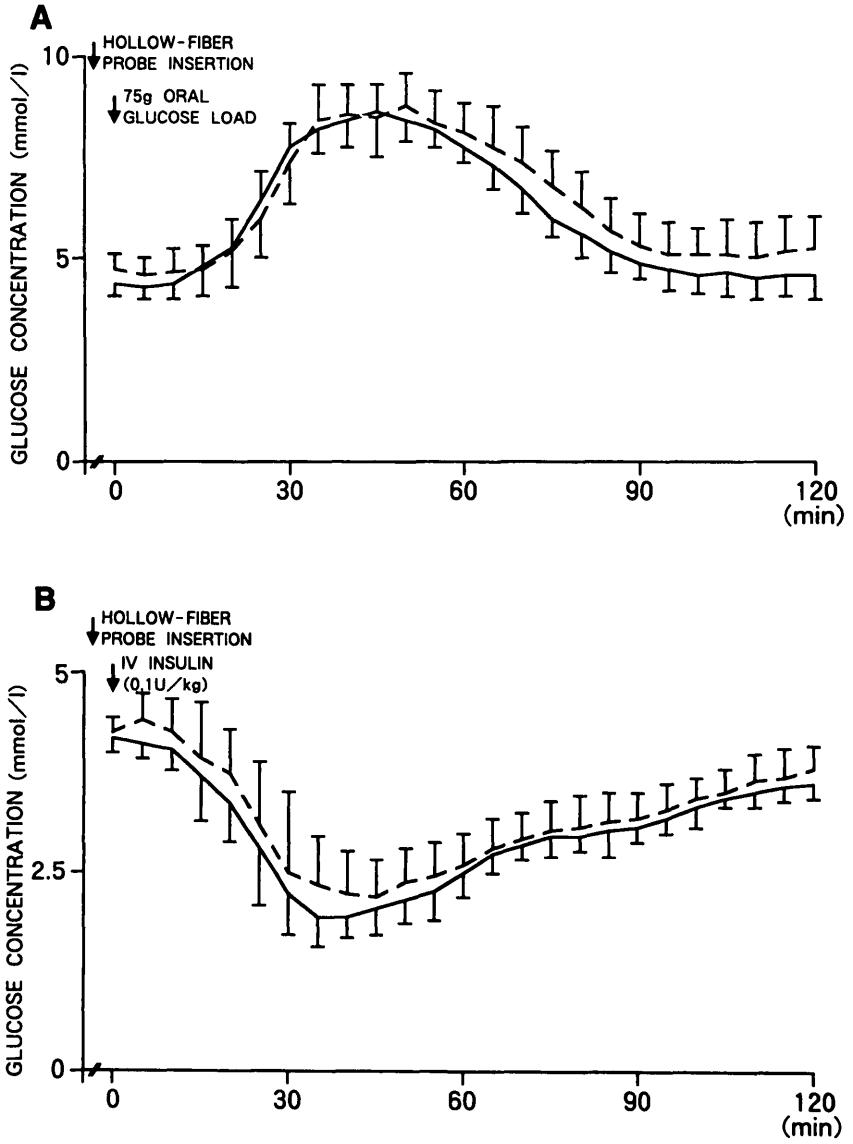

Figure 2—*Continuous monitoring of subcutaneous tissue glucose concentrations measured by the extracorporeal glucose monitoring system with microdialysis sampling methods after 75-g oral glucose load (A) and after intravenous insulin injection (0.1 U/kg) (B) in 5 healthy subjects. Subcutaneous tissue glucose concentrations (- - - -) were compared with blood glucose concentration (——) measured by the glucose monitoring system (STG, Nikkiso). Results are analyzed at 5-min intervals of the continuous monitoring records and expressed as means ± SE.*

of continuous monitoring (Table 3 and Fig. 3*B*), subcutaneous tissue glucose concentrations measured by this system again correlated with the glycemic excursions for 7 days.

By replacing the dialysis hollow-fiber probe at day 5, subcutaneous tissue glucose concentrations could be well monitored without necessitating any in vivo calibrations for 8 days in healthy subjects and diabetic patients, as shown in Table 3 and in Fig. 3*C*.

When the results obtained during oral glucose loads, intravenous insulin injections, and 4 days of monitorings without in vivo calibration in all healthy subjects and diabetic patients studied were calculated together, a close relation ($Y = 1.08X + 0.19$, $r = 0.99$, $n = 1{,}050$ determinations in 5 healthy and 8 diabetic volunteers) was found between subcutaneous tissue glucose concentrations and blood glucose concentrations.

CONCLUSIONS— The development of a wearable or implantable artificial endocrine pancreas remains an elusive goal that awaits the development of a reliable means of blood glucose monitoring on a long-term basis. This study, as an alternative method of subcutaneous tissue glucose monitoring, reports on the clinically applicable extracorporeal monitoring system based on the microdialysis sampling method.

Briefly, the principle of this monitoring system is a dialysis hollow-fiber implanted in subcutaneous tissue, which is perfused with a saline solution. Glucose concentrations in the dialysate are measured continuously with a needle-type glucose sensor with high extracorporeal sensitivity. As only trace amounts of protein were found in the dialysate of subcutaneous tissue fluids, sensor decay attributable to protein fixation may be prevented. Using a needle-type glucose sensor with high sensitivity may be advantageous compared with using a glucose oxidase solution as the perfusion fluid because the possibility of leakage of glucose oxidase, which is toxic to humans, cannot be excluded (7,9,10).

As reported by other investigators (7,8,15), we also demonstrated in our preliminary experiments with dialysis hollow-fiber probes that percentage glucose recovery in the dialysate was inversely proportional to perfusion rate and confirmed the finding of Wages et al. (15) that absolute glucose recovery in the dialysate (expressed as mmol/h) was 0 at 0 flow rate, reached a broad maximum at about 120 to 150 μl/h, and decreased at the higher flow rates. Therefore, in these experiments, a subcutaneous tissue glucose monitoring system with Cuprophan hollow-fiber length of 15 mm and con-

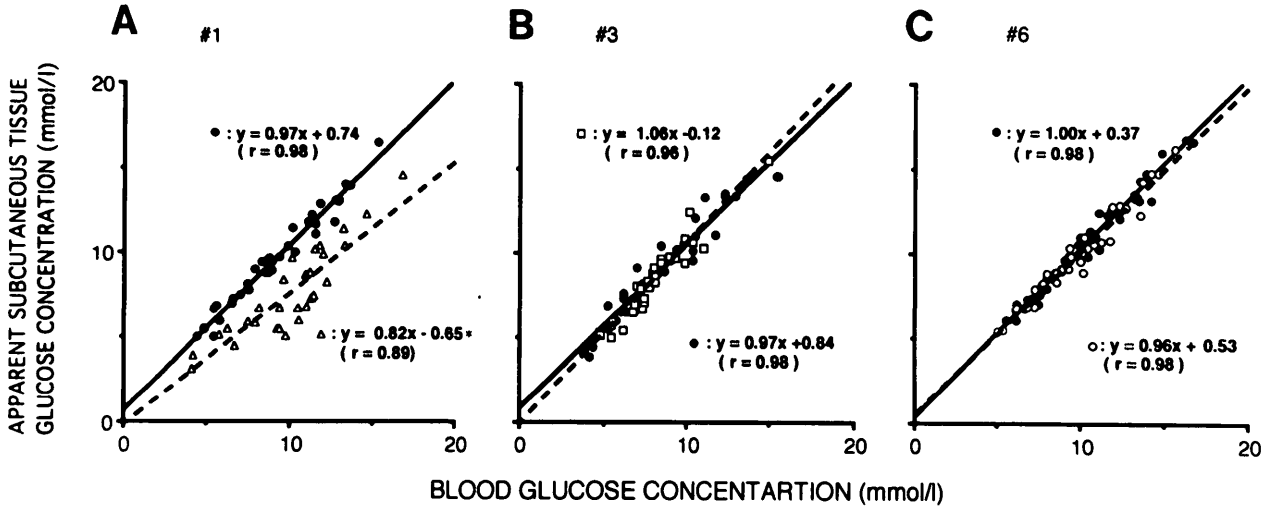

Figure 3—*Linear regression analyses between apparent subcutaneous tissue glucose concentrations measured by the extracorporeal glucose monitoring system with microdialysis sampling method (Y: mM) and blood glucose concentrations determined by glucose oxidase method (X: mM) in representative cases of diabetic patients. A: Subcutaneous tissue glucose monitoring for 7 days without any in vivo calibrations in case 1 (——, ●: data obtained between day 1 and day 4; and - - - -, △: between day 5 and day 7). B: Subcutaneous tissue glucose monitoring for 7 days with in vivo calibrations after day 5 in case 3 (——, ●: data obtained between day 1 and day 4 without in vivo calibration; and - - - -, □: between day 5 and day 7 with in vivo calibration). C: Subcutaneous tissue glucose monitoring for 8 days by replacing the hollow-fiber probe at day 5 in case 6 (——, ●: data obtained between day 1 and day 4 with the first probe; and - - - -, ○: between day 5 and day 7 with the second probe).*

necting tubing of 30 mm between the probe and a needle-type glucose sensor was applied with a perfusion rate at 120 μl/h. Under this dialysis condition, glucose recovery was constant over a wide range of glucose concentrations and variation between different probes was almost negligible. Response time of the monitoring system in vitro with a sudden change in glucose concentrations showed a mean delay of 5.6 ± 0.4 min when glucose concentration increased and 7.4 ± 0.5 min when a decrease in glucose concentration occurred. The time allowed for diffusion exchange processes at the microdialysis membrane was estimated to be 1.6 ± 0.5 min when glucose levels rose and 1.7 ± 0.7 min when the glucose levels fell. This was calculated by subtracting $T_{90\%}$ of the glucose sensor and time lag of connecting tubing (1.9 min) from the response time of this system.

Previous studies with a microdialysis sampling method (16) or implanted wick technique using saline-impregnated cotton threads (17), and with a ferrocene-mediated needle-type glucose sensor (18), demonstrated clearly that the extracellular glucose concentrations in subcutaneous tissue were almost identical to venous plasma glucose concentrations. Our experiments also confirmed that the subcutaneous tissue glucose concentrations measured were consistent with plasma glucose concentrations but were 9.8 ± 0.6% higher than blood glucose concentrations in whole blood samples. This is not surprising because plasma glucose concentrations are 10 to 15% higher than whole blood glucose concentrations.

In our in vivo experiments under the fasting steady-state glucose concentrations, glucose recovery as calculated by the in vivo calibration factor was almost the same as the observed dialysis glucose recovery in vitro. However, other investigators (5,6,9–11) reported that glucose recovery from the extracellular compartment of subcutaneous tissue to the tissue dialysate was significantly smaller than the observed dialysis glucose recovery in vitro. Meyerhoff et al. (11) also demonstrated the significant intra- and interindividual variability of the recovery. The underlying mechanisms are not known. System-related problems, especially different dialysis conditions, might be responsible for the difference between the in vivo and in vitro calibrations. Local influences such as vascularization, limited tissue glucose diffusion, and temperature might also influence dialysis (19). Therefore, the hollow-fiber should be inserted into a suitable site to allow easy drainage of the extracellular fluid. For these reasons, to measure the extracellular concentrations adequately, it is recommended that the dialysis probe is properly calibrated in vivo.

Data concerning the delay between subcutaneous tissue glucose concentrations and plasma glucose concentrations after an oral glucose load and an insulin injection were described previously by Aalders et al. (10), with a 4.4-min delay noted when blood glucose rises and an 8.2-min delay when blood glucose falls. A significant delay (>8 min) in the rise of the intercellular glucose concentration was found in diabetic patients under the unphysiological condition when hy-

Table 2—*Changes to in vivo calibration factors during continuous subcutaneous tissue glucose monitoring for 7 to 8 days*

Experimental design (number)	Study subjects (*n*)	
	Healthy (case number)	Diabetic (case number)
1. Subcutaneous tissue glucose monitoring for 7 days without in vivo calibration	3 (1,2,3)	2 (1,2)
2. Subcutaneous tissue glucose monitoring for 7 days with in vivo calibration after 4 days continuous monitoring	2 (4,5)	3 (3,4,5)
3. Subcutaneous tissue glucose monitoring for 8 days by replacing the hollow-fiber probe at day 5	2 (4,5)	3 (6,7,8)

Data are mean ± SE. The in vivo calibration factor was calculated as the quotient of subcutaneous tissue glucose concentrations and blood glucose concentrations in the fasting state in each morning. At days 5, 6, and 7 in the series 2, the in vivo calibration procedure was introduced in each morning and resulted in the calibration factor being recalibrated to 1.10. *$P < 0.01$ compared with the in vivo calibration factor on day 1. †$P < 0.01$, compared with the in vivo calibration factor recalibrated on the previous day.

perglycemia was rapidly induced with a high glucose infusion rate (9,10,13). In our experiment, response times to reach the peak values after oral glucose loads and the nadir values after intravenous insulin injections were 6.9 ± 1.2 and 8.8 ± 1.6 min. To shorten the response time, improvements in sensor function ($T_{90\%}$ response time), dialysis function (length of hollow-fiber probe), and system design (shortening the connecting tube) are required.

During continuous subcutaneous tissue glucose monitoring of blood glucose excursions, a highly significant correlation between subcutaneous tissue glucose concentration (Y), and blood glucose concentration (X) was observed (overall correlation is $Y = 1.08X + 0.19$ $r = 0.99$). However, the precisely determined differences between subcutaneous tissue glucose and blood glucose concentrations were not the same in cases of blood glucose increasing ($Y = 0.97X + 0.22$) or

Table 3—*Linear regression analyses between apparent subcutaneous tissue glucose concentrations measured by the extracorporeal glucose monitoring system with microdialysis sampling method and blood glucose concentrations determined by glucose oxidase method during continuous subcutaneous tissue glucose monitoring for 7 to 8 days*

Experimental design	Study subjects (*n*)		Subcutaneous tissue glucose monitoring (1–4 days)		Subcutaneous tissue glucose monitoring (5–7 [8] days)	
			Regression line		Regression line	
	Healthy (case number)	Diabetic (case number)	Slope	Coefficient of correlation	Slope	Coefficient of correlation
1. Subcutaneous tissue glucose monitoring for 7 days without in vivo calibration	3 (1,2,3)	2 (1,2)	1.02 ± 0.02	0.96 ± 0.01	0.80 ± 0.02*	0.86 ± 0.02
2. Subcutaneous tissue glucose monitoring for 7 days with in vivo calibration after 4 days continuous monitoring	2 (4,5)	3 (3,4,5)	1.03 ± 0.02	0.95 ± 0.01	1.05 ± 0.01	0.95 ± 0.01
3. Subcutaneous tissue glucose monitoring for 8 days by replacing the hollow-fiber probe at day 5	2 (4,5)	3 (6,7,8)	1.04 ± 0.01	0.97 ± 0.01	1.03 ± 0.01	0.97 ± 0.01

Data are mean ± SE. The overall regression lines between apparent subcutaneous tissue glucose concentrations and blood glucose concentrations are calculated from the results obtained between days 1 and 4, and between day 5 and day 7 or 8 ($n = 15$ determinations). *$P < 0.01$ compared with the results obtained between days 1 and 4.

Table 2—*Continued*

Days after hollow-fiber probe insertion							
1	2	3	4	5	6	7	8
1.08 ± 0.01	1.10 ± 0.01	1.05 ± 0.01	0.99 ± 0.01	0.84 ± 0.03*	0.80 ± 0.02*	0.71 ± 0.03*	
1.09 ± 0.01	1.11 ± 0.01	1.06 ± 0.02	1.03 ± 0.02	0.89 ± 0.02* (1.10)	0.90 ± 0.01*† (1.10)	0.86 ± 0.03*† (1.10)	
1.07 ± 0.01	1.06 ± 0.01	1.04 ± 0.01	1.03 ± 0.01	1.08 ± 0.02	1.06 ± 0.01	1.03 ± 0.01	1.01 ± 0.01

Data are mean ± SE. The in vivo calibration factor was calculated as the quotient of subcutaneous tissue glucose concentrations and blood glucose concentrations in the fasting state in each morning. At days 5, 6, and 7 in the series 2, the in vivo calibration procedure was introduced in each morning and resulted in the calibration factor being recalibrated to 1.10. *$P < 0.01$ compared with the in vivo calibration factor on day 1. †$P < 0.01$, compared with the in vivo calibration factor recalibrated on the previous day.

decreasing ($Y = 1.10X + 0.18$), as observed in the experiments on 75 g oral glucose loads and intravenous insulin injections in healthy subjects. These differences might be explained by the time lag of extracorporeal measurements of subcutaneous tissue glucose concentrations. We have not applied this monitoring system to patients in a diabetic coma who show extremely higher blood glucose levels with dehydration or to patients with brittle diabetes who show significant swings in blood glucose levels. However, with this monitoring system, hypoglycemia (1.7–2.4 mM) observed during the intravenous insulin injection test was precisely detected and notified by the sound of an alarm.

Glucose recoveries through the dialysis hollow-fiber probe in vivo, as observed in the changes of in vivo calibration factors, were not significantly different during 4 days of monitoring but then decreased significantly at day 7. A major obstacle of the microdialysis hollow-fiber probe during continuous dialysis of tissue fluids in vivo, therefore, might be the failure of dialysis function with time. The adhesion of protein (not only albumin but also fibrinogen, IgG and minor components) on the surface of the hollow-fiber membrane and the formation of protein plugs inside the hollow-fiber membrane are two of the major causes responsible for the hollow-fiber decay, as suggested by the findings when the hollow-fiber probe was placed in the glucose solution with albumin in vitro. Scanning electron microscopic examination clearly demonstrated protein adhesion on the membrane surface after 4 days of continuous application in vivo. The impermeable barrier between the implanted probe and the extracellular fluid system, which is usually observed as a tissue reaction to implants, might also be responsible.

With this monitoring system, the subcutaneous tissue glucose concentrations could be monitored precisely for 4 days without any in vivo calibration, and the introduction of the in vivo calibration technique allowed us to compare FBG concentrations every morning during days 5 to 7. Aalders et al. (10) reported previously that 9 days after insertion of the dialysis system, it was still functioning well. In their experiment, subcutaneous tissue glucose concentrations were monitored only during oral glucose loads, though the dialysis probe remained in the tissue for 9 days. However, they indicated that a persistent problem was the significant day-by-day variation of in vivo calibration factors. In addition, glucose recovery in the dialysate on day 9 was rather higher than that reported on days 6 and 7. This might not be the case because dialysis function gradually decreased as a result of protein fixation on the dialysis membrane surface. These variations might not be patient-related problems, and therefore system-related problems should be clarified further.

Using a needle-type glucose sensor that we developed previously, subcutaneous tissue glucose concentrations could be monitored continuously for 1 to 3 days (1,2,20,21). However, the major obstacle in extending the length of study to determine long-term applicability is the failure to develop a stable, reliable, and long-life implantable glucose sensor. Poor reproducibility of a sensor with excellent sensor characteristics is another problem because the sensor is individually fabricated in a laboratory rather than manufactured. Therefore, the monitoring system with the microdialysis sampling method presented here appears the most feasible and useful method for continuous subcutaneous tissue glucose monitoring at present, when a stable, reliable, and implantable glucose sensor is not available.

In conclusion, subcutaneous tissue glucose concentrations could be mea sured precisely and continuously using this extracorporeal glucose monitoring system by combining a needle-type glucose sensor with a microdialysis sampling technique for at least 4 days without any in vivo calibration. Then, after day 4, with the introduction of in vivo calibration, subcutaneous tissue glucose concentration was able to be well monitored for 7

days. By replacing the hollow-fiber probe every fifth day, this system can be used for the subcutaneous tissue glucose monitoring in ambulatory diabetic patients for longer periods.

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References

1. Shichiri M, Kawamori R, Yamasaki Y, Hakui N, Abe H: Wearable artificial endocrine pancreas with needle-type glucose sensor. *Lancet* ii:1129–1131, 1982
2. Shichiri M, Kawamori R, Hakui N, Yamasaki Y, Abe H: Closed-loop glycemic control with a wearable type artificial pancreas: variations in daily insulin requirements to glycemic response. *Diabetes* 33: 1200–1202, 1984
3. Delgado JMR, DeFeudis FV, Roth RH, Ryugo DK, Mitruka BK: Dialytrode for long term intracerebral perfusion in awake monkeys. *Arch Int Pharmacodyn* 198:9–21, 1972
4. Johnson RD, Justice JB: Model studies for brain dialysis. *Brain Res Bull* 10:567–571, 1983
5. Lönnroth P, Jansson PA, Smith U: A microdialysis method allowing characterization of intercellular water space in humans. *Am J Physiol* 253:E228-E231, 1987
6. Bolinder J, Hagström E, Ungerstedt U, Arner P: Microdialysis of subcutaneous adipose tissue in vivo for continuous glucose monitoring in man. *Scand J Lab Invest* 49:465–474, 1989
7. Schoonen AJM, Schmidt FJ, Hasper H, Verbrugge DA, Tiessen RG, Lerk CF: Development of a potentially wearable glucose sensor for patients with diabetes mellitus: design and in vitro evaluation. *Biosensors Bioelectronics* 5:37–46, 1990
8. Keck FS, Kerner W, Meyferhoff C, Zier H, Pfeiffer EF: Combination of microdialysis and glucose sensor permits continuous (on line) s.c. glucose monitoring in a patient operated device. I. In vitro evaluation. *Horm Metab Res* 23:617–618, 1991
9. Schmidt FJ, Aalders AL, Schoonen AJM, Doorenbos H: Calibration of a wearable glucose sensor. *Int J Artif Organs* 15:55–61, 1992
10. Aalders AL, Schmidt FJ, Schoonen AJM, Broek IR, Maessen AGFM, Doorenbos H: Development of a wearable glucose sensor; studies in healthy volunteers and in diabetic patients. *Int J Artif Organs* 14: 102–108, 1991
11. Meyerhoff C, Bischof F, Sternberg F, Zier H, Pfeiffer EF: On line continuous monitoring of subcutaneous tissue glucose in men by combining portable glucosensor with microdialysis. *Diabetologia* 35:1087–1092, 1992
12. Shichiri M, Kawamori R, Goriya Y, Yamasaki Y: Needle type glucose sensor, Vol 137: immobilized enzymes and cells. In *Methods in Enzymology*. Mosbach K. Ed., New York, Academic, 1988, p. 326–334
13. Shichiri M, Kawamori R, Abe H: Normalization of the paradoxic secretion of glucagon in diabetics who were controlled by the artificial beta cell. *Diabetes* 28:272–275, 1979
14. Kawamori R, Shichiri M, Kikuchi M, Yamasaki Y, Abe H: Perfect normalization of excessive glucagon responses to intravenous arginine in human diabetes mellitus with the artificial beta-cell. *Diabetes* 29: 762–765, 1980
15. Wages SA, Church WH, Justice JB: Sampling considerations for on line microbore liquid chromatography of brain dialysate. *Ann Chem* 58:1649–1656, 1986
16. Jansson PA, Fowelin J, Smith U, Lönnroth P: Characterization by microdialysis of intercellular glucose level in subcutaneous tissue in humans. *Am J Physiol* 255:E218-E220, 1988
17. Brückel J, Kerner W, Zier H, Steinbach G, Pfeiffer EF: In vivo measurement of subcutaneous glucose concentrations with an enzymatic glucose sensor and a wick method. *Klin Wochenschr* 67:491–495, 1989
18. Sakakida M, Ichinose K, Fukushima H, Nishida K, Kajiwara K, Hashiguchi Y, Taniguchi I, Shichiri M: Development of ferrocene-mediated needle-type glucose sensor as a measure of true subcutaneous tissue glucose concentrations. *Artif Organs Today* 2:145–158, 1992
19. Hagström E, Arner P, Engfeldt P, Rössner S, Bolinder J: In vivo subcutaneous adipose tissue glucose kinetics after glucose ingestion in obesity and fasting. *Scand J Clin Lab Invest* 50:129–136, 1990
20. Shichiri M, Kawamori R, Goriya Y, Yamasaki Y, Nomura M, Hakui N, Abe H: Glycemic control in pancreatectomized dogs with a wearable artificial endocrine pancreas. *Diabetologia* 24:179–184, 1983
21. Shichiri M, Asakawa N, Yamasaki Y, Kawamori R, Abe H: Telemetry glucose monitoring device with needle-type glucose sensor: a useful tool for blood glucose monitoring in diabetic individuals. *Diabetes Care* 9:298–301, 1986