Subcutaneous Glucose Concentration in Humans

Real estimation and continuous monitoring

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OBJECTIVE — To determine the real subcutaneous glucose concentration in healthy volunteers to help in the development of new calibration methods for subcutaneous glucosensors.

RESEARCH DESIGN AND METHODS — We developed a new method to estimate the real subcutaneous glucose concentration based on the recirculation of phosphate-buffered saline (PBS) in a microdialysis probe inserted into the subcutaneous tissue. Tissue glucose diffuses into the probe until complete equilibration between the glucose concentration outside and inside the microdialysis probe is achieved. Later, the glucose content of the recirculated PBS is assessed in vitro. We applied the method in 10 healthy volunteers under fasting state and during a hyperglycemic clamp. In addition, we monitored the subcutaneous glucose with an enzymatic-amperometric glucosensor combined with a microdialysis probe.

RESULTS — The subcutaneous glucose concentration measured by the recirculation method was 72 ± 6 and 78 ± 6% of the blood glucose measured in the fasting state and during the hyperglycemic clamps, respectively. On the other hand, the glucosensor's signal correlated significantly with the blood glucose.

CONCLUSION — The recirculation method estimated the real subcutaneous glucose concentration, opening the way to develop new calibration procedures for subcutaneous glucosensors. However, a suitable calibration procedure is still lacking.

Many attempts have been made to calculate the real subcutaneous glucose concentration. Recent works (1–3) showed discordant results regarding this subject. Schmidt et al. (1) obtained subcutaneous glucose values corresponding to 44 ± 8 and 46 ± 9% of the blood glucose in healthy volunteers by using a glucosensor (calibrated in vitro) and two reference methods based on subcutaneous filtrate collection and an equilibration method using ultrafiltration membranes. Fischer et al. (2) obtained values of glucose in the subcutaneous tissue approaching that of circulating blood plasma by implanting cotton threads in normal and diabetic dogs. Similar values were obtained by Brückel et al. (3) by applying the same method to sheep.

The above results do not permit a final conclusion in that ongoing discussion. Our study was designed to estimate the real glucose concentration in the subcutaneous tissue in healthy volunteers and the in vivo recovery of tissue glucose using microdialysis. These data could help in developing a calibration procedure for subcutaneous glucosensors.

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GOD, glucose oxidase; PBS, phosphate-buffered saline; POD, peroxidase.

RESEARCH DESIGN AND METHODS

Healthy volunteers

Ten healthy volunteers (two women, eight men) participated in our study. The age of the subjects was 22.9 ± 1.2 years (range 20–25 years), and the body mass index (BMI) was 22.6 ± 1.7 kg/m^2 (range 19.3–24.6 kg/m^2). The study was approved by the local committee of ethics, and the subjects gave their written consent after the aims, methods, and risks of the study were described to them.

Measurement of real subcutaneous glucose concentration

A microdialysis probe (CMA/Microdialysis AB, Stockholm, Sweden; polycarbonate-polyether-copolymer membrane, molecular cutoff 20,000 Da, membrane length 16 mm) with its inlet and outlet tubes connected to each other (total vol. = 9.2 μl; Fig. 1) was inserted in the subcutaneous tissue. Phosphate-buffered saline (PBS) was recirculated in the microdialysis probe with a peristaltic pump (recirculation speed = 2.5 μl/min, cycle duration = 3.7 min). Glucose diffused from the subcutaneous tissue into the probe until reaching an equilibrium between the subcutaneous glucose concentration and the glucose content of the recirculated PBS. The glucose content of the recirculated PBS was measured in vitro

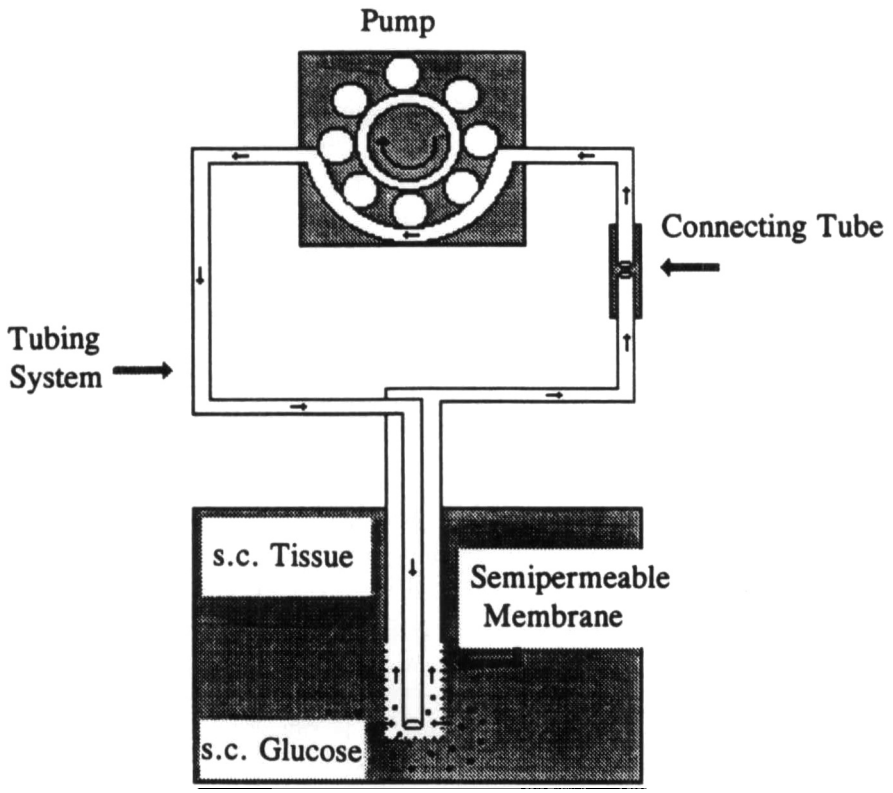

Figure 1—*Recirculation method. Subcutaneous glucose diffuses into the recirculator following a concentration gradient. PBS is recirculated by means of a peristaltic pump in the tubing system; arrows indicate the perfusion direction. The connecting tube allows the recirculator to empty and refill.*

with the glucose oxidase (GOD) and GOD/peroxidase (POD) method (4).

We named the method the recirculator. Before applying the recirculator in humans, we tested it in different glucose concentrations (25–50–100–200 mg/dl) and in plasma at 37°C.

Continuous subcutaneous glucose monitoring

Continuous subcutaneous glucose monitoring was achieved using an enzymatic-amperometric glucosensor combined with a microdialysis probe perfused with PBS at 6.9 μl/min (5–7). Tissue glucose was diffused into the microdialysis probe and was pumped to a flow chamber consisting of a GOD sandwich-type membrane (Ames, Miles, Elkhart, IN) covered by a silver/platinum electrode that was polarized at 700 mV. In the flow chamber, glucose diffuses into the GOD membrane, where, in the presence of oxygen, it is oxidized to gluconolactone and hydrogen peroxide. The latter is oxidized at the electrode system and produces a current proportional to the glucose concentration in the flow chamber. The resulting current is amplified and digitized, and each minute is recorded. A one point–in vitro, one point–in vivo calibration of the subcutaneous glucosensor was performed.

After it passed the flow chamber, the dialysate was collected in 15 min intervals. The glucose content of the dialysate was measured externally later using the GOD/POD method (4).

Study protocol

The volunteers were in at least a 10-h fasting state. The microdialysis probes of the recirculator and the glucosensor were implanted without local anesthesia in the periumbilical region as described previously (6). In the recirculator, PBS was recirculated for a period of 159 ± 5 min under the fasting state. Before the glucose clamp was started, the recirculator was emptied to assess the glucose content of the recirculating PBS in vitro with the GOD/POD method (4). After this, the recirculator was filled with fresh PBS and a hyperglycemic clamp was performed for 132 ± 9 min. Blood glucose was clamped at 48 ± 4 mg/dl over the basal blood glucose value following the technique of DeFronzo et al. (8). Before the end of the hyperglycemic clamp, the recirculator was emptied and the glucose content of the recirculated PBS was assessed in vitro using the same method as before (4).

Tissue glucose was continuously monitored with the glucosensor and blood glucose was measured every 15 min

Table 1—*Blood glucose and subcutaneous glucose in the fasting state and during the hyperglycemic clamp*

Fasting state			Hyperglycemic clamp		
Blood glucose (mg/dl)	Subcutaneous glucose (mg/dl)	Subcutaneous glucose/blood glucose (%)	Blood glucose (mg/dl)	Subcutaneous glucose (mg/dl)	Subcutaneous glucose/blood glucose (%)
84.7 ± 4.5 (75–90)	60.8 ± 5.7 (52–70)	72 ± 6% (60–80)	133 ± 11.3 (119–152)	104 ± 9.5 (92–124)	78 ± 6% (69–90)

Data are means ± SD (range); n = 10. Blood glucose and subcutaneous glucose measured with the recirculator and the quotient of subcutaneous glucose and blood glucose are compared in the fasting state and during a hyperglycemic clamp in 10 healthy volunteers.

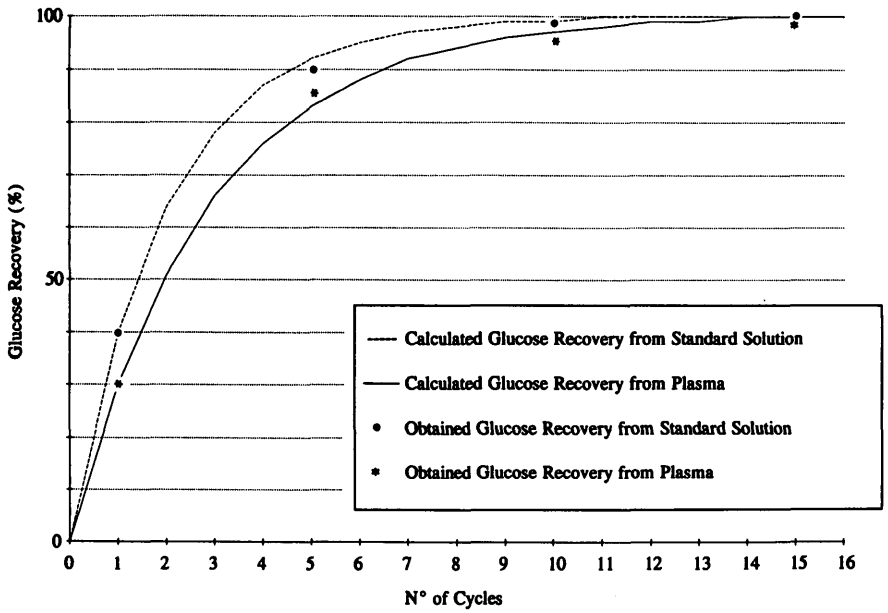

Figure 2—*Glucose equilibration using the recirculator in glucose standard solutions and in plasma.*

in the fasting state and every 5 min during the clamp with a photometric reference method. (Reflotron, Boehringer Mannheim, Mannheim, Germany).

RESULTS

In vitro experiments

Eleven cycles were needed to achieve complete equilibration between recirculated PBS and the glucose concentration of standard solutions, and 15 cycles were needed to achieve complete equilibration between recirculated PBS and plasma glucose (Fig. 2).

In vivo measurement of real subcutaneous glucose

During the fasting state, PBS was recirculated 44 ± 8 times, while under the hyperglycemic clamps, it was recirculated 36 ± 3 times. The glucose content of the recirculated PBS was 72 ± 6% of the blood glucose in the fasting state and 78 ± 6% of the blood glucose during the hyperglycemic clamp (Table 1).

Continuous subcutaneous glucose monitoring

Continuous subcutaneous glucose monitoring was effectively achieved using the glucosensor in combination with the microdialysis technique. The glucose contents of the dialysates that were collected in 15-min intervals (using the subcutaneous glucosensor) were 6.0 ± 0.6 and 6.1 ± 0.6% of the glucose content of the recirculated PBS (using the recirculator) under the fasting state and during the hyperglycemic clamp, respectively.

The signal of the subcutaneous glucosensor correlated significantly with the blood glucose profile. The delay time between changes in the blood glucose and in the subcutaneous tissue was 12 ± 3 min.

CONCLUSIONS — In this study, we used one method to measure the real subcutaneous glucose concentration and one method to monitor the subcutaneous glucose changes. The recirculator's perfusion speed turned out to be adequate during in vitro experiments to achieve complete equilibration between the different media, although PBS had to be recirculated more times in plasma samples than in glucose standards to achieve complete equilibrium. This is probably due to viscosity of the medium.

The recirculator seems to be reliable when measuring the real subcutaneous glucose concentration because of the following: *1*) Tissue damage caused by introducing the microdialysis probe in the subcutaneous tissue does not disturb the glucose measurement, considering that diffusion takes place on a normal basis after 30 min (1). 2) The PBS volume exposed to dialysis is too small to produce any significant alteration (regarding undisturbed medium, see microdialysis theory [9]). 3) The dialysate is continuously stirred. 4) The size of the dialysis probe permits optimum equilibration between the media. 5) Use of the semipermeable membrane avoids blood contamination.

During the fasting state and the glucose clamps, subcutaneous glucose was 72 ± 6 and 78 ± 6% of the blood glucose, respectively. The difference between these concentrations is not statistically significant. This shows that whether recirculating PBS 36 ± 3 or 44 ± 8 times, comparable results were obtained, suggesting that complete equilibrium of glucose concentration between the subcutaneous tissue and recirculated PBS was achieved in every case. However, we cannot affirm that steady state was reached in the clinical in vivo experiments.

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