Continuous metabolic monitoring techniques

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Chapter 1

General introduction

1.1 Clinical motivation to monitor metabolites

An interruption of energy supply to organs such as the brain and the heart can develop quickly into a life threatening situation. Myocardial infarction and hypoglycemic coma are two examples of such complications. These complications of atherosclerosis and diabetes mellitus patients differ in many respects, but both share the danger of the energy supply being cut off. Cellular functions can be rapidly disturbed by shortages of glucose in the brain during hypoglycemia, or accumulations of hydrogen ions during myocardial ischemia. The stores of energy in myocardial cells are exhausted within 3 to 4 seconds[(1)]. Transport of metabolites by diffusion through the fluid between cells is limited to short distances (micrometers)[(1)]. For long-distance transport the human body is dependent on blood circulation (fluid convection). Fine tuning of the uptake and release of metabolites in different organs and regulation of circulation is essential to prevent the build up of concentration differences beyond the limits acceptable for life (i.e. homeostasis). Disturbances in this vital proces can be observed often in the transporting body fluids. Already in ancient Greece, Hippocrates recognised a misbalance in the four fluids of the body, blood, phlegm, yellow bile and black bile, as a sign of disease. Much later, in 1679, the observation of sweet tasting urine led physicians to identify patients with the diagnosis diabetes mellitus (from Greek / Latin: pass through / sweet as honey). The sweet taste of urine is from glucose, one of the most important energy substrates in the organism. Normally, glucose is not lost in urine, but taken up by cells and metabolised predominantly with oxygen to carbon dioxide and water (aerobic metabolism). In the absence of oxygen, glucose is metabolised to hydrogen and lactate ions (anaerobic metabolism). Nowadays, physicians attempt to anticipate sudden pathological events by continuously monitoring patients at risk. Body fluids of critical care patients are often monitored continuously for energy metabolism substrates and products such as oxygen, carbon dioxide and hydrogen ions (pO_2, pCO_2 and pH). An arsenal of effective therapies is currently available to treat imminent energy metabolite transport derangements and treatment of acute homeostatic disturbances has proven rewarding provided a timely and accurate diagnosis is made. Apart from acute risks, homeostatic derangements also carry long-term risks, such as the late complications from frequent high glucose levels in diabetes (see paragraph below).

This thesis focuses on development of continuous glucose and lactate monitoring, because such techniques are not yet clinically available, and these metabolites are of major importance for large patient groups as diabetes

mellitus, critical care and cardiovascular patients. These three patient groups will be discussed separately in the next paragraphs, as they have different monitoring needs. The subsequent section 1.2 reviews the current state of the art of continuous glucose and lactate monitoring techniques. Section 1.3 deals with the possible backgrounds of problems arising when these techniques are applied in vivo. The aims and scope of this thesis are presented in section 1.4.

Diabetes mellitus

The number of diabetic patients in the Netherlands, with 15.8 million inhabitants, is estimated to be 450,000. Diabetes mortality amounts to 3,200 out of 136,000 deaths annually, which means diabetes is reported in 2.4 % of the population as the primary direct or indirect cause of death (CBS 1997). Diabetes is a major cause of blindness, end stage renal disease and limb amputations. There is also a 2 to 4 times increased risk of heart disease or stroke, and higher glucose levels are associated with a larger infarction area. Extensive trials have succeeded in lowering elevated blood glucose levels of diabetic patients by intensive treatment (DCCT'93[2-4], UKPDS[5]). In the DCCT trial, a reduction in microvascular complications of over 60% was achieved compared to standard treatment. The UKPDS trial showed improvements in the number of both microvascular and macrovascular complications. However, blood glucose levels were still about 40% above normal limits with the intensive treatment[2-4], and the risk of hypoglycaemia increased threefold, despite an increased frequency of blood glucose self-monitoring. This may be related among other things to the highly variable insulin levels resulting from subcutaneous injections. Therefore the risk of sudden hypoglycaemia between measurements increases with intensive treatment, thus slowing down the motivation to intensify treatment. Tight blood glucose control not only demands intensive support from a health care team, but also calls on the ability and the commitment of the patient to be continually managing his glucose levels. This means a great effort and implies, among other things, a very disciplined life style, and frequent finger pricking for the rest of the patient's life. The motivation for frequent finger pricking is not easy to sustain, possibly because high blood glucose is often not felt by the patient, who has the consequent complications usually only years later. Currently, only 33-50% of diabetic patients performs at least daily self-monitoring of blood glucose (SMBG)[6;7]. The accuracy reached through self monitoring using current devices appears also far from optimal[8;9]. The limitations of the current standard of intensive therapy can be summarised by the fact that glucose levels cannot be controlled completely , there may be long-term complications, and an overly regulated life is imposed on patients.

Improvements can be achieved by a more continuous and automated method of blood glucose monitoring. A continuous or semi-continuous monitoring technique may give a timely signal of hypo- and hyperglycaemic events. Automation may relieve the patient from tasks as finger pricking, blood sample handling (introducing inaccuracy), and, eventually, insulin dosing. The future acceptance and application of new monitoring techniques will depend on the overall advantage compared to the limitations of the existing practice. Before the general introduction of a new glucose monitoring device, this balance has to be made up. The reliability of the proposed techniques needs special attention because both the improvement of glucose control and the liberation from self-monitoring of blood glucose depend on it. The major steps in development and the present state of the art of continuous glucose monitoring techniques will be discussed in paragraph 1.2 (Sampling and sensing devices).

Myocardial ischemia

Mortality from cardiovascular diseases accounted for 50,545 deaths in the Netherlands in 1997 (CBS). Cardiovascular disease is reported as the primary direct or indirect cause in 37% of all deaths. Coronary heart diseases are responsible for the majority of deaths in this group: 26%. Yearly, there are 90,000 hospital admissions for on average 8.1 days for coronary heart disease complications (SIG 1998). The underlying disease is atherosclerosis which narrows the arteries and progressively diminishes the bloodstream, and therefore the supply of energy metabolites to the myocardial tissue. Atherosclerosis can be complicated by plugging of the arterial stricture with thrombus formation, after which myocardial tissue shows gradual changes and eventually dies (acute myocardial infarction). A patient with an acute myocardial infarct currently has a 35-50% chance to die[(10)]. However, acute insulin-glucose infusions have been shown to improve this prognosis in diabetic patients[(11)]. Intervention with defibrillation after circulatory arrest may give a 72% chance of survival[(12)]. Intravenous thrombolysis therapy has been shown to salvage the ischemic myocardium, and the faster it is applied, the more effective it is[(13)]. Therefore, secondary prevention is possible, provided early diagnosis is made. Death or myocardial infarction occurs in only 8.1% of all patients admitted to coronary care units for unstable angina[(14)]. The diagnosis in the early stages of a developing myocardial infarction depends largely on patient history and ST-segment depression[(15)], both of which may be ambiguous. For example, one-third of heart attack victims feels no chest pain, so-called “silent” ischemia[(16)], and the electrocardiogram changes may be absent in the early hours. Routinely used markers of myocardial ischemia, e.g. troponin and

creatine kinase, are released after cell death, thus these markers may be present only after several hours and can not help prevent infarction[17]. Identification of patients at risk for death or myocardial infarction is difficult with current diagnostics, yielding a sensitivity of 80% and a specificity of only 33%[14].
In the early stage of ischemia, before irreversible cell death has occurred, poorly perfused tissue shifts from aerobic to anaerobic metabolism, the latter characterised among other things by an increased lactate production. Major lactate concentration shifts may occur in a matter of minutes both after the onset and at the end of myocardial ischemia[18]. There is also a quantitative relation between the extent of ischemia in the myocard and its release of lactate[19;20]. Therefore, a lactate monitoring technique would, if available, offer the opportunity to detect a tissue oxygen deficit in time for therapeutic intervention, and thus contribute to the management of patients at cardiac risk.

Emergency medicine
Diverse other critically ill patients may also benefit from lactate monitoring. In critical care units, high lactate levels in the general circulation have a strong prognostic value[21]. Lactate levels over 5 mM (normally 0.6-2.4 mM) are associated with a poor outcome[22]. It is possible to predict organ failure from serial lactate measurements(13) and blood lactate appears to be a better prognostic factor than oxygen[22]. Lactate monitoring has been suggested to be valuable for many patients in emergency medicine: perioperatively for surgical patients[23-27], for trauma patients including battlefield medicine, for patients with head injury or cerebral ischemia[28-32], acute intestinal ischemia[33], liver ischemia[34], transplanted organ surveillance e.g. myocutaneous flaps[35], intrapartum for the foetus[36;37], and patients with septic shock[22;38;39]. Other applications include research on mental stress in healthy subjects[40], physical exercise intop althletes[41;42], and spatial and pharmacological research (e.g. in research of metformin lactacidosis, or to reduce the total blood sample volume).
In summary, large patient groups may benefit from continuous glucose and lactate monitoring devices. The future application of the proposed devices will depend on the risk, patient friendliness and reliability in comparison with concurring techniques and other therapies, e.g. pancreatic islet transplantation[43].

1.2 Sampling and sensing devices

Sensing devices

In 1956, Clark took the first step to metabolic monitoring in vivo by introducing platinum-silver/silver chloride electrodes to measure oxygen (reference). A particular oxygen concentration generates a certain electric current directly at the platinum electrode (amperometric measurement). By adding glucose oxidase enzyme, glucose concentrations can be measured indirectly, because oxygen is metabolised with glucose to hydrogenperoxide in a one to one relation[44]. Ever since, scientists and physicians have dreamt of using this technique to function as an artificial pancreas[45]. Such a technique may control glucose levels through insulin delivery guided by a glucose sensor, without need for action from the patient. Lactate oxidase enzyme can be applied in the same way to measure lactate. However, these sensors measure oxygen disappearance or hydrogenperoxide formation, so they are dependent on both the metabolite (glucose or lactate) and the oxygen concentration. In order to be independent of oxygen levels, mediators such as ferrocene have been introduced. Ferrocene is a substitute for oxygen or hydrogen peroxide by transferring electrons directly from the enzyme to the electrode. It also allows a lower voltage potential to be used for measurements, thus reducing the influence of electrically active substances such as ascorbic acid. This technical principle has been applied in flow-injection analysis[46] (see also chapter 2, figure 2). Limitation of diffusion of the metabolite and interfering substances towards the electrode has been achieved e.g. by a membrane cover[47;48] (see also chapter 5), or by physical entrapment of the enzyme in a polymer[49;50] (see also chapter 6). Thus the concentration range for linear measurement is extended and metabolite depletion at the sensor surface decreased, whilst interference is diminished. Addition of horseradish-peroxidase[46] or catalase[47] can prevent the build-up of toxic levels of hydrogen peroxide, which may denature the enzyme. Apart from the measurement of electric current (amperometry), potentiometric electrodes have also been applied. Potentiometric miniaturised sensors are e.g. ISFETs[51] (ion-sensitive field-effect transistors). A completely different sensor concept has been demonstrated by Ballerstadt et al.[52] Using a hollow fibre. In this method, changes in glucose concentration cause changes in fluorescence emission when free glucose competes with fixed glucose for affinity with a fluorophore.

Sampling devices

Hollow fibres are used in many monitoring techniques as a sampling interface between the sensor and the tissue. Hollow fibres are semi-permeable membranes in a tubular shape and a diameter of about 200-300 µm. Sampling with hollow fibres has several advantages compared to sensor implantation. Hollow fibres are easily applicable and relatively patient friendly. Hollow fibres can be inserted into tissue with a small needle, and there is no need for implantation of complex, potentially hazardous electronics or enzymes. In addition, large molecules and cellular remains are excluded by the membrane, so there is a clean matrix for measurement. Hollow fibres are often applied for microdialysis sampling. In classical microdialysis, a fluid flow through a hollow fibre partially equilibrates with the surrounding tissue, after which the sample is withdrawn and the metabolite content is measured outside the body. Many attempts have been made to determine the exact microdialysis metabolite recovery in vivo, but none of these methods are sufficiently accurate or feasible for clinical application. To obtain samples equilibrated with interstitial fluid, Rosdahl et al. lowered the microdialysis flow to 160 nanolitre per minute[53]. To prevent loss of all perfusion fluid through the membrane into the tissue, he added a colloid. Kaptein et al. employed a suction pump to lower the microdialysis flow to 100 nl/min in rats, obtaining equilibrated samples without need for colloid addition[54].

Ash et al. introduced ultrafiltration as an alternative method to microdialysis[55]. In their method, tissue fluid is ultrafiltered through a hollow-fibre membrane by underpressure. Large needles are required, because many fibres are needed to collect sufficient fluid for off-line analysis. Moscone et al. made ultrafiltration patient friendly by miniaturising the ultrafiltration probe, and introducing a light-weight disposable pump[56]. Kaptein et al. demonstrated the possibility of using ultrafiltration to continuously monitor glucose in the subcutis and the veins of rats[57]. Recently developed, light weight, nanolitre flow-sensors by Rhemrev et al.[50] make it possible to analyse glucose or lactate in a fluid flow of 30-100 nl per minute continuously,. from ultrafiltration or slow microdialysis.

Demands for monitoring

Before clinical application, new metabolic sensors will always be compared with the standard discontinuous technique. Frequent self-monitoring of blood glucose (SMBG) by finger pricking is currently the standard for diabetic patients[58]. A new glucose monitoring technique would need to have an edge over current SMBG with respect to compliance (less pain, automation),

measurement reliability and frequency, less need for selfdiscipline, and improved life expectancy, quality and duration . Essential for any improvement is device reliability, because otherwise, frequent blood sampling for calibration will equal the SMBG burden for the patient.
Blood sample lactate analysis is currently possible with bed-side devices in the operating theatre. Myocardial lactate detection is now limited to scientific research, because of the need to perform heart catheterisation for blood sampling. Analysis of blood samples of critical care patients can be done in the (remote) hospital laboratory. Lactate analysis is rarely practised, mainly due to the slow result turnaround time, preanalytic problems, and blood loss. A lactate monitoring technique would be clinically interesting if it was automated, continuous (for trending data), and operating in real-time like ECG and blood pressure monitoring.

Complications
Continuous application in patients poses specific demands to the newly proposed metabolic sensors. Generally, a device is safer and friendlier to the patient, the less invasive it is. An undisturbed, constant contact between body fluid and sensor surface is however essential for reliable measurements (see further section 1.3). Sensing and sampling devices have been proposed for intravenous, subcutaneous and transcutaneous applications. Transcutaneous devices[52;59-62] often demand skin stripping and local heating, whereas subcutaneous and intravenous devices demand insertion by needle. Pain from needle insertion depends of course on the diameter of the device, and the sensitivity of the site of the skin e.g. the arm is more sensitive than abdomen. Further complications may include damage to nearby structures at insertion (in the thin cutis of neonates), inflammation or infection (if permanent skin penetration is needed), and the risk of release of toxic (e.g. ferrocene), carcinogenic, or allergenic substances.
Experience with intravascular blood-gas monitoring and other catheters has shown that intravascular devices may cause vessel trauma, thrombus formation, blood flow cessation in the particular vessel, ischemic organ damage or loss, bacterial colonization of the catheter surface, infection, and sepsis[63]. An important secondary complication can be caused by inaccurate data, leading to misdiagnosis. Nowadays, morbidity from blood-gas sensors has become rare in critically ill patients[64;65]. Notably the appliance of an anticoagulant, such as heparin, to the surface of catheters has contributed to an improved accuracy. For out patients, such as . diabetes mellitus patients, the less invasive trans- or subcutaneous device remains the preferred option.

Device mass production
Future clinical appliance also demands that from techniques be fit for mass production. Reliable sensors require namely a standardised manufacturing process to guarantee reproducibility. Candidate techniques in this regard are e.g. thin-film sensors[47], ISFET sensors[61], the GlucoWatch® iontopheresis sensor[60], and the CGMS® needle type glucose sensor[66]. Several currently proposed lactate and glucose sensors have been shown to measure reliably in vitro, and some are already being tested in patients for in vivo accuracy (see further paragraph 1.3: Influence on measurements by device and organism). Of the currently proposed sampling techniques for glucose monitoring, ultrafiltration and ultraslow microdialysis are readily applicable in vivo, are minimally invasive, and function accurately in vitro and in blood without recovery calculations. An overview of sampling and sensing techniques currently in development for metabolic monitoring is given in table 1.

1.3 Influence on measurements by device and organism

Relation of interstitial to blood metabolite levels
Currently proposed trans- or subcutaneous glucose sensors often detect a lower concentration in tissue compared to simultaneously measured blood plasma levels, which is the reference for diabetic management. To estimate blood plasma levels, a one-point or multiple-point calibration to blood levels is necessary. This calibration factor is called “in vivo recovery” of microdialysis probes or “in vivo sensitivity” of needle type sensors. Unfortunately, this factor is rather variable, both between subjects, within subjects, and in time. To determine this factor, patients are now urged to calibrate the sensor several times a day against blood samples. To improve accuracy and patient friendliness, a better insight is urgently needed. When the relation of cutaneous and subcutaneous tissue to blood levels can be established, a more widespread application of glucose monitoring devices will become possible.

Glucose and lactate metabolism in the subcutis
Glucose and lactate are constantly metabolised and transported between tissues by the blood circulation. So, nowhere in the living organism, will there be the same concentration at one time or place, however these gradients are not necessarily steep or of long duration.

Table 1. Glucose and lactate sampling and sensing techniques

Device	Sampling interface	Detection technique	Authors
CGMS®	sc. needle membr.	g.o.d. amperometry	Mastrototaro et al.[67;68]
Glucowatch®	transdermal iontopheresis	g.o.d. amperometry	Garg et al.[60;69]
microdialysis	sc. hollow fibre diffusion	off-line	Bolinder et al.[70]
microdialysis	hollow fibre diffusion	off-line	Schoonen et al.[71-73]
needle type (gluc./lac.)	sc. needle membr.	g.o.d./l.o., amperometry	Yang et al.[49;74]
needle type (lac.)	sc. needle membr.	l.o., amperometry	Kyrolainen et al.[48]
open-flow microperfusion	sc. perforated catheter	thin-film (gluc+lac)	Schaupp et al.[75]
optical	transdermal diff. hollow fibre	fluor. competitive affinity	Ballerstadt et al.[52]
suction effusion	transcutaneous	I.S.F.E.T.	Ito et al.[51]
thin-film (gluc.+lac.)	sc. thin-film membrane	g.o.d./l.o.,catalase, amp.	Jobst et al.[47]
u.f.	hollow fibre ultrafiltration	off-line	Ash et al.[76]
u.f.	hollow fibre ultrafiltration	F.I.A. amperometry	Moscone et al.[56]
u.f.	hollow fibre ultrafiltration	flow-sensor amperometry	Rhemrev et al.[77]
u.s.m.d.	hollow fibre diffusion	F.I.A. amperometry	Kaptein et al.[57]
ultrasound	transcutaneous	g.o.d. amperometry	Kost et al.[62]

The arterio-venous differences measured over the adipose tissue of the abdominal wall are found to be very small in the late postprandial period, but increase significantly during an oral glucose load. In this situation glucose is being taken up, and lactate is being produced[78-80]. Both glucose and insulin levels influence lactate levels [81]. More lactate is found subcutaneously in lean than in obese healthy volunteers after an oral glucose load[82]. Adipose tissue also displays regional metabolic differences in lipolysis[83] and in lactate production in reaction to glucose and insulin[84]. The rate of glucose transport into cells also differs between adipose and connective tissues[85], and these are unequally distributed in the subcutis. The arterio-venous gradients mentioned above for the blood circulation constitute the minimal gradients inside adipose tissue, since glucose is exchanged freely over the capillary wall. So, the molar proportion of glucose and water will be the same on both sides of the capillary wall. The glucose concentration (mmol/l), however, will be considerably higher in the interstitium, because the protein content of interstitial fluid is much lower than in blood[1]. Differences in protein content of samples can lead to 15-20% differences in glucose concentration[86].

Abbreviations in Table 1: glucose and lactate sampling and sensing techniques

amp.	Amperometry
CGMS®:	Continuous Glucose Monitoring System®
diff.:	diffusion
F.I.A.:	Flow-Injection Analysis
fluor.:	Fluorescence
g.o.d.:	Glucose OxiDase enzyme
gluc.:	Glucose
I.S.F.E.T.:	Ion-Sensitive Field-Effect Transistor
l.o..:	Lactate Oxidase enzyme
lac.:	Lactate
membr.:	membrane
sc.:	subcutaneous
u.f.:	Ultrafiltration
u.s.m.d.:	Ultra-Slow MicroDialysis

The transit times of glucose in the organism can be identified by compartmental modelling e.g. with the minimal model[87;88]. The transit time is estimated to be a few minutes from the blood plasma, to a second, insulin independent, compartment, such as the brain, splanchnic tissues, erythrocytes and renal medulla[89]. A third, insulin dependent, compartment which equilibrates slowly is thought to exist, and presumed to be predominantly muscle and adipose tissue. Compartmental modelling is generally based on the measurement of parameters in blood; the other parameters are derived from these. The anatomical locations of the different compartments are not known with certainty; so the transit time to subcutaneous adipose tissue remains unknown from these models.

Pathophysiologic alterations in the subcutis of diabetic patients

The knowledge of physiological glucose metabolism, as discussed above, may not be applicable to diabetic patients, as they do not express a physiological insulin and glucose metabolism. In addition, there is considerable variation, between patients in such factors as insulin sensitivity. Generally, microvascular function strongly relates to insulin sensitivity [90]. The microvascular vessel anatomy and function is significantly altered in diabetis patients, as well as the diffusion in tissues [91;92].

Possible device-tissue contact interactions

Apart from the physiologic and pathophysiologic metabolism in tissues, the mere insertion and presence of a measuring device in the subcutis may well change local metabolite concentrations. The response of the organism may also influence the measurements made by the device. In particular changes around the device may especially diminish diffusion from the tissue to the device. Diffusion parameters at the contact site are the available surface area of device for sensing/sampling, diffusion distance from blood capillaries to the device, interstitial diffusion path width, and availability of medium (interstitial fluid). Furthermore, the glucose metabolism may change from predominantly aerobic to more anaerobic, switching from carbondioxide to lactate production. A comparison of the relative sizes of adipose tissue cells and implanted devices helps to illustrate these factors. The diameter of adipose cells is variable and ranges up to 100µm. The intercellular width is about 1µm. The blood circulation in a microlitre of adipose tissue is approximately 0.1-0.03 µl/min. A needle used for implanting a device has an outer diameter of around 1400-1250µm. A hollow fibre has an outer diameter of 200-600µm, and a volume of 1-8 µl. The microdialysis flow may be 0.16-10 µl/min. Other factors to consider

are that the device itself may consume more metabolites than can be restored by the circulation and organic molecules may influence the enzymatic or electric functioning of devices. Changes at the device-tissue contact site may occur as a consequence of primary cell lesions at the moment of insertion resulting in free fat or cellular remains. Primary vessel lesions at the site of insertion may result in (diffusional path lengthening, so that less fluid filtered out of the capillaries into the interstitium. In addition, hematoma may cause local volume expansion and erythrocyte metabolism, or coagulation may result in changes to the device area and surrounding cells. Hypoxia, secondary vessel trauma due to movement of a rigid device in vulnerable tissue, or inflammation may cause effusion of fluid and proteins, cellular infiltration and fibrous encapsulation. Some of these possible influences on measurements in vitro, have been documented, for example, glucose oxidase is significantly inhibited by small molecules from rabbit serum and by high molecular weight granulocyte excretion products[93]. To date there is very little information from in vivo experiments in humans.

Transcutaneous interactions

Experiments with transcutaneous glucose measurements suffer from the water resistance of skin, which, even when stripped from the corneal layer, exhibits a low and very variable permeability for interstitial fluid[94]. The transcutaneous microdialysis recovery may be one-twentieth of the glucose recovery in vitro[95]. Variable evaporation and perspiration can influence transcutaneous measurements as well[96]. Changes in the resistance due to skin water resistance will add to changes in the device-tissue contact. If the instability at the contact site remains unsolved transcutaneous devices will stay heavily dependent on finger stick blood glucose calibrations, seriously limiting the prospects of current transcutaneous devices[51;60-62].

Subcutaneous interactions

Experimental findings with subcutaneous glucose monitoring devices in vivo yield a high within subject variation coefficient of recovery or sensitivity. These differences may originate from regional differences in adipose tissue. Stallknecht found an increasing microdialysis recovery with decreasing skinfold thickness, and with increasing water content of adipose tissue[97]. Microdialysis recovery was not found to depend on blood flow. Glucose was found on average 0.8 mM lower and lactate 1.2 mM higher in adipose tissue than in blood plasma[97]. Thomas et al. found increasing recovery and less variance in subcutis from the forearm than from the abdomen[98]. Classical microdialysis however changes the biological environment by pumping fluid into the tissue (e.g. 0.1µl

of 0.3µl/min microdialysis flow is lost into tissue), by draining metabolites from the tissue, and by inducing a diffusion gradient in the surrounding tissue. This diffusion gradient may stretch for two millimetres into tissue around the probe[99]. Rosdahl et al., using equilibrated microdialysis, found lower glucose levels in muscle tissue, but similar levels in adipose tissue and blood[53].

Long-term interactions
In long-term experiments, Sharkawy found the diffusion in subcutis tissue of rats to be similar, before and after a fibrous capsule had formed around a porous implant. However, fibrous capsules around nonporous implants diminished the diffusion coefficient by one half[100-102]. Considerably reduced glucose diffusion has been observed in tumour tissue, when an only 30µm thick mesenchym layer was formed around the capillary vessels[103]. A three week experiment in healthy volunteers showed an approximately two-fold increase in microdialysis recovery the first six to nine days after subcutaneous implantation, and the estimated glucose concentration to double in the first three days[73].

Intravascular interactions
Arterial blood-gas monitoring catheters have been shown to be sensitive to a number of device-organism interactions. Measurement may be influenced by blood flow stagnation which creates gradients, and from catheter perfusion fluid diluting the blood. Measurement may also be influenced by thrombus formation, yielding declining pH and pO_2, and rising pCO_2 due to metabolism within the thrombus, and an increased response time through decreased diffusion. Other possible influences are contact with the vessel wall, with declining pO_2, and little or no deviation in pH and pCO_2[63]. Thrombus formation can be reduced by heparin coating of the catheter. However, even with anticoagulants in situ proteins in blood can still adsorb onto artificial surfaces, usually forming a monolayer. Such a thin layer may reduce and define the diffusing properties of hollow fibre membranes in artificial kidneys[104;105]. Interestingly, a disturbance in the contact between the intravascular gas sensor and with the bloodstream can be recognised by comparison of several analytes (H^+, O_2, and CO_2)[63]. These analytes are altered by cellular (an-)aerobic metabolism of glucose to CO_2 and/or lactate if the catheter becomes isolated from the blood stream as in the case of monolayer formation.

In summary, the clinical application of subcutaneous sensors is slowed down by the unknown relationship between subcutaneous measurements and blood glucose levels. Since current in vivo calibration techniques are insufficient,

frequent blood sampling is required. The differences found between subcutaneous and blood glucose levels are still subject to debate. Subcutaneous measurements have been variously interpreted to originate from blood levels in the general circulation, to reflect local tissue metabolism, or to be caused by disturbing device-tissue interactions.

1.4 Aims and scope of the thesis

The aim of this thesis was to develop and test in vivo some continuous sampling and sensing techniques for clinical monitoring of glucose and lactate. The performance of ultrafiltration and ultra-slow microdialysis equilibrated sampling techniques was determined with oral or intravenous glucose and lactate. The in vivo time-resolution was assessed with amperometric sensors placed either directly in the interstitium, in the sample flow, or in a sample flow-injection system. Interpretations of the in vivo measurements were explored studying the concurrent glucose and lactate levels in the general circulation, the effect of aerobic or anaerobic metabolism, the influence of insulin levels, different tissue types for device implantation, the temporal changes after implantation, and the effects of ischemia and reperfusion. Computation methods were developed to estimate glucose or lactate in tissues utilising intravascular glucose, lactate and insulin levels.
New ultrafiltration (ch. 2 & 4) and ultraslow microdialysis techniques (ch. 3, 6, 7) were applied for continuous equilibrated sampling in subcutaneous tissue (ch. 2, 3, 6, 7) and intravascular, in the coronary sinus (ch. 4). Application of a wearable glucose flow-sensor with a high time resolution was studied, when connected on-line with ultraslow microdialysis sampling in man (ch. 6). A novel continuous monitoring catheter was developed and studied for detection of myocardial ischemic events (ch. 4).
The performance of devices implanted intravenously (ch. 4), was evaluated in muscle, in adipose, and in connective tissue in swines (ch. 5); and in adipose and connective tissue in man (ch. 6) to find the optimal contact in vivo. A bed-side flow-injection analysis was developed and applied (ch. 2 & 3) to measure glucose and lactate simultaneously and continuously in nanolitre samples, allowing the study of glucose-lactate metabolism in tissue near an implanted device. A thin-film glucose-lactate sensor was applied directly in tissue for the same purpose (ch. 5). To explore the course of aerobic and anaerobic metabolism around a newly implanted device, the glucose and lactate concentrations were compared at the time of device insertion and 24 hours later (ch. 3). Also, the influence of insulin levels on the subcutaneous glucose

concentration (ch. 6 & 7) was studied. Computation methods were explored to estimate blood glucose levels directly from subcutaneous measurements (ch. 3) and to estimate myocardial lactate production during ischemia from venous levels (ch. 4). Finally, several kinetic models were developed and tested to identify a model for subcutaneous glucose level estimation (ch. 7).

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