Continuous metabolic monitoring techniques

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Chapter 3

Dynamic monitoring of glucose and lactate in the subcutis with ultraslow microdialysis

Abstract

Introduction: Subcutaneously implanted glucose sensors have been proposed for diabetes monitoring. We determined how close subcutaneous levels of glucose follow blood glucose and whether high subcutaneous lactate points to anaerobic metabolism by implantation damage at 2 subsequent days. We used slow microdialysis and a novel sensor method, to monitor glucose and lactate.
Methods: We placed microdialysis probes in the abdominal fat and applied low perfusion rates, resulting in similar concentrations of lactate and glucose in the perfusate and subcutaneous space (100% recovery). The perfusate was analyzed every 2 minutes with a biosensor. Venous glucose levels were estimated from perfusate levels and a single venous glucose assay. Oral glucose tolerance tests (OGTT) were conducted in 11 healthy volunteers and repeated the day after probe insertion.
Results: Subcutaneous glucose levels were lower than venous levels (1.47 ± 1.20 mM) at steady state and the average delay of the increase following OGTT was 7.3 ± 1.2 minutes. Lactate levels remained low in most experiments. The accuracy of the venous glucose estimates was moderate (0.85mM), both with a steady state and a non-steady state blood sample.
Conclusions: The subcutis must be considered as a kinetic compartment distinct from the vascular compartment. Subcutaneous glucose can be related to blood glucose by taking into consideration time-delay and local glucose consumption. The lactate levels indicated little implantation damage. Subcutaneous factors should be further analyzed, in particular after long-term implantation of a dialysis probe, to yield a method for accurate estimations of diabetes monitoring devices.

Abbreviations: SMBG: self monitoring of blood glucose, MD: Microdialysis, usMD: ultraslow microdialysis, OGTT: oral glucose tolerance test, sc: subcutaneous(-ly), iv: intravenous(-ly)

Introduction

Diabetes treatment goals are the prevention of acute and long-term complications [(1)]. These goals are approached by (near) normalising blood glucose levels[(2)]. Therefore current treatment is based on self monitoring of blood glucose (SMBG). SMBG has improved therapy considerably, leaving only three major drawbacks. First, a hypoglycaemic episode can not easily be foreseen because of the discontinuous nature of SMBG. Secondly, severe nocturnal or other unrecognised hypoglycaemia's interfere with attempts to normalise high glucose levels. Thirdly, only 33-50% of diabetic patients complies to perform daily SMBG[(3;4)]. Thus diabetes management will improve by the introduction of a continuous, patient friendly hypoglycaemia alerting device.

Several devices for continuous glucose monitoring have been proposed. Intravenous (iv) devices face thrombotic and infectious risks[(5)]. Alternatively, sensors placed in the abdominal subcutis carry little risk, and are easy to handle by the patient. Needle-type glucose electrodes have been applied subcutaneously (sc)[(6-8)], but suffer from a decreased signal and drift once implanted. Microdialysis (MD) could serve as an interface between a sensor and tissue. This technique obtains a relatively clean matrix for analysis because the tubular membrane is impermeable for proteins[(9;10)]. Conventional microdialysis has two major limitations. First, interstitial glucose diffusing into the probe is diluted with an unknown factor, prompting to apply lengthy calibration procedures in steady state to estimate effective interstitial levels[(11)]. Second, time-resolution is poor, because samples are most often collected for 15 minutes or longer; questioning timeliness of a sensor. The calibration procedures are clinically impractical, because non-steady-state conditions prevail in patients. Because of these limitations, several kinetic issues remain unresolved. The effective sc tissue levels are e.g. estimated between 44 and 92% of blood[(12-19)], and are possibly drifting[(19)]. Also, the distribution of glucose from blood to the subcutis appears to be delayed[(14;18)]. A possible explanation of low sc glucose could be enhanced anaerobic metabolism due to the implantation damage of the probe. If this would be the case, low sc glucose levels may be accompanied by high lactate levels.

To overcome most of the pitfalls of the studies with conventional microdialysis, we have developed a method of ultraslow microdialysis (usMD) for subcutaneous glucose and lactate monitoring. In animal studies we have shown that usMD at flow rates below 100nl/minute gives a (near) 100% recovery thus avoiding time consuming recovery calibration procedures[(20)]. We combined

usMD with a sensitive on-line detection technique of glucose and lactate, thus making our set-up suitable for fast kinetic analysis[18;21].
In the present investigation, sc glucose and lactate were monitored at steady state and during an oral glucose tolerance test (OGTT) in the abdominal subcutis of 11 healthy volunteers to measure the effective steady state and time delay. The OGTT was repeated on the day after implantation of the dialysis probe to determine short-term stability. We compared computations to estimate reference blood levels accurately, using sc levels with consideration for sc glucose consumption and time-delay. Possible clinical implications of the results are discussed with special regard for timely hypoglycemia alerting.

Methods

Oral Glucose Tolerance Test
Eighteen OGTT experiments were performed after informed consent of eleven young adult males and females with a blank history for diabetes (OGTTs in healthy volunteers as described previously[18]) . In seven subjects the OGTT was repeated on the day after insertion of the probe. Following an overnight fast, subjects sat in an easy chair from 8 a.m. till 1 p.m. For usMD was a CMA 60 microdialysis probe placed sc near the umbilicus at least 45 minutes before the start of the OGTT. The glucose and lactate concentrations were measured every two minutes with the sc usMD and in blood samples taken from a forearm vein catheter every 5 to 15 minutes. 100 g glucose dissolved in 200 ml water was ingested at zero time.

Ultraslow Microdialysis
Ultraslow Microdialysis was performed as in animal experiments published previously[20]. A CMA 60 probe consisting of a 3.0 cm long hollow fibre (Polyamide, 620 µm OD, MWCO 20 kD, CMA Microdialysis, Stockholm, Sweden) was filled up with 0.9% saline after insertion. During the experiments, the probes were connected to the system for glucose and lactate detection. The probe outlet tube was glued to a draining fused silica capillary restriction(75cm l, 150µm OD, 35µm ID, Polymicro Technologies inc., Phoenix, AZ, USA). The microdialysis flow rate was generated by the underpressure of a syringe with a fixed piston[22]. The microdialysis flow rate was on average 42 nl/min (range 30-59nl/min) as measured by weight. There was no correlation between the usMD flow rate and the sc steady-state levels ($r = 0.14$). The instrumental lag-time (time to 90% signal change) was on average 53 minutes, due to the dead

volume in wide outlet tube of the probe. The long instrumental lag-time in this set-up may simply be reduced by appropriately shortening and narrowing the tubes connecting to a wearable sensor.

Glucose and lactate analysis

We used a flow injection analysis system to detect glucose and lactate in the same usMD sample as described previously[18;21]. The system injected every 2 minutes 20nl usMD sample using an internal loop.

Two standard glucose/lactate solutions were measured before each experiment, and in vitro calibration curves were made afterwards with solutions containing 0, 1, 2, 4, 6 mM lactate and 0, 2, 4, 8, 12 mM glucose, changed stepwise every 20 minutes. The amperometric signal was linear for these lactate and glucose ranges (both $r > 0.99$, $p < 0.0001$). The 10 % to 90% spreading in the analytical system was 32±5 s (signal change from 10 to 90% after a stepwise concentration change). The spreading (10 to 90%) for the probe and analytical system together was 3-5 minutes, due to mixture in the (wide) probe tubing.

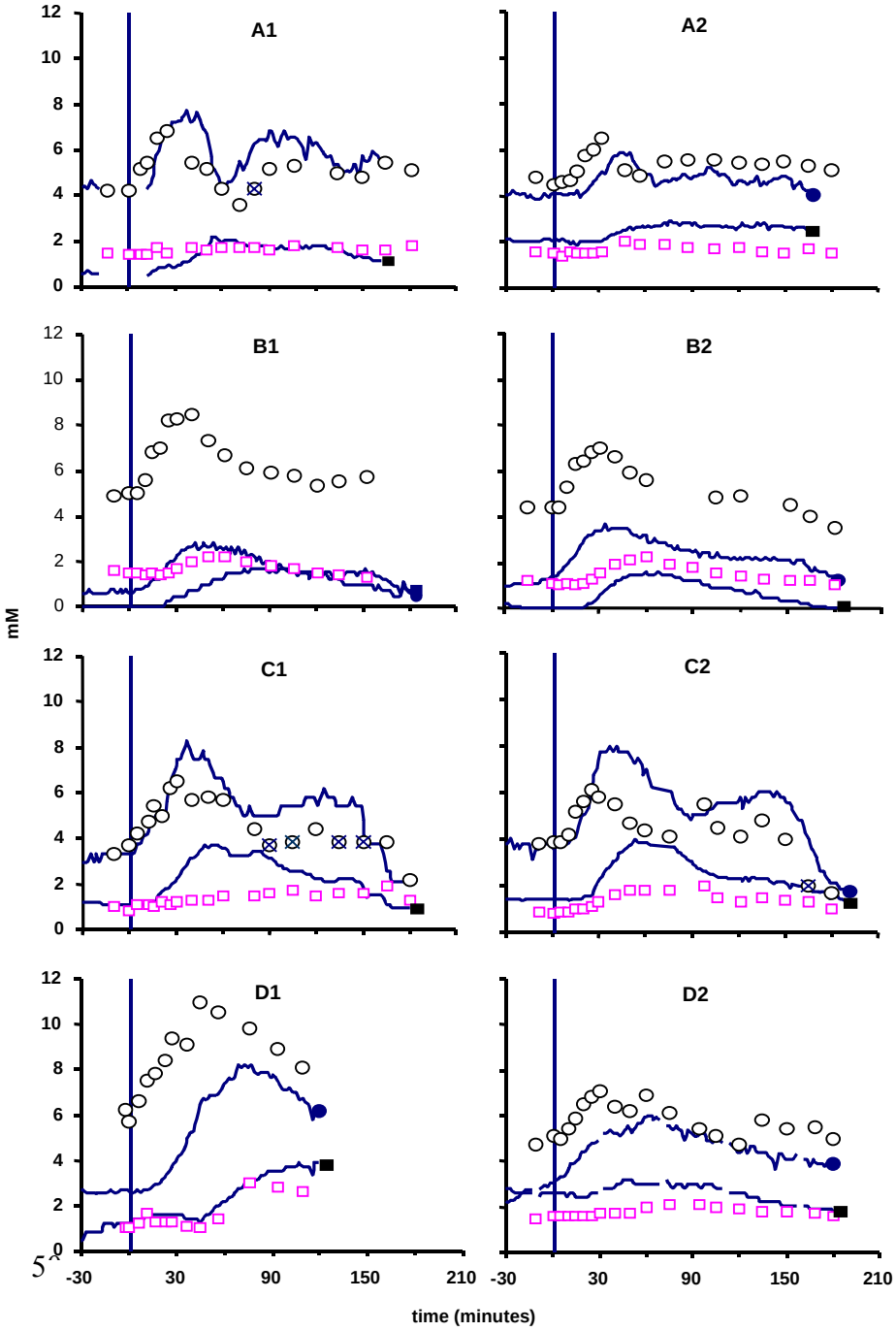
Venous sample concentrations of glucose and lactate were measured with a Vitros 750(manufacturer: Ortho-Clinical Diagnostics, Illkirch Cedex, France). Glucose molarity (mol/l blood) of whole blood is different from the molality (mol/kg water) as about 80% of the blood volume consists of water[23]. The molality of glucose is the same in whole blood, blood plasma, and the extracellular fluid of blood. In order to unite with clinical practice of molarity in full blood, we adjusted UF concentrations by -15% to make a correct comparison with full blood (in accordance with the manufacturer's report). Lactate was determined in blood plasma. No correction for plasma protein volume was done, because no corrective factor is known.

Data analysis and statistics

Glucose and lactate steady-state levels were the average ± SD of the first three iv samples (t=-15,0,5 min) and the concurrent sc levels. Iv and sc steady-state levels were compared with a paired t-test because the values appeared to have a normal distribution. A 95% confidence interval for averages (95% c.i.) was also calculated. The stability of the iv-sc steady-state differences was assessed with a paired t-test between day one and two. The relation between sc steady-state lactate concentrations and steady-state glucose iv-sc differences was tested with linear regression analysis. The iv-sc delay time was defined as the time difference of iv and sc glucose to reach 50% of the maximal glucose values following OGTT.

Computations were made to determine how accurate venous glucose levels may

be estimated from sc sensing. For these computations, we made use of only one blood sample, because we feel that a need for more than one would annihilate the clinical advantage of a sc sensor over SMBG. For the calculation of estimates we had 3 subsequent approaches. Firstly, we continued the difference at steady state,



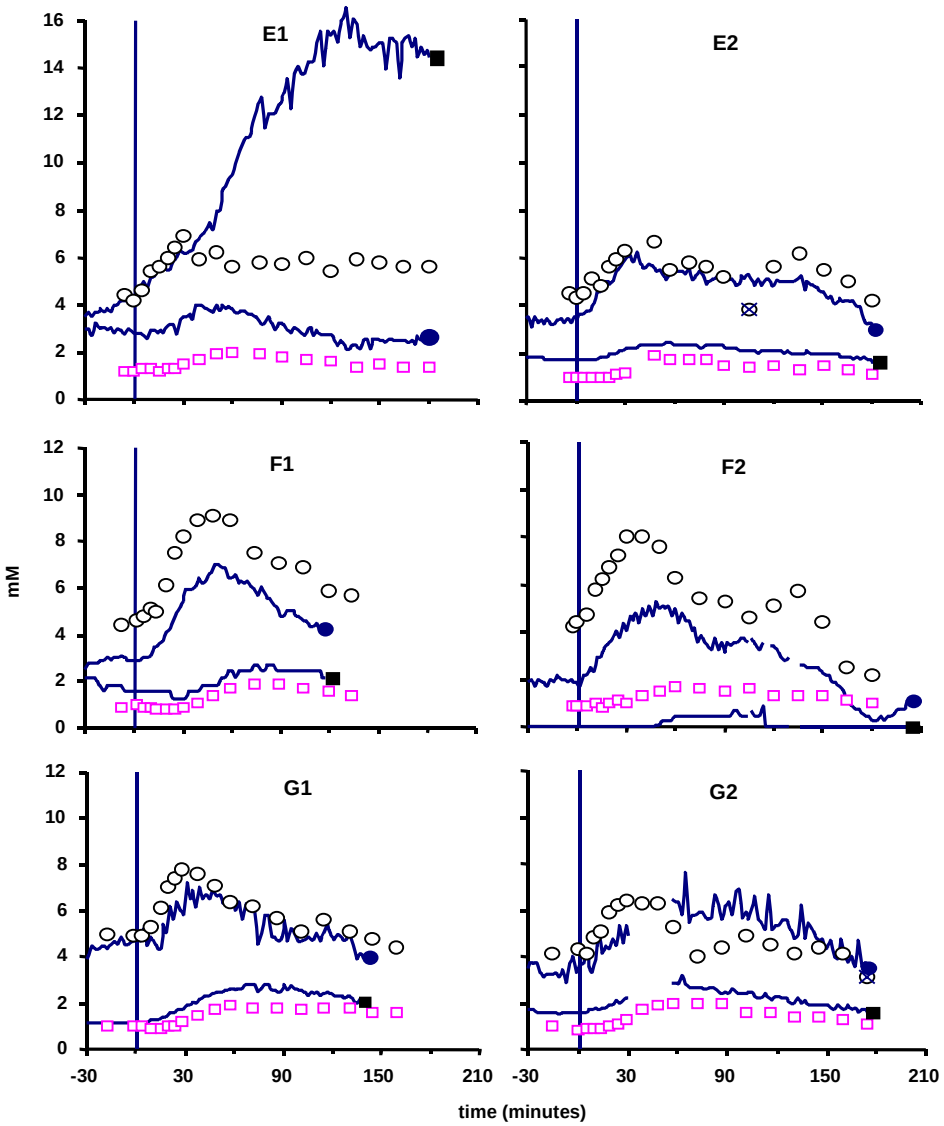
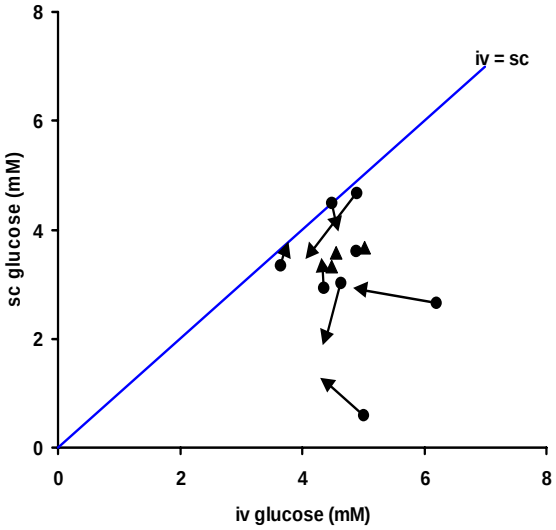


Fig. 1. Sc and iv glucose and lactate measurements during an OGTT in 7 healthy volunteers (A/G) on two successive days (day one, left; day two, right); start OGTT at zero time (|)
○ = iv glucose, top line —● = sc glucose; (in E1 is sc lactate the top line)
□ = iv lactate, bottom line —■ = sc lactate; (in E1 is sc lactate the top line)
⊗ = unacceptable iv glucose concentration estimations in the error grid analysis (iv estimations based on the sc concentrations with addition of the iv-sc difference at zero time)

so we added the difference between the sc and iv glucose concentrations at time zero to all sc values. Secondly, we made estimations departing from non-steady state, so we added the sc-iv difference at t=30 minutes. In a third approach, we calculated the best estimations possible by choosing freely a difference or rate with a time-shift delivering optimal accuracy. To evaluate the stability of the sc space 24 hours after implantation, we continued to use the t=0 and t=30 min. calculation factors of day one for the estimations on day two.
The accuracy of the estimations compared to the measured iv glucose levels was calculated with the method of residuals[24]. In this method is the accuracy the mean absolute difference between estimate and reference, and the precision is the standard deviation. We evaluated the clinical implications in an error-grid as proposed by Clarke et al.[25]. Here, estimations which fall in zone A of this grid are accurate, those in zone B are acceptable, and those in zone C, D, and E are unacceptable because the differences between reference and estimate would lead to dangerous treatment decisions. We also evaluated qualitatively the course in time through the acceptable and unacceptable zones, taking advantage of the high frequency of the sc measurements.

Results

In seven of the eleven volunteers a complete set of data was obtained. Of four persons only data of one day was collected, mainly because air bubbles in the probe blocked the downstream analysis system. This problem occurred in the first experiments only and was resolved in the course of the experiments. One volunteer appeared to have an Hb of 6.5 mM (normal 7.5-9.9 mM) (subject D in fig.1) and another had a high OGTT response 9.48 mM venous glucose was achieved after two hours (graph not shown). Both were informed and referred to their general practitioner. We evaluated the data of these three experiments as the other data.
The sc glucose concentrations in steady state versus iv levels at subsequent days showed no correlation (see fig. 2). Sc concentrations were lower than iv levels on both day one (●) and on day two (▲). We found 4.62 ± 0.53 mM iv and 3.15 ± 1.01 mM sc (mean ± SD). The iv-sc difference was 1.47±1.20 mM (95% c.i. 0.88 to 2.07 mM in a paired t-test). Expressed as a percentage sc levels were 68.8±21.9 % of iv levels. The differences were on average 1.62±1.66mM on day one and 1.35±1.10 mM on day two. The change in difference from day one to day two is -0.59 to 1.07 (95% c.i. in a paired t-test)(paired days indicated in figure 2 as arrows ●—►). In case the iv-sc



difference was small, only little

Fig. 2. Sc versus iv steady-state glucose concentrations.
● = glucose concentrations on day one
▲ = glucose concentrations on day two
●—► = day 1 and 2 measurements with same probe/person
⁄ = equality of concentrations line (iv=sc)

change in this difference was observed. Large differences were accompanied by rather big changes (n was too small for testing the significance of this trend).
Figure 1 shows the results of the seven subjects who completed the study.
The delay-time between the 50% OGTT glucose rise sc and iv was 7.3 ± 1.2 minutes (mean ± SEM). The sc curves appear to be flattened, as compared with the iv curves.
Average steady-state lactate concentrations were 1.18 mM sc, and 1.43 mM iv (95% c.i. of difference: -0.77 to 0.27mM). Again, sc-steady-state-lactate concentrations showed no correlation with steady-state-glucose-iv-sc differences (r=0.26). In most cases, lactate rose in parallel to glucose. The sc lactate concentration was well above the iv level in two volunteers on day 1 (see fig. E1, graph of second volunteer not shown).
Table 1 summarises the results of different computations reflected in the

accuracy reached and the clinical implications of the error grid analysis. This analysis is shown in figure 3 for experiment B1. The raw data, the estimates after calculation with the difference at t=0, the estimates after optimal difference and time-shift recalculation and the estimates after optimal rate and time-shift recalculation are plotted here in the error grid.

Estimates using the t=0 (steady state) difference yielded an overall accuracy and precision of 0.85 mM and 0.62 mM respectively for the 18 OGTTs. Error-grid analysis yielded 73.1% of estimates in zone A (accurate), 23.7% in zone B (acceptable), and 2.9% in zone D (unacceptable). The values in zone D are indicated with a ⊗ in figure 1.

Estimates based on the t=30 min. (non-steady state) difference yielded an accuracy and precision of 0.98 mM and 0.67 mM respectively. Error-grid analysis yielded 63.6% of estimates in zone A, 33.4% in zone B, and 2.6% in zone D.

Estimates made on the second day using the t=0 difference of the first day (testing stability of the sc-iv relation) yielded an accuracy and precision of 1.15 mM and 0.63 mM respectively. Error-grid analysis yielded 54.8% of estimates in zone A, 42.1% in zone B, and 3.2% in zone D.

Table 1. Accuracy of iv glucose concentration estimates from sc measurements analysed by the method of residuals and by the error grid method
(table on two opposite pages)

Computation method of estimates	**Analysis by the method of residuals**		
	Accuracy (mean absolute difference in mM)	Precision (SD absolute difference in mM)	Correlation
Crude sc concentration	1.73	0.65	0.67
With addition of the steady state difference of T=0 min.	0.85	0.62	0.67
With addition of non-steady state difference of T=30 min.	0.98	0.67	0.67
With addition of T=0 difference of day 1 on second day	1.15	0.63	0.64
With addition of T=30 difference of day 1 on second day	2.12	0.61	0.64
With optimal delay and addition	0.42	0.37	0.84

With optimal delay and rate	0.47	0.40	0.84

The accuracy of the estimations could be optimised to 0.42 mM by choosing an iv-sc difference of 1.20 mM mean with a time-shift of 8.7 min. mean. Error-grid analysis yielded 93.2% of estimates in zone A, 5.0% in zone B, and 1.8% in zone D.
The accuracy of the estimations could be optimised as well (to 0.47 mM) by choosing a sc-iv rate of 1.20 mean combined with a time shift of 10.8 min. mean. Error-grid analysis yielded 91.0% of estimates in zone A, 7.6% in zone B, and 1.4% in zone D.

Conclusions

The major observations of the present study are a variably lower sc steady-state glucose level and a short time-delay as well as flattening of the sc OGTT curve, as compared to iv levels. These lower sc levels are only exceptionally

during 18 OGTTs in healthy volunteers; different iv estimate computations

		Error grid analysis				
Mean added difference (mM)	Mean added delay (min.)	Zone A (%)	Zone B (%)	Zone D (%)	Reference <3.88mM (%)	Estimates (n)
		40.6	57.1	2.3	5.5	308
1.39		73.1	23.7	2.9	5.5	308
1.94		63.6	33.4	2.6	5.5	308
1.58		54.8	42.1	3.2	6.3	126
2.72		33.3	63.5	3.2	6.3	126
1.20	8.7	93.2	5.0	1.8	5.0	280
1.20	10.8	91.0	7.6	1.4	5.1	277

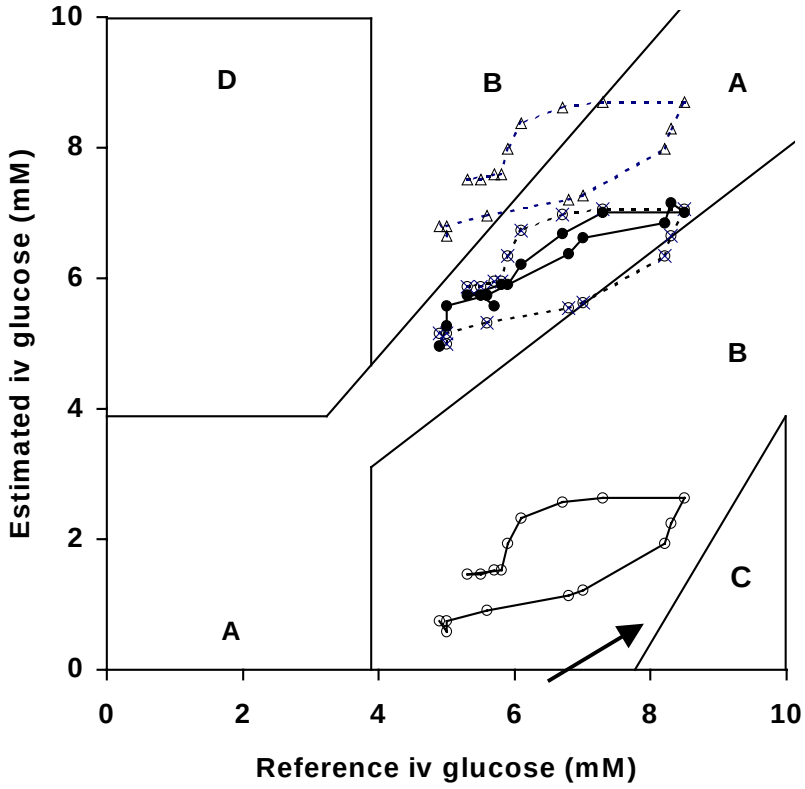


Fig. 3. Error grid analysis of the iv glucose estimations from OGTT experiment B1 in figure 1. Crude, steady-state, non-steady-state, and optimal iv glucose estimations based on the measured sc glucose concentrations are plotted against the reference iv glucose concentrations.
○ = crude sc concentrations (● is on t=0min. and on t=30min.),→ = OGTT course
⊗ = sc concentrations+4.41mM = iv estimations based on addition of the iv-sc difference at t=0 minutes, so at steady state.
Δ = sc concentrations+6.05mM = iv estimations based on addition of the iv-sc difference at t=30 minutes, so at non-steady state.
● = sc concentrations of 11 minutes later+4.36mM = optimal iv estimations based on sc time delay and addition
Error grid zones: zone A, accurate estimates; zone B, acceptable estimates; zones C and D, unacceptable iv estimates because the differences between

reference and estimate would lead to dangerous treatment decisions. Zone E is an unacceptable zone out of range of this graph.

accompanied by high sc lactate levels. Clinical accuracy of iv estimates is moderate taking into account only the difference between sc and a single blood sample at steady-state or at non-steady-state condition. The accuracy decreases on day two. By adding a time-delay sc monitoring may reach a clinically acceptable accuracy. Lowered, delayed, and flattened sc glucose levels as compared to blood levels found are in accordance with the findings of most previous investigations[12;13;16-19;26;27], and points to local glucose metabolism. The lactate levels found suggest that local anaerobic metabolism is usually not the major cause of low glucose levels in tissue around a probe. So lactate levels indicated little implantation damage.

The observed iv-sc time-delay is in accordance with values found in tissue diffusion experiments[28] and is not likely to pose a problem for the timeliness of a sc sensor hypoglycaemia warning because the development of hypoglycaemia takes most often more than 30 minutes. Here, the time-delay may (partly) be caused by the difference between iv and intra-arterial glucose levels, as seems the case e.g. in experiments A1, C1 and C2 where sc glucose levels were temporally higher than iv. A temporally higher arterial glucose concentration during OGTT has been well documented[29;30]. So, the sc glucose concentrations may thus be more closely related to arterial levels than to iv levels, at least in these cases. This consideration emphasises the relevance of the sc glucose levels found lower than iv levels in this study. Our results and the observations of others indicate that abdominal subcutis must be considered as a kinetic compartment distinct from the vascular compartment.

We tested the accuracy of some simply computed iv estimations. Firstly, the estimations were calculated from the sc levels and a single concurrent blood concentration at steady state and non-steady state. These estimations reveal only moderate accuracy, essentially because of the neglected time-delay and the neglected flattening of sc kinetics. Secondly, a recalculation was made, taking into account a time-delay and difference. Now the accuracy is near to clinical acceptance. A close look at the sc curves in fig. 1 reveals that most estimates in zone D (indicated with *) may be corrected by taking into account a time-delay and/or by the use of arterial glucose levels as a reference rather than iv levels. Unfortunately, accuracy diminishes after one day. It is thus clear that the here proposed methodology has to be applied after long-term implantation. Nevertheless, subcutaneous glucose can be related to blood glucose by taking into consideration time-delay and local glucose consumption.

In conclusion, there is perspective on clinical application of a hypoglycaemia

alerting device, utilising the now available usMD or ultrafiltration[18] techniques which give the sole opportunity to compare absolute tissue concentrations directly to blood levels with a high time-resolution. In clinical practice, we recommend to allow for sc delay and flattening when estimating iv glucose concentrations with a sc sensor. We also propose to control the calculation factor with a blood sample on day two, despite the increase of patient burden.

The remaining limitations of glucose sensing are related to the drift of local tissue kinetic factors in time. Implantation for weeks of sc MD probes is already possible without problems[19].

Future research should concern the reliability of the present approach in ultrafiltration or usMD probes implanted for a long period of time. Further, it should include diabetic patients, because their glucose tissue diffusion can differ from that of healthy subjects[28]. Such investigation may aim at a sc kinetic model, an in vivo parallel measurement of kinetic factors for continuous recalculation or a favourite anatomical place for probes. All this can lead to more accurate diabetes monitoring devices. In addition, tissue levels may even replace blood levels in future as the reference for diabetes management, because tissue levels comprise more specific metabolic information.

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