Glucose Concentration in Subcutaneous Extracellular Space

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OBJECTIVE — To compare the subcutaneous glucose sensor measurements with two reference methods. Previous studies provide conflicting findings about the real glucose concentrations in subcutaneous tissue. Some suggest substantially lower concentration, whereas others measure proportionally higher glucose concentrations compared with the blood compartment. Before these results can be taken seriously as an expression of the real glucose concentration in the extracellular space, the measurements must be validated by an independent method.

RESEARCH DESIGN AND METHODS — We applied a microdialysis-based enzyme sensor to measure glucose concentration in subcutaneous tissue. We also developed two reference methods: subcutaneous filtrate collection and an equilibration method using ultrafiltration membranes to support the earlier findings. We provided an anatomical model to explain the results.

RESULTS — The mean overall intercellular filtrate glucose concentration, sampled with the filtrate collector and taken after a 6-h stabilization time, including the values during the glucose clamp period, was 46 ± 9%. The mean subcutaneous glucose concentration measured with the glucose sensor, calibrated in vitro, was 44 ± 8% of the mean venous blood glucose concentration. Mean overall intercellular equilibrate glucose concentration, i.e., the mean glucose concentration in the subcutaneous extracellular space, taken after a 4-h stabilization time, was 46 ± 15% of the mean venous blood glucose concentration.

CONCLUSIONS — The close agreement between the mean values of subcutaneous glucose concentrations, obtained with three independent methods—filtration, equilibration, and dialysis (sensor)—shows the real glucose concentration in subcutaneous interstitial fluid is ~50% the blood glucose value in normal humans. Our results clarify some of the conflicting evidence presented in previous studies.

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TYPE I DIABETES, INSULIN-DEPENDENT DIABETES MELLITUS.

Since the introduction of glucose sensors small enough to be implanted in subcutaneous tissue, uncertainty has arisen about the real glucose concentrations in that body compartment. Shichiri et al. (1–4) measured values 6–22% lower subcutaneously than intravenously in type I diabetic patients and 15% lower in dogs. Abel et al. (5,6) measured values 30–70% lower, if the sensor was calibrated independently. Pickup et al. (7) found a mean ratio of blood glucose to the in vitro calibrated sensor tissue glucose concentration in humans of 1:8, with a range of 0:6–4:1. Ertefai and Gough (8) found the sensor response in rats suggested even proportionally higher glucose concentrations than in the blood compartment. All these sensors work on the same principle: an oxidation of glucose, catalyzed by the enzyme glucose-oxidase in situ. Before these results can be taken seriously as an expression of the real glucose concentration in the extracellular space, the sensor measurements must be validated by an independent method. This is not easy, because the sampling of extracellular fluid must not be contaminated by intravenous fluid.

When Wolfson et al. (9) implanted dialysis bags and membrane-covered devices in rabbits and baboons, extracellular fluid filled these devices spontaneously. They sampled the devices over several months and found lower glucose concentrations in the extracellular space (49–77%), whereas other substances remained at levels near those of blood plasma.

Lönnroth et al. (10,11) used a microdialysis method in which they placed a hollow fiber in the periumbilical subcutaneous tissue of humans. They perfused the system with different glucose concentrations in isotonic saline at a constant rate. If the hollow fiber was perfused with a glucose concentration equal to the concentration in blood, equilibrium was reached between the perfusion medium and the extracellular

fluid. If a solution was perfused with a higher or lower glucose concentration, an efflux or influx of glucose could be detected. When perfused with isotonic saline, however, the amount of glucose that diffused into the fiber in vivo was ~50% of the amount of glucose that diffused into the fiber in vitro. The authors could offer no explanation for this phenomenon.

Fischer et al. (12,13) used an implanted-wick technique, employing saline-impregnated cotton threads in dogs to obtain samples from the subcutaneous extracellular fluid. At the same time, they measured subcutaneous glucose concentrations with a sensor. Both values were compared with intravenous glucose concentrations. The glucose concentrations measured with the wick technique closely followed the intravenous concentrations, whereas the sensor measured tissue glucose concentrations of 70–90% of glycemia.

The above cited studies do not allow a straightforward conclusion on the actual extracellular glucose concentration. This study presents the results of glucose measurements in the subcutaneous extracellular space with three different methods: *1*) glucose sensor measurements, based on microdialysis; *2*) sampling of extracellular fluid, based on a filtration technique; 3) glucose equilibration with extracellular fluid, applying ultrafiltration membranes.

Earlier studies showed that the microdialysis-based glucose sensor, when calibrated in vitro, measured only 43% of the concomitant blood glucose level (16). The purpose of this study was to compare the subcutaneous glucose sensor measurements with the two reference methods.

RESEARCH DESIGN AND METHODS

Glucose sensor

A glucose sensor was used to measure glucose in the subcutaneous tissue with a microdialysis system, described elsewhere (14–16). Briefly, we placed a hollow fiber in subcutaneous tissue and perfused it with a glucose-oxidase solution at a fixed flow rate (1.2 ml/h). Glucose diffused from the tissue into the hollow fiber and reacted with oxygen, catalyzed by the enzyme. Oxygen concentration of the perfusion fluid was measured on line outside the body with a miniature Clark-type oxygen electrode (14).

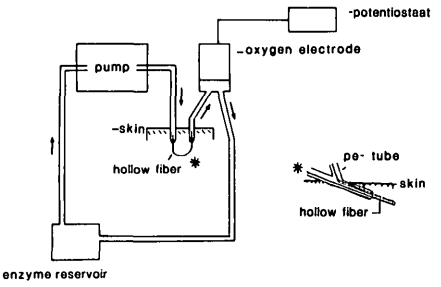

Figure 1—*Schematic drawing of the glucose sensor microdialysis system.*

Sensor functioning is independent of local tissue oxygen concentration because the recirculating enzyme solution is allowed to equilibrate with the environmental oxygen concentration before reuse. Before in vivo use the glucose sensor is calibrated in vitro in different glucose solutions. Sensor output thus can be related to the present glucose concentration using the in vitro calibration factor (16). Figure 1 is a schematic drawing of the glucose sensor microdialysis system.

Filtration study

We glued three polysulfone hollow fiber membranes, internal diameter 0.5 mm, outer diameter 0.8 mm, length 5 cm (AG Technology, Needham, MA) on both ends to polyethylene connecting tubes, internal diameter 0.4 mm, outer diameter 0.8 mm (Talas, Ommen, The Netherlands). One end of each connecting tube was sealed tightly with glue.

The three connecting tubes were glued together into one conducting tube, and a needle was fixed onto the conducting tube, enabling connection of the three hollow fibers to one vacutainer tube (Fig. 2). Three hollow fibers of one filtrate collector can be inserted separately into subcutaneous fatty tissue of the abdomen with the use of 16-gauge needles (Becton Dickinson, Rutherford, NJ). In this way samples of intercellular subcutaneous tissue fluid can be obtained with the vacuum of the vacutainer tube as driving force.

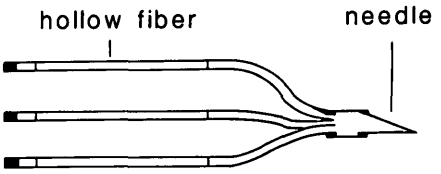

Figure 2—*Filtrate collector as used in this study*

Filtrate collectors with a molecular weight cut-off of 500,000 M_r were studied in 7 healthy volunteer subjects, 6 men and 1 woman, 19–32 yr of age; and with a cut-off of 30,000 M_r in 6 healthy volunteer subjects, 4 men and 2 women, 20–26 yr of age. All volunteer subjects consented to take part in the study after an explanation of its rationale. The Local Ethical Committee approved the study.

The tests began at 2000 h. We inserted the three fibers of the filtrate collector into subcutaneous fatty tissue on one side of the abdomen and the glucose sensor microdialysis probe (16) on the opposite side. The subcutaneous glucose concentration was measured continuously during the test with the glucose sensor. Intercellular fluid samples, obtained with the filtrate collector, were measured over a 1-h sampling period.

Intermittent blood sampling was taken through a catheter inserted in an antecubital vein. Venous blood samples were taken each hour in the middle of a filtrate collecting period.

Blood glucose values and intercellular fluid glucose values were measured with a glucose analyzer (YSI, Yellow Springs, OH). Subcutaneous glucose

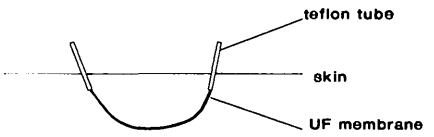

Figure 3—*Equilibration ultrafiltration membrane as used in this study.*

sensor values were calculated from the in vitro calibration factor (16), which was determined before and controlled after each test. All volunteer subjects fasted during the tests.

At 0800 the next day, blood glucose was raised and clamped at 140 mg/dl for 2 h. Thereafter, glucose infusion was stopped. After the venous blood glucose level and the subcutaneous glucose sensor signal had reached steady state, we removed the dialysis probe and filtrate collector.

Equilibration study

Nine healthy male volunteer subjects, 21–48 yr of age, took part in the equilibration study, which was approved by the Local Ethical Committee. The equilibration fibers consisted of a polysulfone ultrafiltration membrane with molecular weight cut-off of 100,000 M_r, internal diameter 1.0 mm, external diameter 1.6 mm, length 10 cm (AG Technology, Needham, MA). The fibers were glued on both ends to connective tubes made of Teflon, internal diameter 0.58 mm, external diameter 1.08 mm (Talas, Ommen, The Netherlands).

Equilibration fibers were inserted into subcutaneous fatty tissue of the abdomen with an intravenous catheter unit 80 mm in length (Intraflon 2, G12, Vygon, France), pushed through a fold of the skin of the abdomen. After equilibration fibers were guided through the catheter, the catheter was removed (Fig. 3).

In 5 volunteer subjects the equilibration fibers were filled with 100 μl saline (NaCl 0.9%), and in 4 others with 100 μl of a 5-mM glucose solution in saline. In both situations the fluid was allowed to equilibrate for 1 h with the surrounding tissue, after which it was sampled. A new amount of equilibration fluid then was brought in.

The time needed to achieve complete in vivo equilibration was determined in preliminary tests, showing no significant change (<5%) in glucose concentration after a 1-h equilibration period. Accurate in vitro equilibration could be achieved within 15 min. We recognized possible ion exchange effects, but soaking the fibers in water obviated any adverse reaction. Glucose concentration in the collected fluid samples and in the simultaneously obtained venous blood samples was measured with the YSI glucose analyzer.

RESULTS

Filtration/glucose sensor study

The results obtained with the filtrate collector with a molecular weight cut-off of 500,000 M_r are shown in Fig. 4. A few hours after insertion of the filtrate collector, the glucose concentration of the intercellular filtrate decreased to a value of 46 ± 9% of the blood glucose value (after 6 h). When the blood glucose was clamped at a higher level, the filtrate value rose accordingly to remain 51 ± 11% of the blood glucose value.

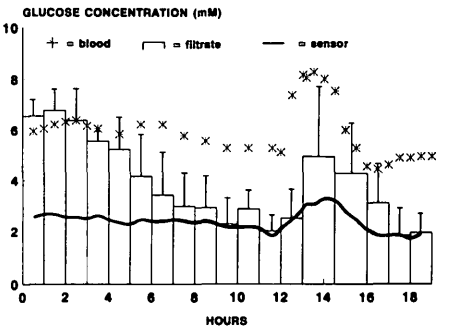

Figure 4—*Mean subcutaneous intercellular-glucose values of filtrate (molecular weight cut-off of 500,000 M_r) and glucose sensor (calibrated in vitro); filtrate data are means ± SD (n = 7).*

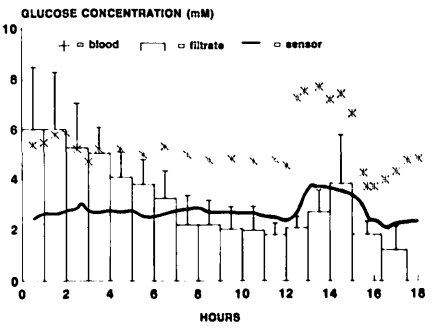

Figure 5—*Mean subcutaneous intercellular glucose values of filtrate (molecular weight cut-off of 30,000 M_r) and glucose sensor (calibrated in vitro); filtrate data are means ± SD (n = 6).*

The glucose sensor needed a 30-min run-in period to reach a steady signal. When calibrated in vitro, the glucose sensor signal was 43 ± 7% of the simultaneous blood glucose concentration over the whole test.

The results obtained with the filtrate collector with a molecular weight cut-off of 30,000 M_r are shown in Fig. 5. The intercellular filtrate glucose concentration decreased to 44 ± 5% of the blood glucose value after 6 h. During the clamp the glucose concentration measured in the intercellular filtrate was 45 ± 10% of the blood glucose value. The in vitro calibrated sensor signal was 49 ± 8% of the simultaneously determined venous blood glucose concentration for all tests in 6 healthy volunteer subjects.

The mean overall intercellular filtrate glucose concentration, sampled with the filtrate collector and taken after a 6-h stabilization time for all 13 volunteer subjects, including the values during the glucose clamp period, was 46 ± 9%. The mean subcutaneous glucose concentration measured with the glucose sensor, calibrated in vitro, was 44 ± 8% of the mean venous blood glucose concentration.

The sample volume of the filtrate collector, measured for 10 volunteer subjects, gradually decreased during the

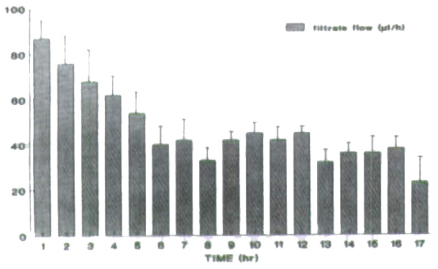

Figure 6—*Sample volume of filtrate collector for 10 healthy volunteer subjects; data are means ± SD.*

test to a constant level of ~40 μl/h after 6 h (Fig. 6). In vitro functioning of explanted filtration fibers was unimpaired after the tests. In vitro flow experienced no change.

Equilibration study

The results obtained with the equilibration method are depicted in Figs. 7 and 8. Figure 7 shows the mean results of the in vivo study in 5 healthy volunteer subjects in which the ultrafiltration membranes were filled with a saline solution and allowed to equilibrate with the surrounding tissue for 1 h.

After a 4-h decline period, the glucose concentration remained stable at 41 ± 10% of the simultaneously measured venous blood glucose level. Filling the ultrafiltration membranes with a solution of 5 mM glucose in saline resulted in a similar pattern, as shown in Fig. 8.

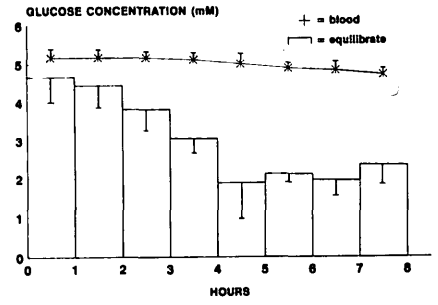

Figure 7—*Glucose concentration in equilibrate (saline solution equilibrated over 1 h) and venous blood in 5 healthy volunteer subjects; data are means ± SD.*

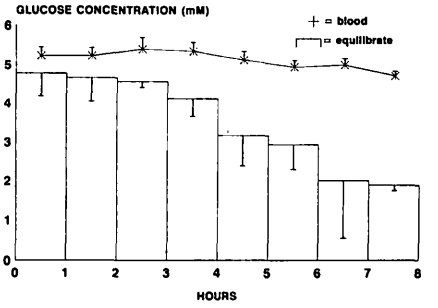

Figure 8—*Glucose concentration in equilibrate (5 mM glucose solution in saline equilibrated over 1 h) and venous blood in 4 healthy volunteer subjects; data are means ± SD.*

After a 4-h decline period, the glucose concentration of the equilibrate remained stable at 50 ± 13% of the venous blood glucose level. Mean overall intercellular equilibrate glucose concentration, i.e., the mean glucose concentration in the subcutaneous extracellular space, taken after a 4-h stabilization time for all 9 healthy volunteer subjects, was 46 ± 15% of the mean venous blood glucose concentration. Table 1 presents an overview of all mean subcutaneous glucose concentrations obtained in this study.

CONCLUSIONS— Our results may clarify some of the conflicting evidence presented in the literature. The close agreement between the mean values of subcutaneous glucose concentrations obtained with three independent methods—46% for filtration, 46% for equilibration, and 44% for dialysis (sensor)—shows that the real glucose concentration in subcutaneous interstitial fluid is about half the blood glucose value in normal humans.

Most striking is the decline in glucose concentration of the equilibration fluid when its initial concentration is close to the venous concentration. The decline of the glucose concentration within the hollow fiber in this situation proves that glucose leaves the fiber to be redistributed in the tissue. Actual glucose concentration of the interstitial fluid surrounding the fiber therefore has to be lower than the blood glucose concentration.

The results show that shortly after application of devices, the tissue glucose is equal to blood glucose. This is caused by disruption of local blood vessels, cells, and capillaries by insertion of the device. The normal situation, in which interstitial and blood compartment are separated, needs to be restored before actual subcutaneous glucose concentrations can be measured. The time needed for resolution of altered capillary permeability differs with the technique used and is influenced by the size of the insertion needle and the size of the implantation site. The hypothesis of disruption of capillaries and small blood vessels just after insertion is confirmed by the decrease in sample volume when resolution of altered capillary permeability is completed.

In the studies of Fischer et al. (12,13) maximum indwelling time of a wick was 60 min for the kinetic study and only 20 min for measurements to follow the pattern of tissue glucose after induced alterations. In principle, the

Table 1—***Glucose concentration in the subcutaneous extracellular space***

	n	GLUCOSE VALUE (%)*
GLUCOSE SENSOR	13	44 ± 8
FILTRATE	7	46 ± 9
500,000 M_r		51 ± 11†
30,000 M_r	6	44 ± 5
		45 ± 10†
EQUILIBRATE		
SALINE	5	41 ± 10
5 MM GLUCOSE	4	50 ± 13

Data are means ± SD.
*Glucose value is the percentage of the concomitant venous blood glucose value.
†Clamp level.

wick method should give results consistent with the filtration or dialysis technique. Therefore, implanting the wicks for several hours could be instructive. Our results suggest that 20 min of indwelling time might be too short to equilibrate with real interstitial glucose concentrations.

Simultaneous measurements with our glucose sensor, using the microdialysis system, did not always show consistent high values at the start of a test. The time needed to reach a constant signal was within 30–45 min. Resolution of altered capillary permeability also should be completed within this time to save the hypothesis that subcutaneous glucose values equal to blood glucose values are measured only if capillary membranes are damaged. Incidentally, we observed a sudden rise in sensor signal if movement of the sensor resulted in bleeding at the site of implantation during an test. When the bleeding stopped, the signal decreased to its initial level.

Until now the most probable mechanism to explain the low glucose values measured with sensors was a rate-limiting diffusion step in the extracellular space: Sensors are consuming glucose, so a transport of glucose must occur into the direction of the sensor. This transport could be limited because little convection occurs in the extracellular space compared with the blood compartment. With this mechanism, we could assume the extracellular glucose concentration is equal to the blood glucose concentration, in agreement with the results of Fischer et al (12,13).

The measurements of the glucose concentration in the equilibration study are based on passive diffusion of glucose in and out of the equilibration fibers. Because we used no suction or other mechanical technique, the glucose concentrations measured in the equilibrate are believed to be true interstitial concentrations. In the filtration study, interstitial fluid itself was extracted so diffusion did not interfere. The real glucose concentration in the extracellular space

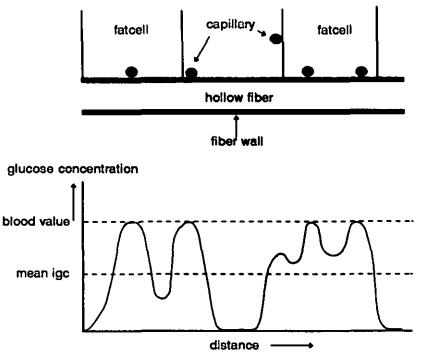

Figure 9—*Anatomical model of the subcutaneous tissue; mean IGC = mean interstitial glucose concentration.*

of fatty tissue may be as low as the sensors are measuring.

Lönnroth et al. (11) found a 20% recovery of glucose in vivo for their dialysis probes. The in vitro recovery was 44%. Thus, if the in vitro calibration factor is applied, they actually measured 45% of the blood glucose concentration subcutaneously, which is consistent with our results. If a solution was perfused with a glucose concentration that equalled the venous blood glucose concentration, however, they found no net influx or efflux of glucose across the fiber membrane. This result strongly suggests an equal concentration of glucose in blood and subcutaneous interstitial fluid, in contrast with the results of our study. This phenomenon should be explained.

The volume of interstitial fluid between fat cells is very small. Each fat cell is surrounded by small capillaries. As the cells consume glucose, concentration gradients should develop in such a narrow space. The surface of a measuring device positioned in this tissue is surrounded by fat cells and capillaries and measures the mean value of all concentration gradients present around its surface. Figure 9 is a schematic drawing of fat cells and capillaries. This anatomical model of the subcutaneous tissue suggests local differences in glucose concentrations along the surface of wick and hollow fiber.

The model also may provide an explanation for the so-called equilibrium glucose concentration and the mechanism for the change in recovery as a function of the concentration gradient across the dialysis membrane, as found by Lönnroth et al. (11). If glucose is added to the perfusion solution in a concentration almost equal to the venous blood concentration, no net influx or efflux of glucose occurs between the hollow fiber and the capillaries. The small volume of hardly convecting interstitial fluid around the fiber is easily equilibrated with the perfusion medium. Little glucose is consumed by the fat cells located close to the hollow fiber. Thus, the venous glucose concentration is approached quite closely in the interstitial fluid around the hollow fiber.

Figure 9 shows a possible concentration profile along the wall of a hollow fiber if the fiber is perfused with isotonic saline. The capillaries deliver glucose to the interstitial space. The fat cells consume only glucose, leading to local differences in glucose concentration.

If a certain amount of glucose is dissolved in the perfusion solution, a different concentration profile develops. The interstitial fluid equilibrates with the perfusion medium, leaving only the areas close to capillaries to exchange glucose with the fiber. The net change in the perfusion medium in vivo is accordingly lower than in vitro, because only part of the fiber surface is available for net exchange. Only when small fibers are used (such as in our sensor) is the net transport of glucose small enough to maintain the real glucose concentration profile in the interstitial fluid through convection. Therefore, the sensor actually measures the real average subcutaneous glucose concentration.

The local distribution of glucose in subcutaneous tissue might be affected by parameters such as heat, insulin, changing blood flow, or long-term high blood glucose concentrations in diabetic patients. With the glucose sensor as a

reliable tool for measuring subcutaneous extracellular glucose concentration, these effects can be investigated.

Furthermore, the knowledge about the actual situation in the subcutaneous extracellular space is important for the possible application of a subcutaneous glucose sensor, integrated in an artificial pancreas for the treatment of type I diabetes.

For now, the major part of the conflicting evidence regarding the subcutaneous glucose concentration can be clarified using the resolution of altered capillary permeability hypothesis and the anatomical model presented in this study.

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