Continuous metabolic monitoring techniques

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Chapter 8

Summary and final comments

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General introduction

The aim of this thesis was to develop and test devices for the continuous in vivo sampling and sensing of glucose and lactate, in clinical monitoring. Currently, glucose levels are not able to be constantly monitored and are therefore not well controlled by patients with diabetes mellitus. Glucose levels vary considerably, provoking serious short and long term complications. Instable cardiac and other emergency patients are at risk of sudden deterioration (as a result of tissue ischemia), which is difficult to diagnose in time for adequate treatment. Ischemia is often accompanied by an increased lactate release from the tissue in jeopardy. To allow for early diagnosis, glucose and lactate should be monitored continuously and automatically in these patients, instead of the current practice which requires frequent blood sampling and off-line analysis.
The introduction of glucose sensors in the clinic has been delayed by poorly understood differences between measurements of glucose in subcutaneous interstitial fluid and blood. Glucose sampling and sensing devices proposed thus far, have been shown to measure reliably in vitro, but, when applied subcutaneously in man, have a low level of accuracy in estimating blood levels. Also, to date there is neither a lactate sensor nor a cardiac lactate monitoring device available for patient use. The differences found between glucose levels in the subcutis and blood, necessitate to calibrate frequently with blood samples. This has precluded the clinical application of glucose sensors. These difficulties have arisen from technical shortcomings, physiological metabolism around the device, or disturbances caused by device-tissue interactions.
In the experiments described in this thesis, equilibrated sampling and sensing techniques have been developed, thus avoiding frequent in vivo calibration. These techniques have been applied to test several hypotheses on device-tissue interactions, as well as metabolism of glucose in the subcutis, and lactate in the myocardium, in order to improve the application of sensing devices for clinical monitoring.

Glucose and lactate ultrafiltration

In **chapter 2**, a newly developed ultrafiltration hollow fibre probe (length 2.5 cm, diameter 340 µm) was inserted into the abdominal subcutis in man to sample interstitial fluid at a continuous flow rate of about 47nl/min. An on-line bedside flow injection system was developed to measure glucose and lactate simultaneously once a minute using 20 nanolitre from the sample flow. This

system contained a splitter in front of parallel enzymatic conversion cells (with glucoseoxidase, lactate oxidase and horse-radish peroxidase enzymes) and electric detection cells (amperometry). The potential for monitoring using this sampling probe was tested, and the subcutaneous and blood glucose concentrations were compared for equality (zero hypothesis). Six healthy volunteers ingested 100g glucose in the fasting state and continuous equilibrated interstitial sampling with ultrafiltration was used for monitoring glucose and lactate. Glucose concentrations in the abdominal adipose subcutis were 1.06 mM (95% confidence interval 0.127-1.98) lower than in venous blood in the fasting state. The maximum glucose concentration was reached in the subcutis between one and thirty minutes later than in blood.
The finding that subcutaneous glucose levels were variable and significantly lower than in blood samples suggests that the abdominal subcutaneous adipose tissue is a kinetic compartment not directly linked to blood.

Monitoring with ultraslow microdialysis

In **chapter 3**, microdialysis (hollow fibre probe length 3.0 cm, diameter 620 µm) was applied at a continuous ultraslow flow rate of 42 nl/min to sample fluid in equilibrium with the abdominal subcutaneous adipose tissue in man. The occurrence of a shift to anaerobic metabolism in the subcutis due to insertion damage and subsequent restoration, was explored. Glucose and lactate levels were compared at probe implantation, and followed during two oral glucose tolerance tests (100 g) on two subsequent days in seven healthy subjects. Venous glucose levels were estimated using dialysate levels and a single venous glucose assay. The accuracy of this method was evaluated.
The results of this experiment were found to be similar to the ultrafiltration experiment (see chapter 2). Fasting subcutaneous glucose levels were often lower than venous levels (1.47 ± 1.20 mM) and there was a delay of 7.3 ± 1.2 minutes between venous and subcutaneous Cmax. In some subjects, the subcutaneous glucose levels were almost equal to venous levels before the glucose tolerance test, higher during the test, and again almost equal at the end. In these subjects, subcutaneous glucose levels appeared to be close to arterial levels. The low glucose levels found, were only occasionally accompanied by elevated lactate levels, which were generally still low. Shifts to anaerobic metabolism were generally not found, suggesting that this mechanism does not explain the glucose level differences found between blood and subcutaneous adipose tissue. The degree of accuracy of estimating venous glucose levels was

moderate (0.85 mM), using blood samples both from the fasting and from the non-fasting condition. The accuracy of the estimates decreased on day two after implantation.
This experiment confirms the results of the previous chapter, showing that subcutaneous adipose tissue must be considered as a kinetic compartment distinct from the vascular compartment. Adipose tissue glucose appears to be related to blood glucose in such a complex way that estimation of blood glucose using simple mathematical methods does not give sufficient accuracy for clinical application.

Monitoring myocardial ischemia

Chapter 4 describes the development of a cardiac catheter, as well as several tests to monitor lactate and glucose in the venous efflux of the myocardium, and the exploration of its diagnostic potential. A hollow fibre (length 4 cm) was built into the tip of a cardiac catheter (length 120 cm, diameter 1.78 mm) for continuous blood ultrafiltration in the coronary sinus of swines. The on-line flow-injection system outside the body detected lactate and glucose every minute with an in vivo response time of 1.33 ± 0.61 minutes (10-90%) and a lag-time of approximately 24 minutes. For a total of 27 hours the swine were monitored. The linear regression in vivo of blood and ultrafiltrate samples was 0.977 for lactate and 0.994 for glucose. Lactate levels rose 0.38 ± 0.10 mM above baseline within five minutes after the start of ischemia by obstruction of the left anterior descending coronary artery. After 5, 15 or 45 minutes obstruction, reperfusion was promptly detected as a lactate peak with the same shape as an intravenous injection (highest level 9.27 $mmol \cdot l^{-1}$). Myocardial stress induced by dobutamine infusion increased glucose but not lactate levels. Once, a wall effect, a well known technical problem with intravascular sensors, occured at the catheter tip disturbing the measurements.
The intravascular ultrafiltration catheter has demonstrated semi-continuous glucose-lactate monitoring of an organ with good accuracy. It appears feasible to detect myocardial ischemia and reperfusion almost instantly through a lactate level rise. The concentration of lactate at the time of reperfusion of the myocardium and the area under the curve can be estimated quantitatively (max. 14.82 $mmol \cdot l^{-1}$ and 3.77 $mmol \cdot l^{-1} \cdot h$ in this experiment).

Implantation of micro-biosensors

In **chapter 5,** alternative sites for glucose and lactate monitoring device implantation were explored to find a closer contact to the circulation than the subcutaneous adipose tissue. Twenty four thin-film amperometric lactate-glucose sensors (film width: 0.7 mm) were inserted in adipose tissue, striated muscle, and loose connective tissue of swines. Sensor baselines and response times were assessed in response to an intravenous lactate plus glucose bolus injection. Baselines and responses were found generally close to zero in adipose and muscle tissue. In connective tissue, lactate and glucose baseline levels were 80.5±19.1% and 90.9±15.7% of blood; and 95% of the response was reached after 5.5±2.1 and 17.1±8.5 minutes respectively. The intravenous bolus injection of the sensor's analyte was found to be a quick way to check whether a newly implanted sensor is in close kinetic contact with blood. The results suggest that a probe in subcutaneous loose connective tissue is in better contact with the circulation than in the commonly used adipose tissue.

Glucose gradients in subcutis

In **chapter 6**, glucose measurements between arteriolized blood, abdominal superficial subcutis (predominantly adipose tissue), and abdominal deep subcutis (predominantly loose connective tissue) were compared during an oral glucose tolerance test in man. Blood insulin levels, body mass index and skinfold thickness were compared with the tissue glucose levels as well. A newly developed nanolitre size glucose sensor was connected to an ultraslow microdialysis probe. Fasting glucose levels were 2.15±0.77 mM lower in adipose tissue than in blood and this difference increased to 5.19±1.57 at maximal glucose concentration after glucose challenge. In contrast, connective tissue glucose levels did not differ significantly from those in blood (-0.06±0.27 fasting and –0.11±1.35 at maximum after challenge) and correlated well (r^2 = 0.962). Between subjects, blood insulin levels, body mass index and skinfold thickness showed no obvious correlation with the tissue glucose levels. The wearable glucose sensor functioned well when connected to the ultraslow microdialysis probe. The abdominal subcutaneous loose connective tissue appears to be a more attractive option than adipose tissue for studying glucose monitoring in intensive diabetis therapy, because of its close approximation to arterial glucose levels.

Quantitative model of glucose transfer

In **Chapter 7**, kinetic models for subcutaneous glucose were tested to find which factors contribute, and to what extent, to subcutaneous glucose levels. A secondary aim was to evaluate the possibility of reversing the algorithm found.
The data from the seven subjects in chapter 6 were used to evaluate three models.
Model A is a two compartment model, using the measured arterial glucose as the linear input into the connective tissue compartment. The latter compartment has one linear glucose output. Model B is like A, but modifies the output using the arterial insulin levels. Model C is like B, but adds a third compartment between the arterial and the connective tissue compartment, and also modifies the input using the arterial insulin levels. To identify the best model, first the model parameters have been estimated by weighted non-linear least squares. Second, to make a comparison among the models in terms of parsimony, the Akaike Information Criterion was used. The parameter precision was acceptable (coefficient of variation below 100%) and the fractional standard deviation was estimated to be below 5%. Model B showed the best fit, followed by model C. However, the results also show parameters k3 and k4 (insulin effect) to virtually disappear in some subjects, suggesting a more simple model is then sufficient. The heterogeneity of the parameters found may be a reflection of the mix in the subcutis of insulin dependent adipose tissue and insulin independent connective tissue, as well as the tissue concentrations varying with the distance to the nearest capillaries.
A future reversal of the best model found will need the insulin levels as input for the algorithm. This will be possible, as the previous insulin administration will be remembered by a future artificial pancreas. However, the model presented here needs to be validated with addional data for this purpose.

Conclusions

In conclusion, the studies described in this thesis have shown that ultrafiltration and ultraslow microdialysis can readily be applied in the subcutis in man for monitoring glucose without recovery calculations. In addition, a newly developed intravascular ultrafiltration catheter can be applied in the continuous measurement of lactate from an internal organ. The little invasive sampling probes, connected on-line with bedside flow analysis or wearable nanolitre size

sensors, have shown to measure glucose and lactate accurately, and with a high time resolution in vivo.
Information has been gathered on the relation between subcutaneous and blood glucose levels, important for the clinical application of sensing devices. The application of novel equilibrated sampling techniques and a diffusion limited sensor prevented the drainage of glucose and lactate from the interstitium as is the case in classical microdialysis. These experiments have demonstrated that drainage is not the only reason for subcutis glucose levels to be lower than in blood.

Only a few sampling device-tissue contact disturbances have been observed in the present experiments. These were implantation effects in adipose tissue marked by high lactate levels from anaerobic glucose metabolism which recovered the next day, and an intravascular wall effect in the coronary sinus. A thin-film amperometric sensor has only little electrolytic contact with the interstitial fluid if inserted in adipose or muscle tissue as compared to implantation in loose connective tissue in swine.
Generally, lower interstitial glucose levels appear to be determined by the balance between the supply through diffusion from the blood capillaries and the specific tissue metabolic characteristics. The considerable variability in glucose levels measured locally in the subcutis can be interpreted as reflecting the physiological gradients naturally present in tissue due to the spatial difference between microvascular supply and cellular metabolism.
Glucose levels were found to be closer to arterial levels in deep subcutis layers (predominantly loose connective tissue) than in superficial layers (predominantly adipose tissue). Known tissue differences such as the rate of aerobic and anaerobic metabolism, the degree of insulin dependence of cellular glucose uptake, and the width of the intercellular space may well explain the difference in interstitial glucose levels between these tissues. Estimation of blood glucose levels by simple calculation from subcutaneous adipose tissue glucose is possible, but only with a limited accuracy (see chapter 3). In the attempt to identify an optimal kinetic model for subcutaneous connective tissue glucose estimation, the different model parameters illustrated the heterogeneity of the subcutis regarding glucose supply and metabolism. The subcutaneous measurements with ultraslow microdialysis sampling are, however, so close to arterial levels, that continued clinical research in this area is justified and should be aimed at the optimal positioning in tissue for long-term measurement, to avoid algorithms for estimation.

Future

The equilibrated glucose sampling technique combined with a nanolitre sensor can be combined to form a wearable device. Future subcutaneous glucose sensing may thus become a reality for diabetes patients. Continuing clinical development of lactate sensing may help to detect and quantify ischemic events in cardiac or other emergency patients at an early stage, which would help prevent secondary infarction. Sudden reperfusion of a major coronary artery releases a remarkable lactate peak, which may be recognized even by a less invasive peripheral lactate sensor.

The techniques studied here avoid direct contact of sensor material with the organism, thereby increasing biocompatibility. Application of the small, flexible hollow fibre probe thus appears interesting for long-term monitoring with ultrafiltration and ultraslow microdialysis.