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METABOLIC MONITORING IN PATIENTS UNDERGOING CARDIAC SURGERY USING INTRAVASCULAR MICRODIALYSIS

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METABOLIC MONITORING IN PATIENTS UNDERGOING CARDIAC SURGERY USING INTRAVASCULAR MICRODIALYSIS

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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Learn from yesterday, live for today, hope for tomorrow.

The important thing is not to stop questioning.

- Albert Einstein

ABSTRACT

Critically ill patients and patients undergoing cardiac surgery often experience hyperglycemia, hypoglycemia and glycemic variability (fluctuating blood glucose concentrations), all of which have been associated with adverse outcomes. Glycemic control aimed at avoiding both hyper- and hypoglycemia as well as at minimizing glycemic variability has been shown to be beneficial in these patients. In order to enable glycemic control, a safe and reliable glucose monitoring system is required. Additionally, monitoring of lactate is beneficial since an elevated lactate level may serve as a warning signal, and the knowledge of lactate concentration is necessary to guide lactate-reducing treatment, which has been shown to improve outcome in critically ill patients.

Intravascular microdialysis is a technique that can monitor small molecules like glucose and lactate in the bloodstream without the need for blood sampling. This thesis aimed at developing intravascular microdialysis into a verified and accepted clinical method for continuous monitoring of glucose and lactate in patients undergoing cardiac surgery requiring postoperative treatment in the intensive care unit. Furthermore, another aim was to incorporate this technology into a standard procedure.

The intravascular microdialysis method is based on a new and innovative technology, which involves a small compartment located between a catheter body and a covering microdialysis membrane. By perfusing this compartment, a dialysate fluid is produced wherein the concentrations of glucose and lactate are theoretically equal to those in the surrounding tissue, i.e. the bloodstream as the catheter is placed in a central vein. In Study I, the intravascular microdialysis concept was applied to a single-lumen catheter. The dialysate fluid was collected and intermittently analyzed in a separate dialysate analyzer for glucose and lactate concentrations and compared to blood samples. This study verified the microdialysis concept as an efficient clinical method for measuring blood glucose and lactate levels. In Study II, the outlet of the compartment of the microdialysis catheter was directly connected to a recently developed sensor, continuously analyzing glucose and lactate concentrations of the dialysate fluid. The continuous microdialysis catheter-sensor system was shown to be very accurate in monitoring blood glucose, with a lag time of only a few minutes in the clinical setting. The technique was improved in Study III, in which the microdialysis membrane was integrated in a standard central venous catheter, which is routinely applied in patients subjected to cardiac surgery. The results further demonstrated the accuracy and reliability of the microdialysis system. The data on lactate from Studies II and III were processed in Study IV, which demonstrated that the microdialysis system was also accurate and efficient in monitoring blood lactate. In Study V, the accuracy and responsiveness of the microdialysis system were confirmed in an animal model during extreme hypoglycemic conditions and rapid blood glucose oscillations, as well as during infusion of a solution with a high glucose concentration via the catheter. Finally in Study VI, the microdialysis system was compared to a certified and clinically verified continuous glucose monitoring system placed subcutaneously. The microdialysis system was found to be superior in terms of accuracy and met the clinical standards in terms of safety in the intensive care unit, while the subcutaneous system failed in this respect.

This thesis demonstrates that intravascular microdialysis is a safe and accurate method for continuous monitoring of glucose and lactate in patients undergoing cardiac surgery, and is superior to a subcutaneous system in this specific setting. Based on the results of the included studies, a safe, reliable and certified standard procedure was developed for use in critically ill patients for the pertinent control of blood glucose and lactate for early detection of metabolic derangement.

SAMMANFATTNING

Svårt sjuka patienter och patienter som hjärtopereras drabbas ofta av hyperglykemi, hypoglykemi och glykemisk variabilitet (fluktuerande blodglukoskoncentrationer), som alla har associerats till komplikationer. Glykemisk kontroll syftar till att undvika både hyper- och hypoglykemi såväl som att minimera glykemisk variabilitet, och har visat sig förbättra resultaten för dessa patienter. För att möjliggöra glykemisk kontroll krävs ett säkert och exakt system för monitorering av blodglukos. Monitorering av laktat har också visat sig vara värdefullt då ett förhöjt laktatvärde kan ses som en varningssignal. Vetskapen om laktatvärdet är nödvändig för att styra intensivvårdsbehandling som syftar till att minska laktatnivån, vilket kan leda till förbättrade resultat för dessa svårt sjuka patienter.

Intravaskulär mikrodialys är en teknik som kan monitorera små molekyler, såsom glukos och laktat, i blodet utan blodprovstagning. Denna avhandling har syftat till att utveckla intravaskulär mikrodialys till en verifierad och accepterad klinisk metod för kontinuerlig monitorering av både glukos och laktat hos patienter som genomgår hjärtkirurgi, och som kräver postoperativ behandling på intensivvårdsavdelning. Vidare var syftet att införliva tekniken i ett standardförfarande.

Intravaskulär mikrodialys är baserat på en ny och innovativ teknik, där grunden utgörs av ett mindre spatium lokaliserat mellan en kateterkropp och ett omgivande mikrodialysmembran. Genom att perfundera detta spatium produceras en dialysat-vätska, som teoretiskt kommer att innehålla samma koncentrationer av glukos och laktat som i omgivande vävnad, vilket i detta fall är blodbanan då katetern placeras centralvenöst. I Studie I applicerades det nya intravaskulära mikrodialyssystemet i en singel-lumen kateter. Dialysat-vätskan samlades upp och analyserades intermittent avseende glukos- och laktatkoncentration i en separat dialysat-analysator. Denna studie verifierade mikrodialyssystemet som en effektiv och noggrann metod för att kliniskt mäta blodglukos och laktat. I Studie II anslöts utloppet från mikrodialyskatetern till en nyutvecklad sensor, som kontinuerligt analyserade glukos- och laktatkoncentrationerna i dialysat-vätskan. Det kontinuerliga mikrodialyskateter-sensorsystemet visade sig vara mycket noggrant och exakt avseende mätning av glukos med en kort tidsförskjutning på bara ett par minuter. Tekniken utvecklades ytterligare i Studie III, då mikrodialysmembranet integrerades i en trippel-lumen centralvenöskateter, ett standardförfarande för alla patienter som hjärtopereras, vilket exkluderar införandet av en separat mikrodialyskateter. Resultaten bekräftade mikrodialyssystemets säkerhet och noggrannhet. Laktatdata ifrån Studierna II och III analyserades i Studie IV, och resultaten visade att mikrodialyssystemet var mycket noggrant även avseende mätning av laktat. I Studie V bekräftades effektiviteten och noggrannheten hos mikrodialyssystemet för glukosmonitorering i en djurmodell under extrema hypoglykemiska tillstånd, liksom vid snabba blodsockersvängningar, samt vid hastig tillförsel av en infusion med hög glukoskoncentration via katetern. Slutligen, i Studie VI jämfördes det intravaskulära mikrodialyssystemet med ett tidigare kliniskt verifierat kontinuerligt monitoreringssystem som mäter glukos i subkutan vävnad. Denna studie visade att mikrodialyssystemet var mer exakt vid mätning av glukos, samt uppfyllde de kliniska krav gällande säkerhet på en intesivvårdsavdelning, vilket inte det subkutana systemet gjorde.

Denna avhandling visar att intravaskulär mikrodialys är en säker och effektiv metod för kontinuerlig mätning av glukos och laktat hos patienter som hjärtopereras, och är överlägsen ett subkutant monitoreringssystem i denna specifika miljö. Baserat på resultaten ifrån dessa studier har en säker, tillförlitlig, och kliniskt verifierad standardprocedur utvecklats för kontroll av blodglukos och laktat hos svårt sjuka patienter för tidig upptäckt av en metabol störning.

LIST OF PUPBLICATIONS

I. Möller F, Liska J, Eidhagen F, Franco-Cereceda A. Intravascular microdialysis as a method for measuring glucose and lactate during and after cardiac surgery.
J Diabetes Sci Technol 2011;5:1099-1107.

II. Schierenbeck F, Franco-Cereceda A, Liska J. Evaluation of a continuous blood glucose monitoring system using central venous microdialysis.
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III. Schierenbeck F, Öwall A, Franco-Cereceda A, Liska J. Evaluation of a continuous blood glucose monitoring system using a central venous catheter with an integrated microdialysis function.
Diabetes Technol Ther 2013;15(1):26-31.

IV. Schierenbeck F, Nijsten MW, Franco-Cereceda A, Liska J. Introducing intravascular microdialysis for continuous lactate monitoring in patients undergoing cardiac surgery: A prospective observational study.
Crit Care 2014;18(2):R56.

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VI. Schierenbeck F, Franco-Cereceda A, Liska J. Accuracy of two different continuous glucose monitoring systems in patients undergoing cardiac surgery: intravascular microdialysis vs. subcutaneous tissue monitoring.
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CONTENTS

LIST OF ABBREVIATIONS

Art-BG	Arterial blood gas
ATP	Adenosine tri-phosphate
CABG	Coronary artery bypass grafting
CPB	Cardiopulmonary bypass
CGM	Continuous glucose monitoring
CVC	Central venous catheter
DM	Diabetes mellitus
EGA	Error grid analysis
FFA	Free fatty acid
GC	Glycemic control
GIK	Glucose-insulin-potassium
GO	Glucose oxidase
GV	Glycemic variability
ICU	Intensive care unit
IIT	Intensive insulin treatment
ISO	International Organization for Standardization
LDH	Lactate dehydrogenase
MARD	Mean absolute relative difference
MD	Microdialysis
MD-CGM	Microdialysis continuous glucose monitoring
NO	Nitric oxide
PDH	Pyruvate dehydrogenase
SC-CGM	Subcutaneous continuous glucose monitoring
SLC	Single-lumen catheter
TGC	Tight glycemic control
TLC	Triple-lumen catheter
Ven-BG	Venous blood gas
VSMC	Vascular smooth muscle cell

1 INTRODUCTION

Intravascular microdialysis is a novel technique that provides continuous monitoring of small molecules in the bloodstream without the need for blood sampling, using a microdialysis catheter placed in a central vein.

This thesis focuses on the development of the intravascular microdialysis method as a procedure for continuous monitoring of blood glucose and lactate in patients undergoing cardiac surgery. The six studies included in this thesis all explore the accuracy, safety, and potential clinical use of intravascular microdialysis from various perspectives. In the first study, intravascular microdialysis was used for monitoring of blood glucose and lactate with intermittent analysis in a special separate dialysate fluid analyzer. The development of a sensor that could be connected to the microdialysis catheter provided a possibility that enabled continuous glucose monitoring (CGM), which was evaluated in the second and third studies. Two different catheters were used, a separate microdialysis catheter (Study II) and a central venous catheter (CVC) with integrated microdialysis membrane (Study III). The fourth study assessed intravascular microdialysis for continuous lactate monitoring. As there were no hypoglycemic events in the second and third studies, a fifth study was performed using an animal model to evaluate the accuracy of CGM in hypoglycemia and in rapidly fluctuating blood glucose concentrations, and to assess possible interactions during glucose administration via the microdialysis catheter. In the sixth study, the intravascular microdialysis system was compared to a less invasive subcutaneous CGM system.

2 BACKGROUND

2.1 MICRODIALYSIS

2.1.1 History of microdialysis

Microdialysis is an *in vivo* sampling technique first described in 1966 by Bito *et al.*, who used a static dialysate sac implanted in brain tissue in dogs to measure amino acids and electrolytes.[1] In 1972, Delgado *et al.* developed the "dialytrode", the first basic microdialysis catheter with a semi-permeable membrane, and used it for long-term intracerebral perfusion in awake monkeys.[2] In 1974, the technique was further improved by Ungerstedt and Pycock, who succeeded in measuring dopamine levels in brain tissue of rats[3]. Microdialysis has since then been used to monitor various molecules in many different tissues. With the development of a microdialysis membrane that can be positioned inside a blood vessel, monitoring of small molecules in the bloodstream without the need for blood sampling has become possible. Intravascular microdialysis has been shown to be useful for blood glucose and lactate monitoring in order to detect myocardial ischemia in patients undergoing cardiac surgery.[4]

2.1.2 Basic principle of microdialysis

The basic principle of microdialysis is to constantly perfuse a "tube" with a dialysate fluid. The tube is a microdialysis catheter that carries a specially designed semi-permeable membrane. Small molecules diffuse through the membrane, creating equilibrium. Hence, the dialysate fluid has the same concentration of permeable molecules as the site where the membrane is placed,[5] mimicking the function of a capillary. The microdialysis technique thus requires a catheter with a microdialysis membrane, dialysate fluid that is pumped through the system, and a device for analyzing the dialysate fluid for the molecules of interest (see figure 1).

The studies included in this thesis have used a specially designed microdialysis membrane that can be placed inside a blood vessel. This enables continuous monitoring of small molecules in the bloodstream without blood sampling. In this thesis, intravascular microdialysis has been used for monitoring of blood glucose and lactate in a central vein.

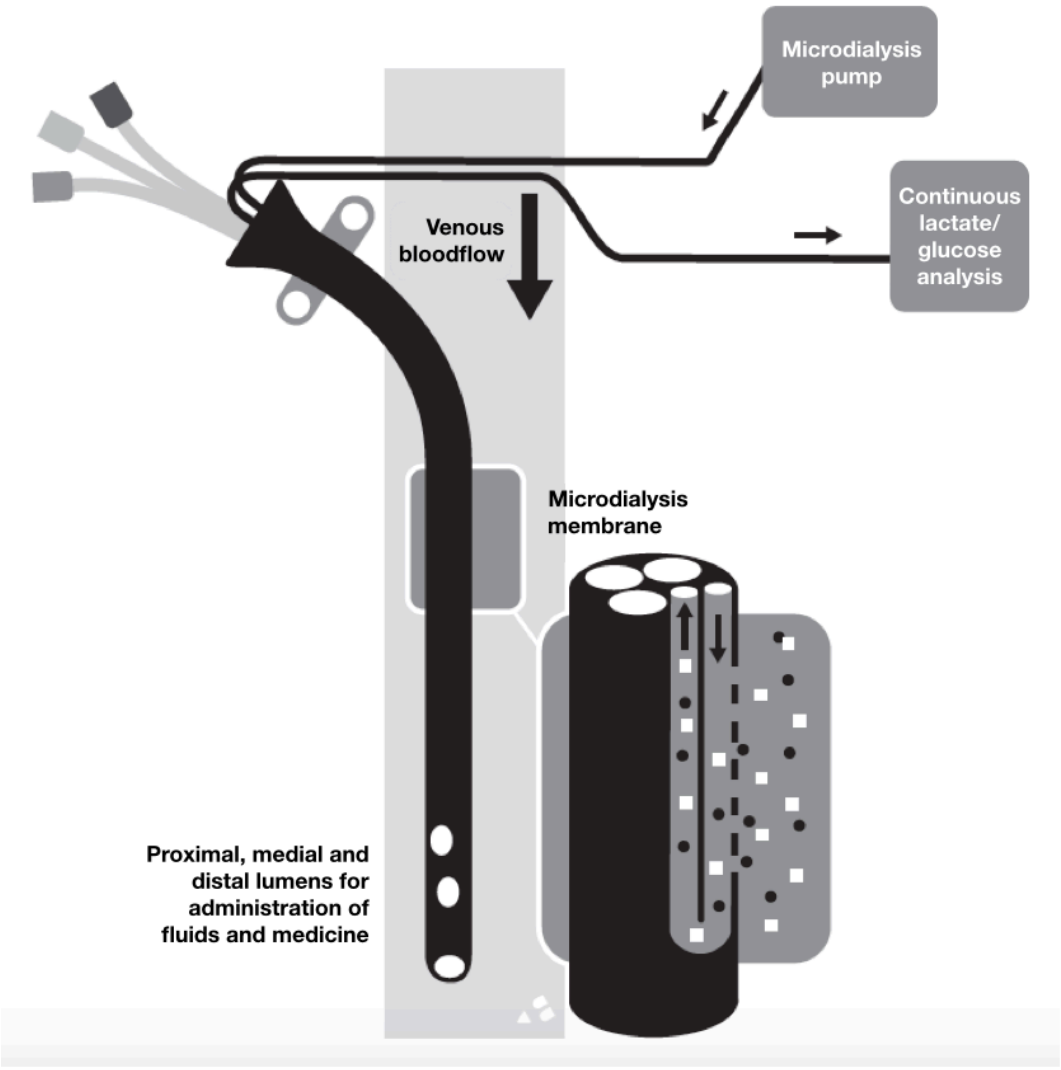

Figure 1. A scheamtic illustation of the intravascular microdialysis system. The microdialysis membrane is located on a catheter positioned inside a central vein. Small molecules diffuse through the membrane, resulting in the same concentration of glucose and lactate in the dialysate fluid (pumped through the system by a special pump) as in the bloodstream. The dialysate fluid can then be analyzed for glucose and lactate concentrations.

2.1.3 Factors affecting the microdialysis technique

Recovery is a term describing the amount of monitored molecule found in the dialysate fluid. Absolute recovery is the amount of this specific molecule during a certain time period, and relative recovery is the concentration of the monitored molecule in the dialysate fluid expressed as a percentage of the real concentration in the studied tissue, which in the case of intravascular microdialysis is the bloodstream. The recovery is affected by the perfusion rate: if the perfusion rate decreases, the relative recovery increases. The surface area of the microdialysis membrane further affects the recovery, as these two factors are directly proportional to each other. A larger surface membrane area will result in higher recovery. Adjusting the microdialysis membrane area and the perfusion rate are two ways of influencing the recovery.

Liquid permeability is an attribute of the microdialysis membrane that describes its permeability. It is determined by measuring the volume of fluid that passes through a pre-defined surface area of the membrane at a certain pressure in a specific time, and is expressed in cm/bar*s.

2.2 GLUCOSE AND LACTATE METABOLISM

2.2.1 Glucose

The blood glucose concentration is tightly regulated by several different cellular events, controlled by various hormones. Elevated blood glucose concentrations increase the release of insulin, a blood glucose-lowering hormone. Insulin acts by increasing glucose uptake in skeletal muscle tissue and suppressing gluconeogenesis. After glucose is taken up by a cell, it can either be used to provide energy via glycolysis in the cytoplasm, or stored as glycogen. When the blood glucose level decreases, counter-regulatory hormones (glucagon, epinephrine, cortisol, and growth hormone) counteract the action of insulin and stimulate the production of glucose by increasing hepatic gluconeogenesis and glycogenolysis (breakdown of glycogen into glucose). Stress increases the levels of these counter-regulatory hormones, leading to increased blood glucose concentration.

Glucose is metabolized via glycolysis to generate pyruvate, NADH, and 2 molecules of ATP (figure 2). Pyruvate may be disposed of either aerobically or anaerobically, depending on the tissue oxygen state. The major fate of pyruvate under aerobic conditions is transport into the mitochondria, where it is decarboxylated by pyruvate dehydrogenase (PDH) into acetyl-CoA. Metabolism of free fatty acids (FFAs) via β-oxidation is another source of acetyl-CoA. Acetyl-CoA enters the citric acid cycle to produce more ATP, NADH and $FADH_2$ along with byproducts such as CO_2, and H_2O. The electron-transport chain then oxidizes the produced NADH and $FADH_2$ for additional ATP synthesis through oxidative phosphorylation.

Under anaerobic conditions, inhibition of oxidative phosphorylation results in accumulation of NADH, which in turn inhibits the citric acid cycle. Pyruvate is then converted into lactate by the enzyme lactate dehydrogenase (LDH) to regenerate NAD^+ to allow the glycolysis to continue. Lactate may then either be transported from the cell and be used as a substrate for hepatic gluconeogenesis, or may be converted back into pyruvate. If blood circulation is decreased and washout of metabolites is reduced, accumulation of lactate occurs.

2.2.2 Lactate

Lactate is produced from pyruvate by LDH, which is the major fate for glucose metabolism in an anaerobic setting. Lactate is also produced in exercising skeletal muscle, if NADH production exceeds the oxidative capacity. An increased $NADH/NAD^+$ ratio favors the reduction of pyruvate to lactate. In well-oxygenated skeletal muscle, heart muscle and brain cells during normal conditions, lactate is oxidized back to pyruvate, which is then converted into acetyl-CoA and used as fuel in the citric acid cycle. Lactate may also be converted back

to glucose via gluconeogenesis in the liver (figure 2) and subsequently released back into the circulation, a process called the Cori cycle.[6]

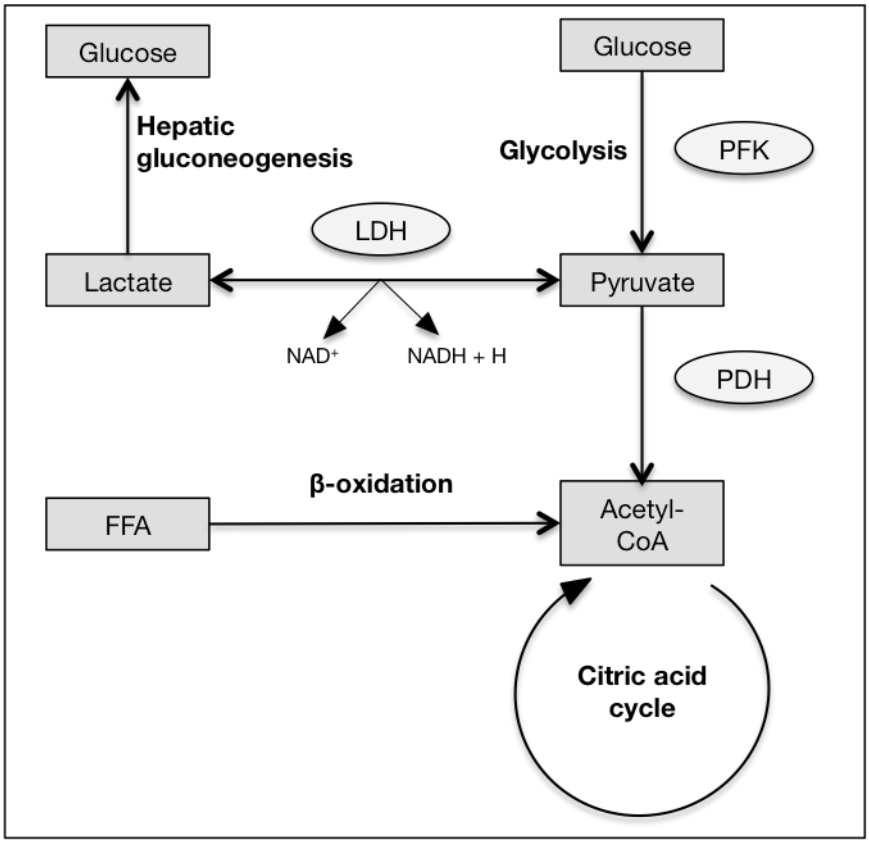

Figure 2. Overview of the metabolism of glucose and lactate.
FFA – free fatty acids, LDH – lactate dehydrogenase, PDH – pyruvate dehydrogenase, PFK – phosphofructokinase, NAD – nicotinamide adenine dinucleotide.

As the rate of glycolysis increases during exercise in order to provide more energy, the produced pyruvate cannot be metabolized via oxidative phosphorylation completely, resulting in its accumulation and conversion into lactate. Thus, lactate serves as an important metabolite allowing glycolysis to continue in the environment of increased energy demand.

Lactate has traditionally been considered a waste product of glycolysis during hypoxia, and hyperlactatemia regarded as a sign of tissue hypoperfusion due to anaerobic metabolism.[7] This hypoxia-induced generation of lactate is presently believed to explain an elevated lactate level only partially, as there is now evidence that lactate is also produced during aerobic conditions.[8] Thus, stimulation of glycolysis increases the blood lactate concentration during normoxia as well. Presently, lactate is increasingly being regarded as an important molecule in numerous metabolic processes and as a transportable fuel for metabolism.[9]

It should also be noted that an elevated blood lactate level may result from both increased lactate production and decreased lactate clearance. Lactate clearance is mainly performed in the liver and is thus dependent on tissue perfusion and liver function. Lactate clearance has

further been shown to be impaired in patients undergoing cardiac surgery involving cardiopulmonary bypass (CPB), possibly owing to a mild liver dysfunction.[10]

2.3 BLOOD GLUCOSE IN CRITICAL ILLNESS

The metabolism of blood glucose in critical illness is complex. Critically ill patients may develop hyper- and hypoglycemia, as well as large fluctuations in blood glucose concentration (glycemic variability, GV), which have all been associated with adverse outcomes. Glycemic control (GC) in the setting of an intensive care unit (ICU) is important, as it has been shown to reduce both mortality and morbidity. In order to achieve adequate GC, it is important that blood glucose monitoring is both correct and practicable.

2.3.1 Blood glucose monitoring in critical illness

How and when glucose is monitored in critically ill patients is of great importance for achieving GC. Glucose can be monitored using several different techniques, ranging from infrared spectroscopy to more manageable enzymatic methods, which most routine analysis methods, including those employed in hospital laboratories, point-of-care (POC) glucometers, and blood gas analyzers, are often based on. Three different enzymatic methods may be used for measuring blood glucose: glucose oxidase (GO), glucose dehydrogenase, and hexokinase.[11] The most common is the GO method, which is summarized in figure 3.

1) Glucose oxidase catalyzes the reaction:

Glucose + H_2O + O_2 → Gluconic acid + H_2O_2

2) H_2O_2 is quantified and used as an estimate for the glucose concentration using either:

- A peroxidase reaction of a chromogen (C) resulting in a color change:
  - $H_2O_2 + C_{red} \rightarrow H_2O + C_{ox}$
- An electrode measuring a generated current:
  - $H_2O_2 \rightarrow 2H^+ + O_2 + 2e^-$

Figure 3. The details of the glucose oxidase method.
C –chromogen, GO – glucose oxidase, H_2O_2 – hydrogen peroxide.

The gold standard of blood glucose analysis is to measure the plasma glucose concentration in the hospital laboratory, often using the GO method described above. Plasma glucose concentration varies with hematocrit level, and it is approximately 11% higher than that in whole blood because of the large fraction of red blood cells.[12]

Sending blood samples to the hospital laboratory is often too time consuming for use in critically ill patients, necessitating a faster method that can be used in the ICU. A commonly used approach for measuring blood glucose in the ICU is to intermittently obtain arterial

blood samples and analyze these in a blood gas analyzer, which often also uses the GO method. Arterial blood gas analysis has been shown to be accurate when used in the ICU as compared to plasma glucose analysis by the hospital laboratory,[13, 14] and it is generally more accurate than POC devices using test strips.[15] Most POC glucose monitoring systems have not been developed for use in the ICU setting and are not applicable,[16, 17] especially if capillary blood samples are used.[14, 18] Analysis of capillary blood samples in hypotensive patients with decreased peripheral circulation is not the optimal approach.[19]

There are several factors that may affect the glucose monitoring accuracy of POC devices, the most important being the hematocrit level. Hematocrit is the fraction of red blood cells in whole blood, and as the glucose concentration of plasma and red blood cells differ, alterations in hematocrit will affect the result of measuring glucose in whole blood. Usually, an increase in hematocrit will cause a decrease in glucose measurement.[11] Another factor that may affect measurements of glucometers using the GO method is treatment with oxygen. In highly oxygenated blood or during hypoxia, this may result in a false glucose reading.[20]

Blood glucose may be measured in plasma, arterial, venous, or capillary blood, and interstitial fluid. Glucose concentrations in arterial and capillary blood are almost identical, whereas glucose concentration in venous blood is usually a fraction of millimoles per liter lower. Glucose molecules diffuse from capillaries into interstitial fluid passively, and therefore the glucose concentration in this compartment depends on blood flow and vascular permeability. Furthermore, the metabolic state of the subcutaneous cells affects the glucose concentration in interstitial fluid. It is plausible to assume that many of these factors are affected by critical illness.[21]

2.3.1.1 Continuous glucose monitoring

Several CGM systems currently exist for use in critically ill patients. These systems have been shown to reduce the workload of ICU-nurses.[22, 23] Most CGM devices used in the ICU setting use the enzymatic GO method for glucose analysis. Two other existing techniques are mid-infrared spectroscopy and a fluorescence-based method. Mid-infrared spectroscopy detects glucose absorption using different wavelength filters, and the fluorescence technique uses chemical fluorescence to measure glucose concentration and requires a light source.

CGM systems using subcutaneous sensors were originally developed for use in stable out-clinic patients with diabetes and are currently a well-accepted and accurate monitoring method in such patients.[24] Several studies have evaluated these subcutaneous CGM systems in critically ill patients and found them to be reliable both in adults[25-30] and in children[31, 32] and to aid in avoiding hypoglycemia while implementing glycemic control.[33] In adult patients undergoing cardiac surgery, subcutaneous CGM has been shown to be safe and accurate,[34] but one study found questionable accuracy during the cardiac surgery phase and early postoperative period owing to false hypoglycemic readings.[35] A transdermal device utilizing a sensor that analyzes interstitial glucose is also being developed.[36]

Impaired circulation creates a theoretical disadvantage when monitoring glucose in subcutaneous tissue in critically ill patients,[21] which may perhaps be alleviated by improved calibrations.[29] Subcutaneous CGM has been shown to be accurate in cardiac surgery patients with mildly decreased microcirculation, but the sensor accuracy was affected by peripheral temperature.[34] More invasive approaches have been developed, including the intravascular microdialysis technique used in this thesis. Intravascular microdialysis in a peripheral vein have previously been used with promising results for glucose monitoring in patients who had acute coronary syndromes and were admitted to a cardiac ICU,[37] as well as in critically ill patients.[38] Another more invasive CGM system is the GluCath (GluMetrics) that utilizes a sensor, which may be placed both in an artery and in a peripheral vein.[39, 40] The sensor uses fluorescence to measure blood glucose optically. The same type of glucose measuring technique is used by the GlySure (GlySure) system that consists of a sensor placed in a central vein. The GlucoClear system (Edwards Lifesciences) is another intravascular device, consisting of a sensor placed in a peripheral vein. The sensor is covered with a GO layer and can measure glucose concentration when contact with blood is allowed.[41]

The use of CGM in the ICU has been shown to reduce the incidence of hypoglycemia, but it is still uncertain if it can contribute to improved overall GC[42] or reduced GV[43]. However, during a 2013 consensus-meeting dedicated to glucose control, there was general agreement that CGM is the future of glucose monitoring in critically ill patients.[44]

2.3.2 Glycemic control in critical illness

Why is monitoring blood glucose important in critically ill patients? Monitoring of blood glucose is a prerequisite for achieving GC, which has been found to be beneficial in these patients. The aim of GC is to avoid hyper- and hypoglycemia as well as to reduce large fluctuations in blood glucose concentrations, i.e. GV.

2.3.2.1 Hyperglycemia

Hyperglycemia is common and may be regarded as a normal response to stress aiming to provide the brain with nutrition (glucose) during flight-or-fight reactions. Patients with known diabetes mellitus (DM) and obesity are predisposed to developing hyperglycemia during critical illness,[45] but hyperglycemia in critically ill patients develops regardless of the presence of DM.[46, 47] The causes of this stress-induced hyperglycemia include several metabolic changes, such as the release of stress-hormones (e.g. cortisol and epinephrine) and various cytokines. This altered metabolic state leads to increased glucose production and peripheral insulin resistance, resulting in hyperglycemia.[48, 49] Iatrogenic causes of hyperglycemia may also contribute, such as administration of corticosteroids and inotropes, and parental nutrition.

Several studies have demonstrated an association between hyperglycemia and adverse outcomes in critically ill patients,[50, 51] as well as in patients admitted to general wards,[52] and in patients undergoing cardiac surgery.[53] Non-diabetic patients are affected by hyperglycemia

during critical illness more than are patients with known DM,[47, 54-56] and patients with hyperglycemia preceding critical illness.[57] Thus, GC prior to critical illness seems to be of importance. Additionally, hyperglycemia has been linked to increased mortality in patients with myocardial infarction,[58, 59] stroke,[60] and trauma.[61] Glucose-lowering measures have been shown to improve the outcome. The DIGAMI study of 1995 found that treatment with a glucose-insulin infusion in patients with diabetes and myocardial infarction resulted in decreased mortality and morbidity.[62] The observation that GC is beneficial was also made in critically ill patients.[63]

The optimal blood glucose target range in critically ill patients still remains a matter of controversy. Initial studies were focused on a tight glycemic control (TGC) approach, while more recent studies adopted a more comprehensive approach to GC including avoiding hyperglycemia but not necessarily aiming for the tightest interval. A landmark study performed in Leuven, Belgium, by van den Berghe *et al.* and published in 2001, demonstrated a significant mortality risk reduction and improved patient outcome in mixed medical and surgical critically ill patients receiving intensive insulin therapy (IIT), aimed at TGC utilizing a narrow target blood glucose range (4.4-6.1 mmol/l). The control group, which underwent the conventional treatment, only received insulin if the blood glucose concentration exceeded 12 mmol/l, with the target glucose range of 10-11.1 mmol/l. Blood glucose was monitored by analyzing arterial blood samples in a blood gas analyzer at 1-4 hour intervals. The blood glucose concentrations differed significantly between the groups, with the intervention group demonstrating lower blood glucose concentrations than the control group (5.7 ±1.1 mmol/l vs. 8.5 ±1.8 mmol/l, $p<0.0001$). The study showed that IIT significantly reduced mortality in patients admitted to the ICU for more than 5 days.[64] Although reduced morbidity has been revealed with IIT in solely medical ICU-patients, studies have not demonstrated the same mortality benefit,[65] suggesting that IIT is more beneficial in surgical critically ill patients. TGC has additionally been shown to result in lowered economic costs.[66]

Following the Leuven studies, many researchers tried to confirm the positive effects of TGC, but did not arrive at the same conclusions. On the contrary, no mortality benefit could be seen, and the incidence of hypoglycemia increased.[67] Two studies were even stopped prematurely because of the high incidence of hypoglycemia in patients treated according to a TGC protocol.[68, 69]

With this conflicting evidence in mind, a large multi-center study including 6022 patients was designed in order to answer the question of whether TGC should be implemented in critically ill patients, the so-called NICE-SUGAR study.[70] The NICE-SUGAR study randomized its patients to either IIT, with the target blood glucose range of 4.5-6.0 mmol/l, or to conventional glucose control, with the target of 10 mmol/l or less. The results directly opposed those of the Leuven study, as the authors reported a significant increase in 90-day mortality (IIT vs. conventional treatment: 27.5% vs. 24.9%, $p=0.02$). Monitoring of blood glucose was conducted using either test-strip POC glucometers or blood gas analyzers, but

there was no standardization as to how often the blood glucose concentration was measured. With the publication of the NICE-SUGAR results, TGC in critically ill patients was widely criticized.

The NICE-SUGAR study results were subsequently included in a meta-analysis of TGC vs. conventional GC in critically ill patients, in which no overall benefit of IIT was established.[71] The meta-analysis included 26 trials with a total of 13567 patients specifically investigating the effect of IIT on mortality. The authors obtained a risk ratio (RR) of 0.93 (95% CI 0.83-1.04), thus concluding that IIT does not result in reduced mortality. There was also an increased incidence of hypoglycemia among patients receiving IIT. However, because of the significant heterogeneity between the included studies, the authors suggested that patients in surgical ICUs may still benefit from IIT.[71]

2.3.2.2 Hypoglycemia

All studies investigating the effect of TGC have demonstrated an increased incidence of hypoglycemia among patients receiving IIT. In the NICE SUGAR study, the incidence of hypoglycemia was 6.8% in patients in the intervention group vs. 0.5% in the control group.[70] Both mild (<4.5 mmol/l) and severe (<2.2 mmol/l) hypoglycemia have been associated with increased mortality in critically ill patients.[72-74]

Several risk factors for developing hypoglycemia in critically ill patients have been identified: DM, septic shock, renal insufficiency, mechanical ventilation, severity of illness, need of inotropic support and treatment with IIT.[73, 75]

2.3.2.3 Glycemic variability

GV reflects how much the blood glucose concentration oscillates in an individual patient (see figure 4). High GV is an independent predictor of adverse outcome in critically ill patients,[76-79] as well as in patients with subarachnoid hemorrhage.[80] GV is better tolerated by patients with DM, as it has been shown that increased GV is only associated with increased mortality in patients without DM.[56]

Several different hypotheses may explain how GV affects patient outcome. Low GV may indicate better nursing care. It is also possible that patients with lower GV are healthier than patients with higher GV, or that GV by itself may have a detrimental effect. There are studies indicating that GV may lead to increased oxidative stress in patients with type 2 DM.[81] It has been suggested that IIT may improve patient outcome by reducing GV,[76] further establishing low GV as a therapeutic target.[82]

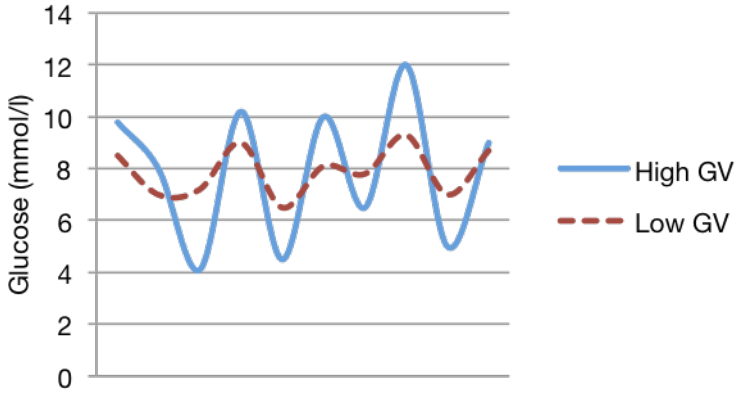

Figure 4. Glycemic variability illustrated by two different glucose concentration curves, with the same mean glucose concentration but varying variability.
GV – glycemic variability.

2.3.3 Glycemic control in cardiac surgery patients

The effect of GC varies in different patient categories. A more positive effect has been demonstrated in surgical ICU-patients,[71] and the benefit from GC is especially obvious in cardiac surgical patients.[83-85]

Perioperative hyperglycemia has been associated with adverse outcomes in patients undergoing cardiac surgery, and found to be an independent risk factor for all complications including death.[86-88] Hyperglycemia has further been shown to increase mortality and deep sternal wound infection, while treatment with continuous insulin infusions aimed at improving GC reduced the risk of death and infection in diabetic cardiac surgery patients.[89, 90] Postoperative hyperglycemia and high glycemic variability have also been linked to an increase in complications following coronary artery bypass grafting (CABG).[91, 92]

Studies dedicated to improving GC during cardiac surgery have arrived at varying conclusions, ranging from unaffected outcome[93] to a beneficial effect.[89, 90, 94-96] Treating patients with a glucose-insulin-potassium (GIK) solution during surgery and 12 hours postoperatively to achieve a blood glucose level in the range of 6.9-11.1 mmol/l, significantly reduced the incidence of atrial fibrillation, infection-related complications, and time on mechanical ventilation and resulted in a shorter postoperative ICU and hospital stay in diabetic patients undergoing cardiac surgery. Notably, a survival benefit for GIK-treated patients was detectable 2 years after surgery, and these patients also had lower incidence of recurrent ischemia.[95] A positive long-term effect of improved GC was also demonstrated in sub-group analysis of the cardiac surgery patients included in the original van den Berghe study.[97]

The use of continuous insulin infusion during surgery and for the first 2 days postoperatively reduced mortality in diabetic CABG patients, primarily due to a decrease in cardiac-related deaths.[98] Patients receiving insulin infusions had significantly lowered blood glucose

concentrations, implying that the benefit in outcome originates from improved GC. Furthermore, improved GC has been shown to reduce the risk of deep sternal wound infections.[99, 100] Targeting a lower glucose threshold of <6.7 mmol/l results in an increase in incidences of hypoglycemia and does not provide an additional clinical benefit compared to a more moderate approach targeting a blood glucose interval of < 10 mmol/l.[101] Thus, GC after cardiac surgery is important, but it should not necessarily be aimed at achieving blood glucose concentrations that are too low.[102]

2.4 MOLECULAR ASPECTS OF GLYCEMIC CONTROL

A question arising when discussing GC in critically ill patients is whether the effect on outcome depends on the achievement of normalized blood glucose concentrations, administration of insulin itself, or a combination of these factors. In a study investigating the effect of insulin infusions during and after cardiac surgery, patients receiving insulin infusions had significantly lowered blood glucose concentrations,[98] implying that the benefit in outcome not only originates from the administered insulin, but also from normalized blood glucose concentrations.

2.4.1 Insulin has anti-inflammatory effects

Significantly reduced levels of acute phase proteins (C-reactive protein and mannose-binding lectin) were found in patients treated with IIT compared to patients subjected to conventional GC in a sub-group of patients included in the original van den Berghe study. This implies that treatment with insulin exerts an anti-inflammatory effect bedsides lowering the blood glucose concentration.[103] IIT has further been shown to lower levels of pro-inflammatory cytokines in patients who have undergone a traumatic incident.[104] Thus the positive effect of IIT cannot solely be explained by normalized blood glucose concentrations.

2.4.2 The danger of hyperglycemia

Vascular disease is the principal cause of death in patients with diabetes. Patients with DM are prone to both macro- and microvasculature complications, for example atherosclerosis, nephropathy and retinopathy. This has resulted in hyperglycemia being regarded as a cause of vascular disease.[105]

There are four main hypotheses explaining how hyperglycemia may cause vascular damage: formation of advanced glycation end-products (AGEs) that activate the AGE-receptor, activation of protein kinase C, and stimulation of the polylol, and the hexosamine pathways (figure 5).[105] All these cellular pathways are activated by hyperglycemia-induced oxidative stress and overproduction of superoxide anion by the mitochondrial electron transport chain. It is believed that hyperglycemia leads to increased superoxide anion production by increasing the proton gradient as a result of overproduction of electron donors, i.e. NADH and $FADH_2$, generated in the citric acid cycle. The high proton gradient prolongs the lifetime of intermediate free radical forms of coenzyme Q (ubiquinone), which reduces oxygen to superoxide.[106]

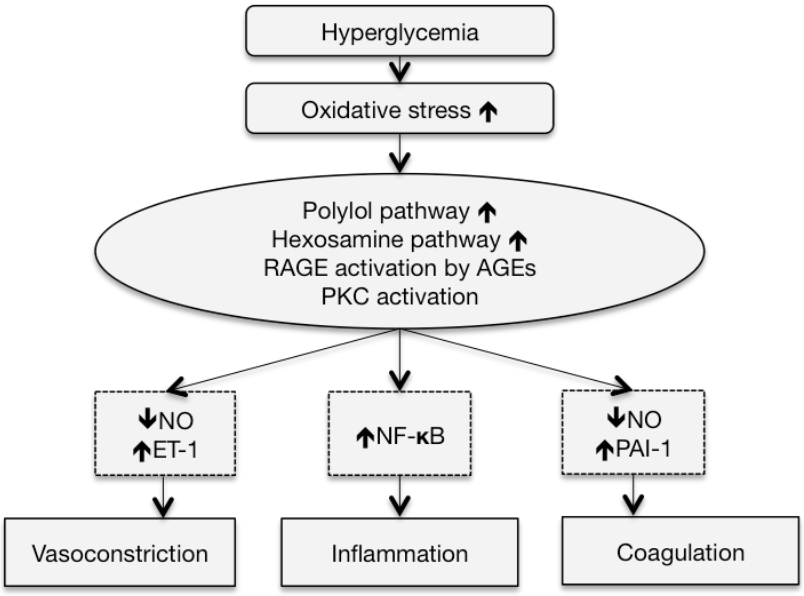

Figure 5. Overview of the molecular pathways of hyperglycemia-induced vascular damage. Hyperglycemia leads to oxidative stress that results in activation of several molecular pathways potentially responsible for vascular damage.
AGE – advanced glycation end products, ET-1 – endothelin-1, NF-κB – nuclear factor kappa beta, NO – nitric oxide, PAI-1 – plasminogen activator inhibitor-1, PKC – protein kinase C, RAGE – receptor for advanced glycation end-products, VSMC – vascular smooth muscle cell.

The activation of these four cellular pathways mediates vascular damage by affecting the endothelium in various ways (see figure 5). In the normal endothelium, several substances are produced and used to control and maintain vascular function. Nitric oxide (NO) is an important substance produced by endothelial NO synthase and responsible for vasodilation, as it affects vascular smooth muscle cells (VSMC).[107] NO protects from atherosclerosis by inhibiting the interaction between the vessel wall and platelets, VSMC migration and leukocyte adhesion.[108, 109] Hyperglycemia decreases endothelial NO concentration, resulting in vasoconstriction and vascular damage.[105] Not only is the hyperglycemic vascular damage characterized by the loss of the vasodilator effect of NO, but there is also an increased synthesis of vasoconstrictor substances, e.g. endothelin-1.

Hyperglycemia further results in increased activity of the pro-inflammatory transcription factor nuclear factor kappa beta (NF-κB), leading to increased expression of leukocyte adhesion molecules and production of cytokines that mediate an inflammatory response within the vessel wall.[110] This promotes atherosclerosis, as recruited macrophages take up cholesterol in the form of oxidized low-density lipoproteins by endocytosis via scavenger receptors, which transforms them into foam cells. The foam cells form fatty streaks that are characteristic of the initial stages of atherosclerosis. Hyperglycemia also affects platelet function, resulting in a pro-thrombotic state with increased synthesis of thrombin and impaired fibrinolysis due to increased levels of plasminogen activator inhibitor-1.[105, 111]

2.4.3 The danger of hypoglycemia

Studies investigating the physiological response to hypoglycemia suggest that it not only serves as a marker of severity of illness, but also exerts a harmful effect by itself. The level of IL-6 was found to be increased in healthy adults with induced hypoglycemia, suggesting that hypoglycemia may increase systemic inflammation.[112] An episode of hypoglycemia has also been shown to affect the autonomous nervous system, resulting in an inadequate sympathetic system response in subsequent hypoglycemia.[113]

2.5 BLOOD LACTATE IN CRITICAL ILLNESS

2.5.1 Monitoring of blood lactate

Blood lactate is an important biomarker in critically ill patients, and its measurement may aid hemodynamic monitoring[114] as well as be of prognostic value, as elevated lactate concentations have been associated with worsened outcome.[115-119] Monitoring of lactate concentrations may thus be useful in patients admitted to the ICU.[120-122] This also applies to patients undergoing cardiac surgery, who often develop hyperlactatemia postoperatively.[123] An elevated blood lactate level may be used to predict postoperative complications and has been associated with increased mortality in adults[124, 125] as well as in children.[126] Furthermore, studies have demonstrated that treatment aiming at normalizing elevated lactate levels is beneficial in critical illness such as septic shock.[127, 128] This has created interest in more standardized lactate monitoring in critically ill patients, and especially in assessing the lactate trend via repetitive measurements over time.[122, 129]

2.5.2 Control of blood lactate in critical illness

Early goal-oriented treatment in septic patients has become the standard care, since it was demonstrated that this resulted in significantly improved outcome and reduced mortality.[130] The Surviving Sepsis Campaign currently recommends monitoring of CVP, MAP, and SvO_2, as well as analyzing the initial blood lactate level, mostly for prognostic purposes. The use of SvO_2 as a hemodynamic parameter is potentially difficult as it requires special equipment, which may not routinely be available, in order to implement the early goal-oriented treatment. Regular analysis of blood lactate levels has instead been suggested to be useful, and it has been shown that this approach is not inferior to SvO_2 monitoring.[131]

Recently, studies have investigated the effect of lactate-guided treatment in critically ill patients. Lactate-guided treatment is a type of treatment aimed at lowering blood lactate levels. This treatment has been shown to be beneficial, and it may lead to improved outcome both in patients admitted to an ICU[132] and in patients undergoing cardiac surgery.[133] Thus, a system continuously monitoring blood lactate in such patients may be of value.

3 AIMS

The aims of this thesis were to develop intravascular microdialysis into a verified clinical method to be used for continuous glucose and lactate monitoring in patients undergoing cardiac surgery.

Specific aims:

I. To evaluate the method of intravascular microdialysis in order to investigate if it is safe and potentially useful for glucose and lactate monitoring using a separate microdialysis catheter and separate analysis of the dialysate fluid.

II. To determine the accuracy of intravascular microdialysis when a separate microdialysis catheter is connected to a sensor that continuously analyzes the glucose concentration in the dialysate fluid.

III. To verify the method of intravascular microdialysis when combing the microdialysis concept into a standard central venous catheter.

IV. To analyze the accuracy of intravascular microdialysis when it is used for continuous lactate monitoring using the data from Study II and Study III.

V. To investigate the performance of intravascular microdialysis during hypoglycemia, to determine if the accuracy is affected by glucose administration, and to test the responsiveness to high oscillations in blood glucose concentrations.

VI. To compare two different continuous glucose monitoring systems, the intravascular microdialysis system and a subcutaneous system, in patients undergoing cardiac surgery.

4 MATERIALS AND METHODS

The studies in this thesis were designed to evaluate the intravascular microdialysis technique for accuracy and safety, when used for monitoring of glucose and lactate both intermittently (in a separate analyzer) and continuously (using a special sensor). As a general approach, glucose and lactate values determined by the microdialysis system (test method) were paired with glucose and lactate values obtained with a reference method. These paired values were then compared to assess the accuracy. Table 1 summarizes the methodology used throughout the studies.

	No of participants	Reference method	Test method	Glucose or lactate analysis	MD-catheter used	Manner of dialysate fluid analysis
Study I	10	Art-BG/ Ven-BG/ P-Glu	MD	Both	DCC	Intermittent in separate analyzer
Study II	50	Art-BG	MD	Glucose	SLC	Continuous
Study III	30	Art-BG	MD	Glucose	TLC	Continuous
Study IV	80*	Art-BG	MD	Lactate	SLC+TLC	Continuous
Study V	9**	Ven-BG	MD	Glucose	TLC	Continuous
Study VI	26	Art-BG	MD/ SC-CGM	Glucose	TLC	Continuous

Table 1. Methodological summary for all studies included in this thesis.
** - Patients from Study II and Study III. ** - Animals (pigs).*
Art-BG – arterial blood gas, DCC – Dipylon cardiac catheter, MD – microdialysis, P-glu – plasma glucose, POC – point-of-care analysis of capillary blood, SC-CGM – subcutaneous continuous glucose monitoring, SLC – single lumen catheter, TLC – triple lumen catheter, Ven-BG – venous blood gas.

4.1 PATIENTS

All included patients were adults (>18 years of age) undergoing cardiac surgery with cardiopulmonary bypass (CPB) at the Karolinska University Hospital, Stockholm, Sweden. All participants had to sign a written consent form before inclusion, after having received both oral and written information about the study. Exclusion criteria were ongoing infection, a state with high risk of blood coagulation, anatomy unsuitable for safe insertion of a CVC, or other ongoing diseases rendering the patient unfit for inclusion.

No postoperative anticoagulation therapy was initiated during the first 24 hours after surgery. Patients that stayed in the ICU after the first 24 hours received the standard anti-thrombotic prophylactic therapy with low molecular weight heparin.

4.2 STUDY TECHNIQUES

4.2.1 Intravascular microdialysis

The microdialysis membrane was perfused with sodium chloride at a velocity of 5 ml/min. In Study I, the dialysate was collected in special containers, called microvials, and analyzed intermittently in the separate ISCUS Clinical Microdialysis Analyzer (CMA Microdialysis AB, Solna, Sweden). In all other studies, the microdialysis catheter was connected to the Eirus (Maquet Critical Care, Solna, Sweden) intravascular microdialysis system (figure 6), which continuously analyzes the dialysate fluid for glucose and lactate concentrations using a patient-specific disposable sensor and presents these values on a monitor. The glucose and lactate concentrations are analyzed every minute. The system is not able to measure glucose levels <1.0 mmol/l. The pump for delivery of perfusion fluid to the microdialysis catheter is integrated into the monitor.

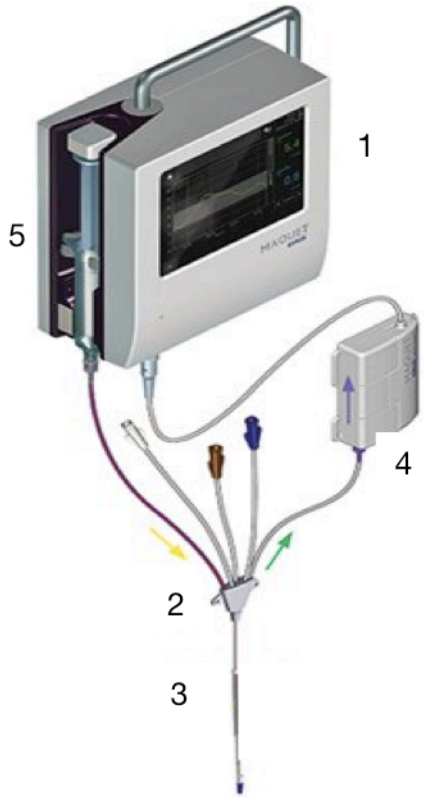

Figure 6. The Eirus intravascular microdialysis system; consists of 1) the monitor, 2) the microdialysis catheter with 3) the microdialysis membrane at its distal end, and 4) the sensor that continuously analyzes the glucose and lactate concentrations in the dialysate fluid perfused through the system by 5) the pump. In this illustration, a triple-lumen catheter is demonstrated, which provides a regular central venous access function as well as the microdialysis membrane.

The sensor analyzes the glucose concentration using the GO method previously described. Similarly, lactate concentration is measured by using lactate oxidase. The sensor is connected to a sensor reader, which converts the currents from the electrodes to digital signals that are handled by the monitor. The monitor converts the sensor signals to concentration values and presents these values to the user as a trend graph and a numerical value, with a time lag of 5 minutes, which is the time necessary to perfuse the system.

4.2.1.1 Microdialysis catheters

The semi-permeable microdialysis membrane is located on a specially designed catheter. Three different microdialysis catheters have been used throughout the studies (table 2). The catheters used in Study I (the Dipylon cardiac catheter – DCC) and Study II (the Eirus single lumen catheter – SLC) were separate microdialysis catheters with a single lumen, whereas in Studies III-VI the microdialysis catheter (the Eirus triple lumen catheter – TLC) had multiple lumens, providing central venous access as well as the microdialysis function. This technical development of the microdialysis catheter aims to minimize the number of catheters necessary, as all patients undergoing cardiac surgery are in need of central venous access both for blood sampling and for drug administration. The Eirus TLC has three infusion channels: one end hole and two side holes. The end hole is situated 20 mm from the side holes, which are placed 10 mm apart. All infusion channels are distal to the microdialysis membrane.

Catheter	Used in Study	Lumens (no)	Diameter (Fr)	Length (cm)	Manufacturer
DCC	I	1	4	67	CMA Microdialysis AB, Solna, Sweden
SLC	II	1	4	30	Dipylon Medical AB, Solna, Sweden
TLC	III, IV, V, VI	3	7	20	Maquet Critical Care, Solna, Sweden

Table 2. Specification of the microdialysis catheters used throughout the studies.
DCC – Dipylon cardiac catheter, SLC – single lumen catheter, TLC – triple lumen catheter.

4.2.1.2 Calibration

The ISCUS Clinical Microdialysis Analyzer is calibrated using solutions with known glucose and lactate concentrations at specified time intervals supplied by the machine itself. The Eirus intravascular microdialysis system is calibrated every 8 hours by manually entering the

reference glucose and lactate concentrations. If calibration is not performed, a warning box will appear on the monitor notifying the user that the displayed values are not calibrated.

4.2.2 Subcutaneous continuous glucose monitoring system

The FreeStyle Libre (Abbott Diabetes Care Inc., Alameda, CA, USA) is a subcutaneous CGM (SC-CGM) system. The system consists of a small sensor (approximately 2 cm in diameter) that is placed subcutaneously in the upper arm and a sensor reader that scans the sensor within a distance of 1-4 cm, and displays the glucose value (figure 7). The sensor automatically analyzes the glucose concentration in the subcutaneous interstitial space every 15 minutes, utilizing the GO method. The sensor needs to be scanned every 8 hours and is approved for use for up to 14 days. The sensors are pre-calibrated by the manufacturer and cannot be re-calibrated. The system requires a 1-hour warm-up period after the sensor is inserted before blood glucose can be analyzed. The sensor reader has an additional function as a POC glucometer, and can be used to analyze glucose in capillary blood using special analyzing strips that are inserted into the sensor reader.

Figure 7. The FreeStyle Libre subcutaneous continuous glucose monitoring system consists of 1) the sensor-reader and 2) the sensor.

4.2.3 Blood gases

Analysis of arterial and/or venous blood samples in a blood gas analyzer was used as the reference method to compare the glucose and lactate values obtained by microdialysis.

The blood gas analyzer used was ABL800 FLEX (Radiometer Medical, Copenhagen, Denmark), which utilizes the GO method for glucose measurement.

4.2.4 Laboratory analyses

In Study I, the hospital's laboratory measured plasma glucose every 4 hours. The plasma glucose level was analyzed by the GO method using the Beckman Coulter DxC 8000 instrument (Beckman Coulter Inc., Brea California, United States of America).

4.3 STUDY PROTOCOLS

4.3.1 Study I

Aim: To evaluate the method of intravascular microdialysis in order to determine if it is safe and potentially useful for glucose and lactate monitoring using a separate microdialysis catheter and separate analysis of the dialysate fluid.

Patients: Ten patients undergoing cardiac surgery with CPB between April and May 2009 were included.

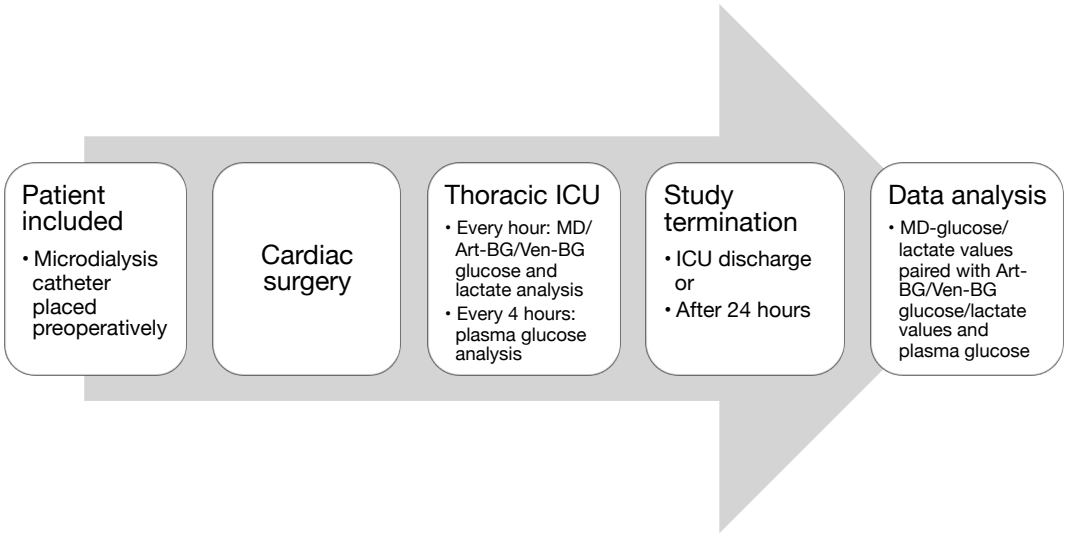

Figure 8. Flow chart of Study I.
Art-BG – arterial blood gas, ICU – intensive care unit, MD – microdialysis, P – plasma, Ven-BG – venous blood gas.

Protocol: The DCC separate microdialysis catheter was preoperatively placed in the superior vena cava. Microvials were inserted in the outlet of the catheter every hour to collect dialysate fluid that was subsequently analyzed for glucose and lactate concentrations in the ISCUS Clinical Microdialysis Analyzer. Arterial and venous blood samples were obtained simultaneously with dialysate fluid collection, and glucose and lactate concentrations were measured by a blood gas analyzer. In addition, a blood sample was sent to the hospital's laboratory for analysis of plasma glucose every four hours (in nine patients). Analysis of glucose and lactate started with the patient's arrival in the ICU after surgery and was terminated after a maximum of 24 hours or upon discharge from the ICU, depending on which event occurred first. A flow chart is presented in figure 8.

Evaluation: Microdialysis glucose and lactate values were paired with reference values obtained from arterial and venous blood gas analysis. All paired samples were then analyzed

for accuracy. Additionally, glucose and lactate values obtained by arterial blood gas analysis were compared to results of venous blood gas analysis in order to determine if these two methods could be used interchangeably as reference methods in future studies. All data obtained with glucose monitoring methods were also compared to the plasma glucose levels determined by the hospital's laboratory.

4.3.2 Studies II and III

Aim: To determine the accuracy of intravascular microdialysis when a separate microdialysis catheter is connected to a sensor that continuously analyzes the glucose concentration in the dialysate fluid (Study II) and to verify the accuracy of the method when the microdialysis concept is implemented in a standard CVC (Study III).

Patients: Fifty patients undergoing cardiac surgery with CPB between March and July 2010 (Study II) and 30 patients undergoing cardiac surgery between May and August 2011 (Study III) were included.

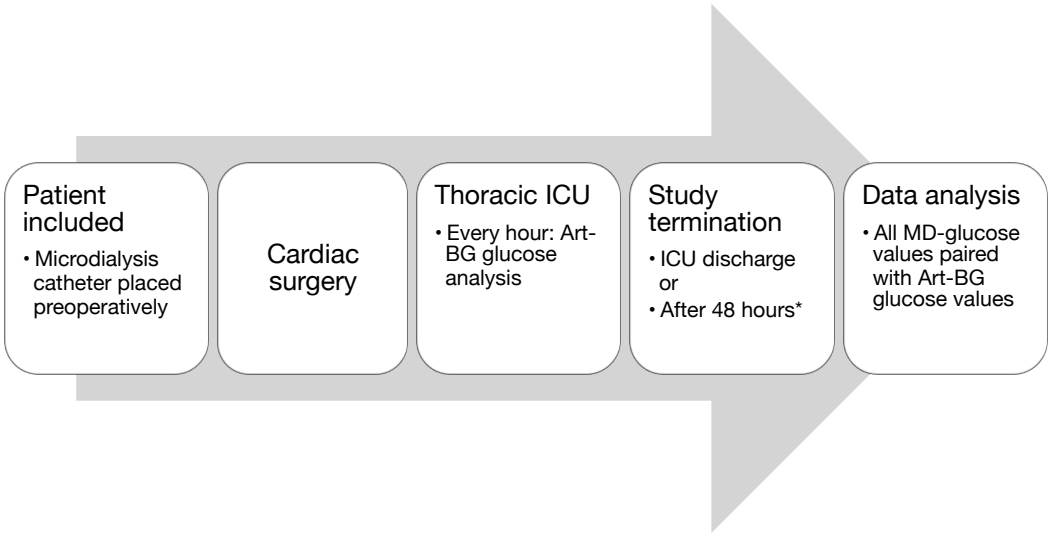

Figure 9. Flow chart of Study II and Study III.
** = In Study III, the monitoring period could be extended beyond 48 hours as the patients had the microdialysis catheter in place longer. Monitoring beyond 48 hours was conducted occasionally.*
Art-BG – arterial blood gas, ICU – intensive care unit, MD – microdialysis.

Protocol: The microdialysis catheter (Study II: Eirus SLC, Study III: Eirus TLC) was preoperatively placed in the superior vena cava/right atrium and connected to the Eirus intravascular microdialysis system. CGM was initiated after calibration of the microdialysis system. Reference glucose values were determined every hour by analyzing an arterial blood sample in a blood gas analyzer, and the time when the blood was sampled was documented. Patients were monitored for up to 48 hours or until they were discharged from the ICU, depending on which event occurred first. A flow chart is presented in figure 9. The glucose values obtained by the intravascular microdialysis system were not used for guiding treatment with insulin. All patients routinely received 5% glucose infusion (1 ml/kg/h) in the ICU and

insulin infusions were used if needed, with the target blood glucose concentration range both during and after cardiac surgery set at 5-10 mmol/l.

In Study III, the patients had the microdialysis catheter with CVC function in place as long as a central line was necessary. Thus, in some patients, the microdialysis system could be used for glucose analysis after the 48-hour study period. This analysis was done occasionally at random times in 4 patients, resulting in a total of 6 paired MD/Art-BG glucose values. The Eirus TLC could be used for blood sampling and drug administration in the postoperative period. After removal of the catheter, the microdialysis membrane was checked for potential blood clotting.

Evaluation: Microdialysis glucose values were paired with reference arterial blood gas glucose values and analyzed for accuracy. Continuous glucose values are presented on the monitor with a lag time equal to the time required to perfuse the microdialysis system. This time lag was estimated to be 10 minutes in Study II and 5 minutes in Study III, the difference being caused by difference in lengths of the microdialysis catheters (see table 2). To describe the technical accuracy of the microdialysis system better, the microdialysis glucose values were adjusted for the time lag when paired with respective arterial blood gas glucose values.

4.3.3 Study IV

Aim: To analyze the accuracy of intravascular microdialysis when it is used for continuous lactate monitoring using data from Study II and Study III.

Patients: All patients from Study II (n=50) and Study III (n=30) were included.

Protocol: No new study was conducted; instead existing data from Study II and Study III were analyzed. Study protocols for Study II and Study III are outlined above.

Evaluation: The microdialysis lactate values were paired with reference arterial blood gas lactate values and analyzed for accuracy. If a high lactate level (>3 mmol/l) was detected, a possible association with an adverse event was investigated.

4.3.4 Study V

Aim: To investigate the performance of intravascular microdialysis during hypoglycemia, to determine if the accuracy is affected by glucose administration, and to test the responsiveness during high oscillations in blood glucose concentrations.

Animal model: As there were no hypoglycemic incidences in Studies I, II, or III, the intravascular microdialysis system was not adequately evaluated in the setting of low blood glucose concentrations. Thus, an animal model using pigs was developed, and set up as displayed in figure 10. In total, 10 pigs were used (the first pig was a pilot object used to establish the study protocol; corresponding data were not included in the results). The animals were equipped with the Eirus intravascular microdialysis system using the Eirus TLC, which was used for all blood sampling and drug administration.

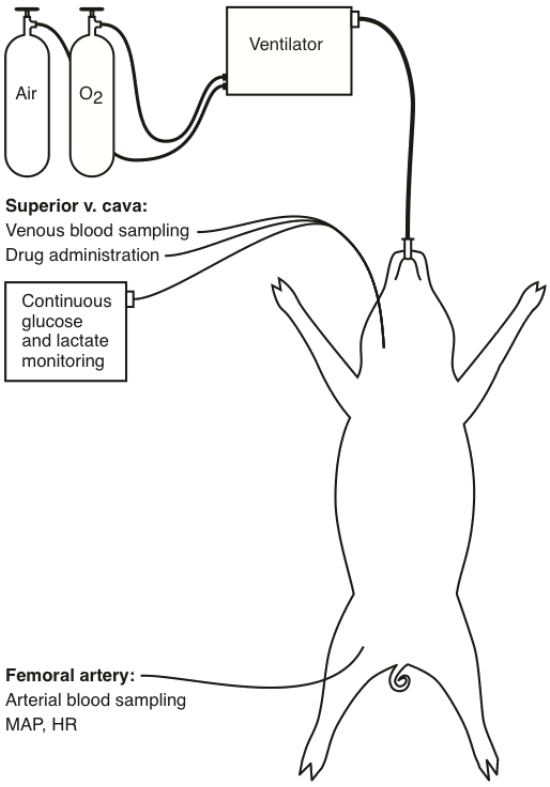

Figure 10. Illustration of the animal model used in Study IV.

After induction of general anesthesia, the microdialysis catheter was inserted in the superior vena cava, and CGM was initiated. Hypoglycemia was then induced with an insulin bolus dose (8 units of NovoRapid (Novo Nordisk Scandinavia AB)); this insulin administration was considered the beginning of the analysis period (time 0). After 30 minutes, 30 ml bolus dose of 30% glucose was given to reverse the hypoglycemia and at 40 minutes, glucose infusion at 100 ml/h was initiated. At 100 min, another insulin bolus dose consisting of 8 units of NovoRapid was given, and CGM was conducted for another 20 minutes (see figure 11). At the end of the study period, the animals were sacrificed. The position of the microdialysis catheter was controlled post mortem.

Arterial and venous blood samples were collected every five minutes and analyzed in a blood gas analyzer for glucose concentrations. The microdialysis system was calibrated once at the beginning of the monitoring period (before hypoglycemia was induced) using the arterial blood gas glucose level.

1	0 min	Insulin (NovoRapid), 8E bolus
2	30 min	Glucose 30%, 30 ml bolus
3	40-100 min	Glucose 30%, 100ml/h infusion
4	100 min	Insulin (NovoRapid), 8E bolus

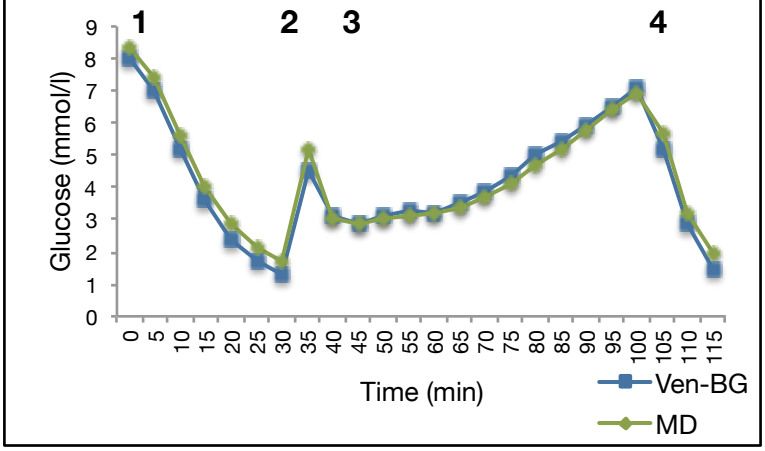

Figure 11. The study protocol for Study V is illustrated using a typical time course of glucose concentration. The different phases of the study are numbered 1-4.
MD – microdialysis, Ven-BG – venous blood gas.

Evaluation: The microdialysis glucose values were paired with reference venous blood gas glucose values and analyzed for accuracy. To describe the technical accuracy of the microdialysis system better, the microdialysis glucose values were adjusted for the time lag (5 minutes) when paired with respective venous blood gas glucose value. Glucose level time courses were plotted to evaluate the responsiveness of intravascular microdialysis during rapid glucose fluctuations. To further evaluate the responsiveness of the microdialysis system and to assess the potential influence of glucose administration, the mean absolute relative difference (MARD) between the different study phases was compared.

4.3.5 Study VI

Aim: To compare two different continuous glucose monitoring systems, the intravascular microdialysis system and a subcutaneous system, in patients undergoing cardiac surgery.

Patients: Twenty-six patients undergoing cardiac surgery with CPB between October and December 2015 were included.

Protocol: All patients were equipped with both the Eirus intravascular microdialysis CGM (MD-CGM) system and the FreeStyle Libre subcutaneous CGM (SC-CGM) system. The sensor of the FreeStyle Libre system was inserted the day before surgery subcutaneously in the left upper arm. The microdialysis catheter (Eirus TLC) was inserted in the operating room in the superior vena cava/right atrium after the induction of general anesthesia, and connected to the microdialysis monitoring system. An arterial blood sample was obtained every hour, and the glucose concentration was analyzed in a blood gas analyzer. A capillary blood sample

was analyzed using the POC function of the FreeStyle Libre sensor reader every 4 hours. The SC-CGM system was scanned every 4 hours. The MD-CGM system was calibrated every 8 hours.

Evaluation: Both the MD-CGM and the SC-CGM glucose values were paired with reference arterial blood gas glucose values and analyzed for accuracy. To describe the technical accuracy of the microdialysis system better, the microdialysis glucose values were adjusted for the time lag (5 minutes). The MD-CGM system provides a glucose value every minute, while the SC-CGM system stores the glucose value every 15 minutes. In order to match the arterial blood gas glucose value taken at a specific time with the correct SC-CGM glucose value, a glucose level time course was plotted using the SC-CGM glucose values. The SC-CGM glucose value was then extracted from this graph. POC glucose values were also compared with reference arterial blood gas glucose values.

4.4 STATISTICAL ANALYSES

All studies included in this thesis compared different techniques of glucose and lactate monitoring. Glucose monitoring devices can be evaluated in different ways and for two different aspects of accuracy: technical and clinical. Clinical accuracy is sufficient when the test glucose monitoring method does not report a glucose concentration leading to a clinical action different from the action that would have been taken based on the reference method. Technical accuracy evaluates how much the values determined by the test method actually differ from the reference values.

In this thesis, the glucose monitoring accuracy of the intravascular microdialysis system was compared to a chosen reference method using the following analyses: Bland-Altman [134], Clarke error grid analysis (EGA),[135, 136] International Organization for Standardization (ISO) criteria,[137] and correlation coefficients.[138] Accuracy of microdialysis lactate monitoring was compared to a chosen reference method using Bland-Altman analysis and correlation coefficients. Furthermore, the MARD was calculated.

Statistical analyses were performed using IBM SPSS Statistics, version 22 (IBM, N.Y., USA) in all studies, except for Study VI, in which version 23 was used. Bland-Altman and EGA analyses were performed using Microsoft Excel (Microsoft, W.A., USA). Overall associations between intravascular microdialysis and the reference method were evaluated using the Pearson correlation coefficient. Additionally, in Study IV, the overall association between microdialysis and arterial blood gas lactate concentrations was assessed based on a regression coefficient, which was calculated using a random coefficient mixed model in SAS Statistical software, version 9.3 (SAS Institute Inc., N.C., USA). A Kruskal-Wallis test was performed in Study V to compare the MARDs in different study phases, and in Study VI to compare the MARDs in different blood glucose ranges.

4.4.1 MARD

MARD is often used when evaluating a test measurement method. It is calculated by the following formula:

$$+ \frac{(\text{Test method} - \text{Reference method})}{\text{Reference method}}$$

A MARD <14% has been suggested to indicate a good agreement between test and reference methods.[139] Depending on the distribution of data, it is sometimes more correct to calculate the median absolute relative difference instead of the MARD. In Study VI, both the median absolute relative difference and the MARD were employed.

4.4.2 Bland-Altman analysis

Bland-Altman analysis is a method commonly used in medical science to compare results of two different measurement methods.[134] The analysis plots the difference between the test and reference methods against their average and may be used to calculate the mean difference and limits of agreement (mean difference ±1.96SD). The correctness of use of Bland-Altman analysis in comparative studies that include multiple paired samples per patient, as is the case in our studies, has been questioned. To avoid possible inaccuracies, we double-checked the results from the Bland-Altman analyses by using the software MedCalc Statistics, version 12.7.5 (MedCalc Software, Ostend, Belgium) that takes this problem into account.

4.4.3 Clarke error grid analysis

The Clarke EGA evaluates the clinical accuracy of the test glucose monitoring method in comparison with the reference method by plotting paired values in five distinct zones of different significance (see figure 12).[135, 136] Paired values in zone A are within 20% of the reference value and their use has no clinical implications. Values in zone B exceed 20% from the reference value, but their use still leads to appropriate clinical decisions. Values in zone C may lead to unnecessary but harmless corrections. Values in zones D and E represent overestimation for hypoglycemia (failure to detect) or underestimation for hyperglycemia that may lead to incorrect clinical actions. In short, the more paired values located in zones A and B, the better is the clinical accuracy of the test method.

The EGA was originally constructed to compare intermittent glucose values, and its use in critically ill patients, and the correctness of this approach when utilized for analyzing continuous glucose data, has been questioned since it does not consider the rate at which blood glucose concentration is changing. Although an updated EGA for CGM devices has been developed, we chose to use the original EGA because our chosen reference method (analysis of arterial or venous blood gases) was employed intermittently.[140] In addition, the continuous EGA is difficult to use and therefore not very popular. Because of this, it is likely easier to compare the results between different studies when they are analyzed with the original EGA.

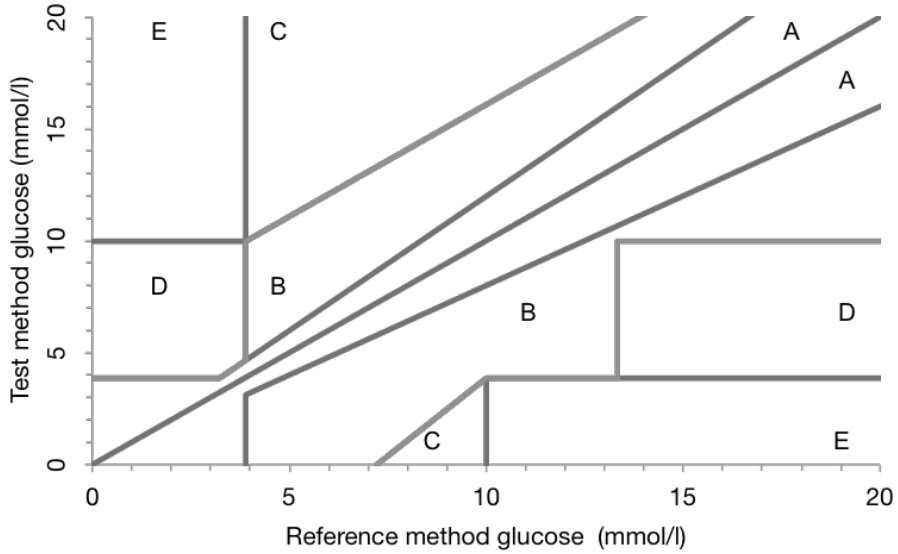

Figure 12. Example of the Clarke error grid for evaluation of the clinical accuracy of a test glucose monitoring method in comparison with a reference method. Paired values in zone A are within 20% of the reference value, whereas values in zone B differ by more than 20% but their use leads to the same clinical actions. The use of values in zone C may lead to harmless but incorrect clinical actions, while utilization of values in zones D and E may lead to harmful and incorrect clinical actions.

4.4.4 International Organization for Standardization criteria

The ISO criteria (ISO15197:2003) for glucose monitoring devices states that 95% of test glucose values have to be within 20% of the reference value, if the reference value is >4.1 mmol/l, and within 0.8 mmol/l, if the reference value is <4.1 mmol/l.[137] After an update in 2013 (ISO15197:2013), the criteria require test glucose values to be within 15% of the reference value, if the reference value is >5.5 mmol/l, and within 0.8 mmol/l, if the reference value is <5.5 mmol/l.[141] During a consensus meeting discussing GC in the ICU, new criteria for accuracy of glucose monitoring devices were suggested, requiring 98% of test glucose values to be within 12.5% of the reference value (or within 0.55 mmol/l for readings <5.5 mmol/l) and the remaining 2% of test glucose values to be within 20% of the reference value.[44] In this thesis, microdialysis glucose values have been evaluated according to the original ISO criteria of 2003, but as the update of 2013 arrived, this was also employed for the analysis of the results from Study VI.

4.5 ETHICAL CONSIDERATIONS

All patients provided written and oral informed consent prior to study participation after being given both written and oral information. All studies were approved by the Regional Ethics Review Committee in Stockholm, application no 2007/1268-31 with acceptance as of 2007-12-14. The following amendments to the original ethical application have been accepted: 2008/742-32, 2008/1608-32, 2008/1897-32, 2009/143-32, 2009/1622-32, 2010/0109-32, 2011/467-32, 2011/1776-32/1, 2014/1479-32, and 2015/952-32.

In Study V, the ethical permission for the animal experiments was obtained from the Stockholm Regional Ethical Committee, application no N397/09 with acceptance as of 2009-12-17. All animals received humane care in compliance with the European Convention on Animal Care, and the investigation conformed to the Guide of Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1985).

5 RESULTS

The results from the studies included in this thesis demonstrate that intravascular microdialysis is a safe and accurate method for continuous monitoring of both glucose and lactate in critically ill patients undergoing cardiac surgery.

5.1 PATIENTS

Characteristics of the patients included in the studies in this thesis are outlined in table 3.

	Study I	Study II	Study III	Study VI
Sex (male/female)	8/2	37/11	19/11	21/3
Age (years)	65 ±11	68 ±14	67 ±12	66 ±9
BMI (kg/m^2)	24.8 ±3.0	26.6 ±4.4	27.1 ±4.8	27.2 ±3.9
History of diabetes (no)	3	10	11	6
Treated with insulin	1	3	4	3
Treated with oral anti-diabetics	2	7	7	3
EuroSCORE (%)	1.23 ±0.6	6.9 ±7.3	5.1 ±4.5	1.79 ±0.8
Surgical procedure (no)				
CABG	9	25	8	6
Valve surgery	1	20	19	14
Other	0	3	3	4
Time on ventilator (h)	4.2 ±3.4	7.5 ±3	11 ±11.5	7 ±1.5
Length of ICU stay (h)	40 ±48	60 ±169	73 ±129	29 ±20
Length of hospital stay (days)	8.0 ±2.4	6.9 ±7.3	7.9 ±5.8	5.5 ±2

Table 3. Characteristics of the patients included in the studies in this thesis. All values are presented either as numbers or mean ±SD.
BMI – Body mass index, CABG – coronary artery bypass grafting, ICU – intensive care unit.

5.2 ACCURACY OF INTERMITTENT MONITORING OF GLUCOSE AND LACTATE WITH MICRODIALYSIS – RESULTS FROM STUDY I

The main finding from Study I was that the intravascular microdialysis system was found to be a potentially useful method for monitoring of both glucose and lactate in critically ill patients. Table 3 demonstrates the results of accuracy evaluation.

Both arterial and venous blood gas glucose values were accurate when compared to plasma glucose values analyzed in the hospital's laboratory. A comparison between arterial and venous blood gas measurements revealed low mean difference and good agreement regarding both glucose and lactate analysis.

	MD/Art-BG	MD/Ven-BG	MD/P	Art-BG/P	Ven-BG/P	Ven-BG/Art-BG
Glucose						
No of samples	174	174	36	37	37	177
Bland-Altman (mmol/l)						
Mean difference	-0.07	-0.09	-0.19	0.27	0.29	0.02
Limits of agreement (±2SD)	1.4	1.8	1.6	0.6	1.2	1
MARD (%)	8.6	9.8	7.6	4.1	6.6	4.3
EGA (%)						
Zone A	93	88	92	100	98	99
Zones A+B	100	99	100	100	100	100
ISO 2003 criteria met (%)	93	89	92	100	97	99
Pearson	0.93	0.89	0.94	0.99	0.96	0.96
Lactate						
No of samples	174	174	-	-	-	177
Bland-Altman (mmol/l)						
Mean difference	0.33	0.24	-	-	-	0.09
Limits of agreement (±2SD)	0.6	0.6	-	-	-	0.2
MARD (%)	28.8	21.2	-	-	-	11
Pearson	0.92	0.93	-	-	-	0.97

Table 3. Results from Study I.
Art-BG – arterial blood gas, EGA – Clarke error grid analysis, ISO – International Organization for Standardization criteria for glucose accuracy, MAD – mean absolute difference, MARD – mean absolute relative difference, MD – microdialysis, MRD – mean relative difference, P – plasma glucose, Pearson – Pearson correlation coefficient, Ven-BG – venous blood gas.

5.3 ACCURACY OF CONTINUOUS GLUCOSE MONITORING WITH MICRODIALYSIS

In general, the intravascular microdialysis system was shown to be useful, safe, and accurate for CGM in patients undergoing cardiac surgery.

5.3.1 Accuracy in normo- and hyperglycemia – results from Study II and Study III

Intravascular microdialysis was used for CGM in both Study II and Study III. Comparison of microdialysis glucose values with the reference method (analysis of an arterial blood gas) revealed good accuracy. A summary of the results is shown in table 4.

	Study II	Study III
No of paired samples	994	607
MD glucose values (mmol/l)		
Mean	8.27	8.52
Min	4.5	4.65
Max	15.4	23.25
Art-BG glucose values (mmol/l)		
Mean	8.25	8.64
Min	4.8	4.9
Max	14.4	22.5
Bland-Altman analysis (mmol/l)		
Mean difference	0.02	-0.12
Limits of agreement (±2SD)	1.1 to -1.1	1.25 to -1.5
MARD (%)	5	5.6
EGA (%)		
Zone A	99	97
Zones A+B	100	100
ISO 2003 criteria met (%)	99.2	97.2
Pearson	0.92 (p<0.0001)	0.92 (p<0.0001)

Table 4. Results from Study II and Study III.
Values are presented either as counts or as means (range, median).
Art-BG – arterial blood gas, EGA – Clarke error grid analysis, ISO – International Organization for Standardization criteria for glucose accuracy, MAD – mean absolute difference, MARD – mean absolute relative difference, MD – microdialysis, MRD – mean relative difference, Pearson – Pearson correlation coefficient.

The Bland-Altman analysis demonstrated low mean difference in both Study II and Study III (see figure 13), and the limits of agreement were near ±1 mmol/l in both studies. EGA analysis revealed a high clinical accuracy, with 100% of paired values within zones A and B (see figure 14).

None of the patients in Study II or Study III experienced hypoglycemia. Thus, the accuracy of intravascular microdialysis used for CGM in the hypoglycemic range could not be evaluated in these studies. The target blood glucose concentration range in the ICU is 5-10 mmol/l at our clinic, and the glucose levels in were within this range 88% (Study II) and 85% (Study III) of the time.

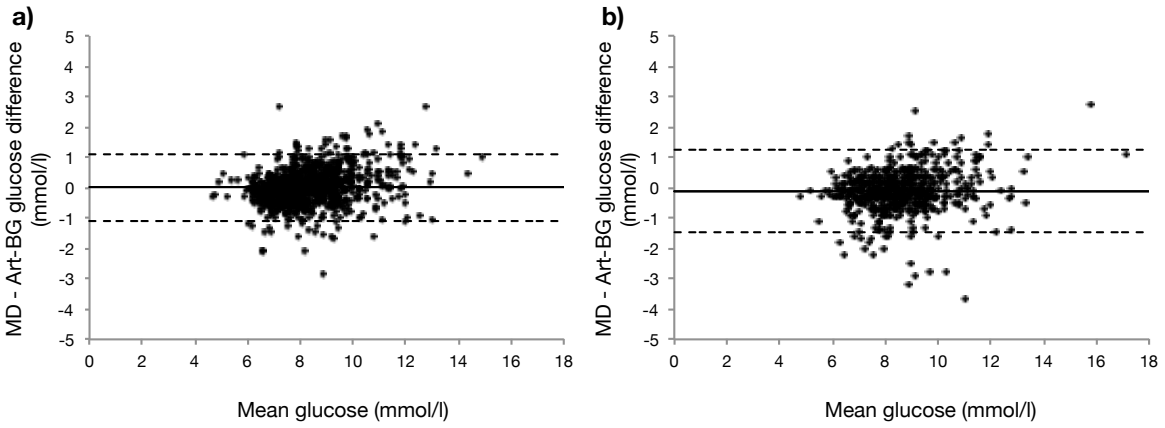

Figure 13. Bland-Altman analysis of paired microdialysis and arterial blood gas glucose values from a) Study II and b) Study III. The straight line represents the mean difference, and the dotted lines are the limits of agreement (mean difference ±1.96SD).
Art-BG – arterial blood gas, MD – microdialysis.

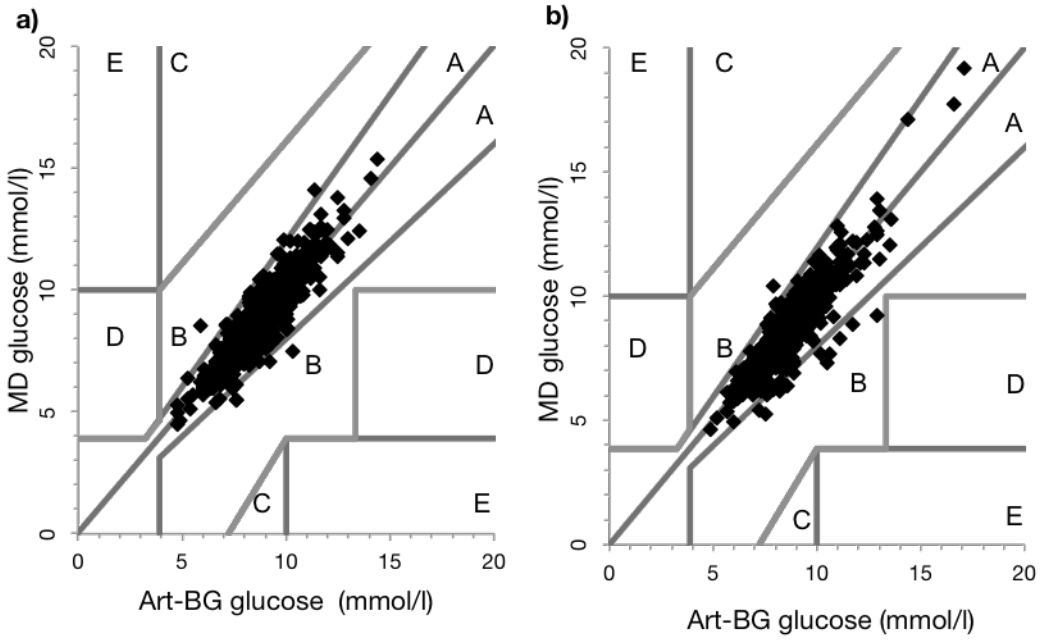

Figure 14. Clarke error grid analysis of paired microdialysis and arterial blood gas glucose values from a) Study II and b) Study III. All paired samples were within zones A and B.
Art-BG – arterial blood gas, MD – microdialysis.

5.3.2 Accuracy in hypoglycemia – results from Study V

In Study V, the glucose monitoring accuracy of the intravascular microdialysis system was evaluated in hypoglycemia using an animal model. A total of 213 paired microdialysis and venous blood gas glucose values were obtained. Of these, 59.2% (n=126) were in the hypoglycemic range (<4.1 mmol/l). The mean microdialysis glucose value was 3.89 mmol/l, and the mean arterial blood gas glucose value was 3.91 mmol/l. The lowest and highest microdialysis glucose values were 1.0 mmol/l (the method cannot measure glucose levels <1.0 mmol/l) and 8.9 mmol/l respectively, and the corresponding venous blood gas glucose

values were 0.4 mmol/l and 9.1 mmol/l. Bland-Altman analysis showed a low mean difference of 0.01 mmol/l, with limits of agreement of ±0.9 mmol/l (see figure 15).

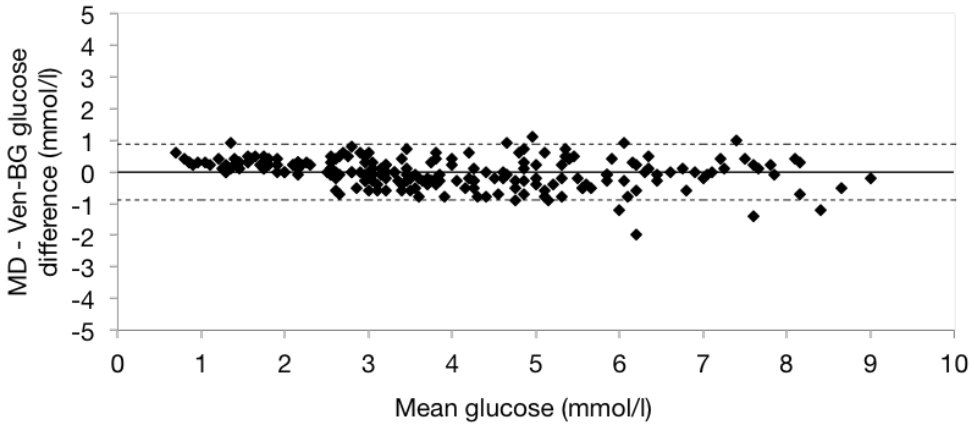

Figure 15. Bland-Altman analysis of paired microdialysis and venous blood gas glucose values from Study V. The straight line represents the mean difference, and the dotted lines are the limits of agreement (mean difference ±1.96SD). The mean difference was 0.01 mmol/l, and the limits of agreement were 0.87 to -0.89 mmol/l.
MD – microdialysis, Ven-BG – venous blood gas.

The MARD was 11.8%. In Clarke EGA, all paired values were within zones A and B, and 99% of them were situated in zone A (see figure 16). The ISO criteria were fulfilled, as 97.7% of the paired values were within the reference guidelines. The Pearson correlation coefficient was 0.97 ($p < 0.0001$).

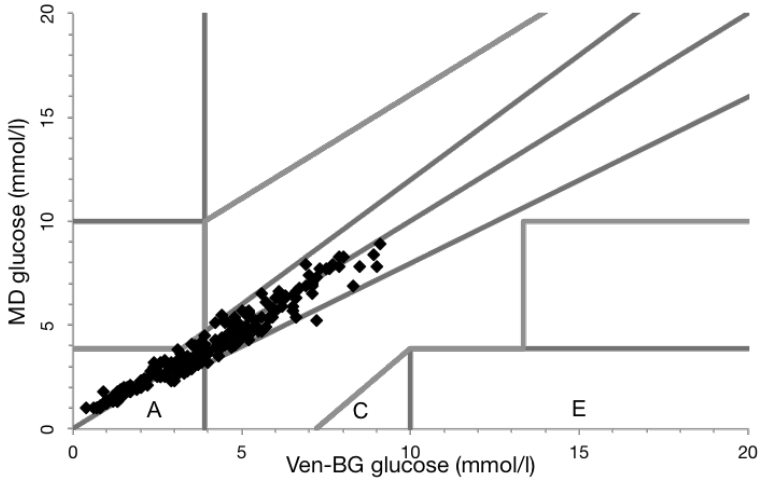

Figure 16. Clarke error grid analysis of paired microdialysis and venous blood gas glucose values from Study V. All paired values were within zones A and B, and 99% of them were within zone A.
MD – microdialysis, Ven-BG – venous blood gas.

5.3.3 Responsiveness and influence during glucose administration – results from Study V

The responsiveness of the intravascular microdialysis system during rapid blood glucose oscillations and the potential influence of glucose administration were also investigated in Study V. The system was found to be very responsive, as illustrated in figure 17, which presents the time course of glucose levels determined with different glucose monitoring methods. Figure 17 also visualizes another recurrent finding in all animals: arterial blood gas glucose values were consistently higher than microdialysis and venous blood gas glucose values during glucose administration (both for bolus dose and infusion), presumably because of the very high concentration of glucose administered in a central vein.

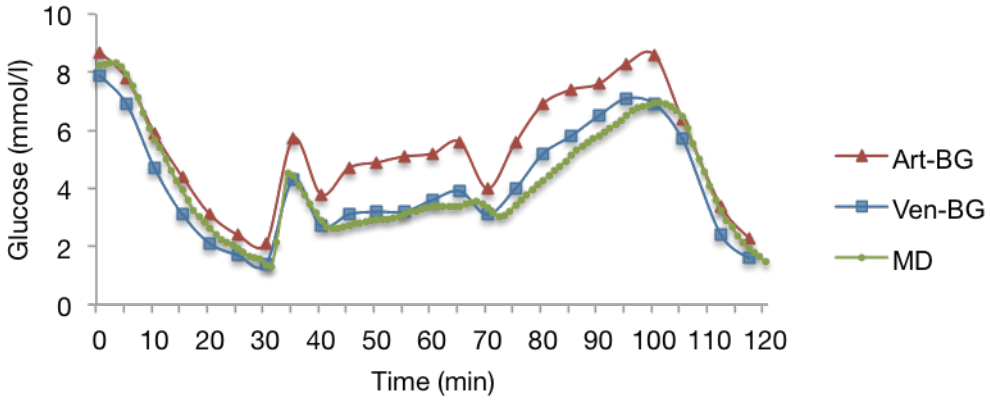

Figure 17. The time course of glucose levels in a single animal in Study V. The Art-BG glucose concentration was higher than both Ven-BG and MD glucose concentrations during glucose administration. The study phases are outlined in figure 11.
Art-BG – arterial blood gas, MD – microdialysis, Ven-BG – venous blood gas.

Furthermore, the glucose monitoring accuracy of the microdialysis system was not influenced by glucose administration via the microdialysis catheter (either for bolus dose or glucose infusion), as there was no significant difference between the MARDs of the microdialysis and venous blood gas glucose values during the four different study phases (see table 5), as revealed by a Kruskal-Wallis test.

Study phase	**Mean MD glucose** (mmol/l)	**Mean Ven-BG glucose** (mmol/l)	**Mean difference** (MD – Ven-BG in mmol/l)	**MARD** (%)
1) Insulin bolus dose	4.33	4.05	0.28	12
2) Glucose bolus dose	3.75	3.34	0.41	20
3) Glucose infusion	3.76	4.03	-0.27	8
4) Insulin bolus dose	2.26	2.54	0.06	18

Table 5. Blood glucose values during the different phases of Study V. The study phases are outlined in figure 11. No significant difference was found between the MARDs of the different phases.
MARD – mean absolute relative difference, MD – microdialysis, Ven-BG – venous blood gas.

5.4 ACCURACY OF CONTINUOUS LACTATE MONITORING WITH MICRODIALYSIS – RESULTS FROM STUDY IV

The intravascular microdialysis system was found to be safe and accurate for continuous lactate monitoring in patients undergoing cardiac surgery. The microdialysis lactate values were paired with arterial blood gas lactate values obtained from Study II and Study III. Accordingly, a total of 1601 (Study II = 994 and Study III = 607) paired microdialysis-arterial blood gas lactate values were included in analysis.

The mean microdialysis lactate value was 1.40 mmol/l, and the mean arterial blood gas lactate value was 1.38 mmol/l. The lowest and highest microdialysis lactate values were 0.35 mmol/l and 6.07 mmol/l respectively, and the corresponding arterial blood gas lactate values were 0.4 mmol/l and 6.7 mmol/l. Bland-Altman analysis demonstrated a low mean difference of 0.02 mmol/l, and the limits of agreement were ±0.4 mmol/l (see figure 18). The MARD was 10.3%. The regression coefficient was 0.98 ($p < 0.0001$) and the Pearson correlation coefficient was 0.96 ($p < 0.0001$).

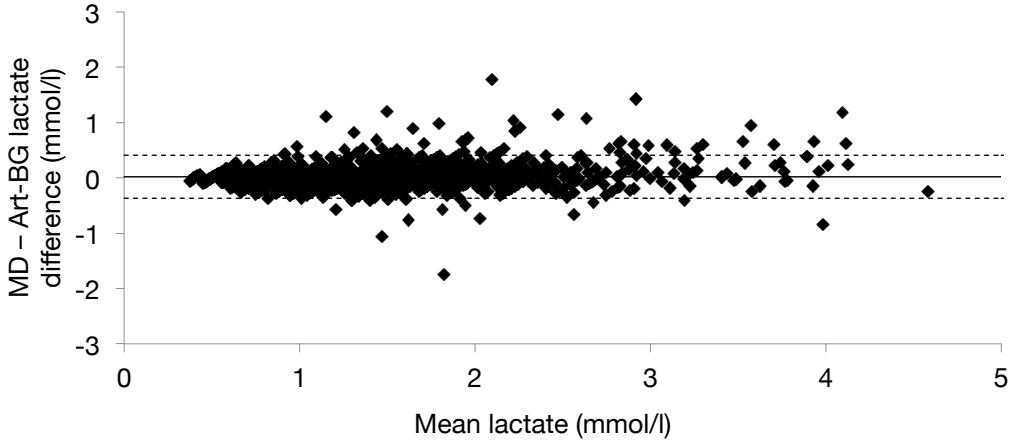

Figure 18. Bland-Altman analysis of paired microdialysis and arterial blood gas lactate values from Study IV. The straight line represents the mean difference, and the dotted lines are the limits of agreement (mean difference ±1.96SD). The mean difference was 0.02 mmol/l, and the limits of agreement were 0.41 to -0.37 mmol/l.
Art-BG – arterial blood gas, MD – microdialysis.

In 18 patients, a microdialysis lactate value >3.0 mmol/l was observed, and adverse events occurred in 7 of these patients (reoperation for bleeding (n=5), perioperative myocardial infarction (n=1), circulatory instability with transient visceral ischemia (n=1)). If the cut-off point had been set to 2.5 mmol/l, another patient who underwent reoperation for bleeding would have been identified, but this would have also resulted in inclusion of another 9 patients who did not experience any complications. A cut-off point lower than 2.5 mmol/l would not have added more patients with adverse events.

5.5 COMPARISON OF INTRAVASCULAR MICRODIALYSIS AND SUBCUTANEOUS CGM – RESULTS FROM STUDY VI

In the last study included in this thesis, the Eirus intravascular microdialysis CGM system was indirectly compared to a subcutaneous tissue CGM system, the FreeStyle Libre. Accuracy of both CGM systems was evaluated compared to the chosen reference method, analysis of arterial blood in a blood gas analyzer. Twenty-six patients were included, of which 24 had successful CGM with both monitoring systems. The FreeStyle Libre system functioned well in all patients, while the microdialysis system could not be used in two patients for different reasons. In one patient this was due to a late start of surgery and the fact that no personnel were available at that time to apply the system. In the other patient, this was due to a malfunction of the microdialysis catheter, most likely because of damage during insertion.

All FreeStyle Libre subcutaneous sensors functioned well, and there were no problems with the insertion procedure. The sensor had to be replaced in one patient because of excessive sweating, which caused the first sensor to detach. The microdialysis system was calibrated every 8 hours using the reference blood glucose concentration obtained by arterial blood gas analysis.

Glucose monitoring method	**Mean glucose** (mmol/l)	**Min glucose** (mmol/l)	**Max glucose** (mmol/l)
Art-BG	8.18	3.5	16
MD-CGM	8.25	3.73	16.17
SC-CGM	5.65	2.2	11.5

Table 6. Glucose values measured by the different glucose monitoring methods used in Study VI. Art-BG – arterial blood gas, MD-CGM – microdialysis continuous glucose monitoring, SC-CGM – subcutaneous continuous glucose monitoring.

The study period continued for a mean (range) of 23.7 (21-24) hours. Both CGM systems were removed after 24 hours in the ICU (n=19) or after the patient was discharged from the ICU (n=5). Mean, minimal and maximal glucose values measured by each monitoring method are presented in table 6. In concordance with these numbers, the SC-CGM system often reported lower glucose values than the reference method and the MD-CGM system. The results from accuracy analysis of both CGM systems are detailed in table 7.

The MARD for individual MD-CGM systems ranged from 2.2% to 13.2%, and the MARD for individual SC-CGM systems ranged from 12.0% to 52.1%. The MARDs were similar in all glucose ranges (i.e. in, below and above the target range of 5-10 mmol/l) for the MD-CGM system, but not for the SC-CGM system, for which the MARD improved (decreased) in higher reference blood glucose ranges. This difference in MARD was found to be significant in a Kruskal-Wallis test.

A total of 87.9% of the reference glucose values were within the target blood glucose range in the ICU (5-10 mmol/l). Only 7 reference glucose values were below this range, and only 1

glucose value was classified as being in the hypoglycemic range (i.e. <4.1 mmol/l). That specific reference glucose value was 3.5 mmol/l, and the corresponding MD-CGM and SC-CGM values were 3.7 mmol/l and 2.2 mmol/l respectively.

	MD-CGM	SC-CGM
Number of paired test-reference values	513	578
Reference value in target range (5-10 mmol/l)	451	508
Reference value below target range (<5 mmol/l)	7	7
Reference value above target range (>10 mmol/l)	55	63
Overall MARD ±SD (%)	6 ±7.9	30.5 ±12.4
MARD ±SD in target range (5-10 mmol/l)	5.8 ±7.8	30.5 ±12.0
MARD ±SD below target range (<5 mmol/l)	6.9 ±7.6	41.5 ±9.6
MARD ±SD above target range (>10 mmol/l)	7.8 ±7.9	29.7 ±15
Overall median ARD (%)	3.4	28.2
Median ARD in target range (5-10 mmol/l)	3.3	27.3
Median ARD below target range (<5 mmol/l)	5,6	43.9
Median ARD above target range (>10 mmol/l)	5,2	25.6
ISO 2003 criteria met (%)	95	18

Table 7. Results of accuracy analysis of the Eirus intravascular microdialysis and the FreeStyle Libre subcutaneous CGM systems. The reference method was arterial blood gas analysis.
ARD – absolute relative difference, CGM – continuous glucose monitoring, ISO – International Organization for Standardization criteria for glucose accuracy, MARD – mean absolute relative difference, MD-CGM – microdialysis continuous glucose monitoring, SC-CGM – subcutaneous continuous glucose monitoring, SD – standard deviation.

The results of Bland-Altman analysis are illustrated in figure 19. The MD-CGM system had a low mean difference (SD) of 0.07 (0.77) mmol/l, with the upper and lower limits of agreement of 1.64 and -1.5 mmol/l. The SC-CGM system had a higher mean difference (SD) of -2.41 (1.12) mmol/l, with the upper and lower limits of agreement of -0.25 and -4.57 mmol/l. The negative mean difference and limits of agreement of the SC-CGM system indicate that this system constantly measured a lower blood glucose value than the reference method.

The EGA (figure 20) revealed good clinical accuracy of the MD-CGM system, with 100% of the paired values within zones A and B, and 95% of them within zone A. The EGA of the SC-CGM system revealed that 0.9% of the paired values (n=5) were within in zones C, D or E.

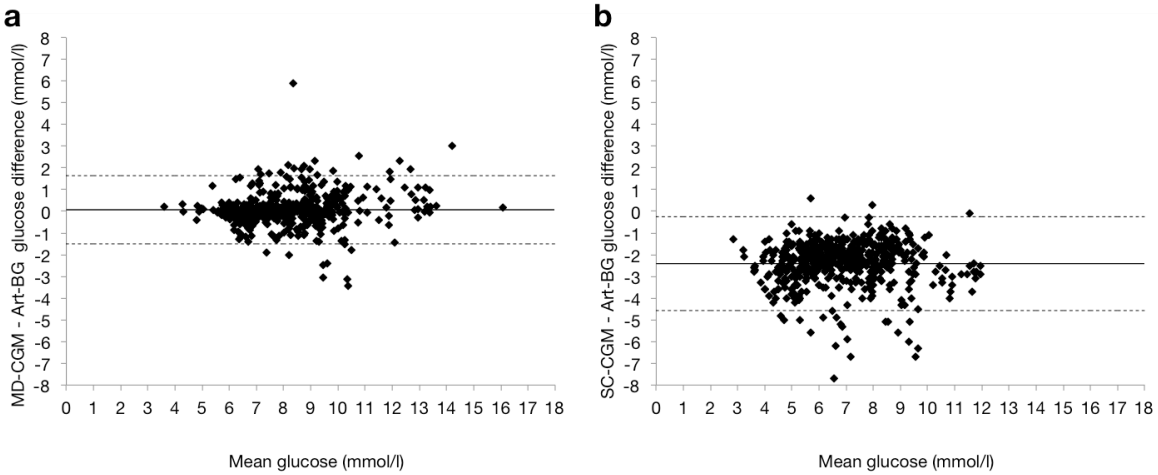

Figure 19. Bland-Altman analysis of a) MD-CGM vs. reference glucose values and b) SC-CGM vs. reference glucose values. The solid line represents the mean difference (MD-CGM: 0.07mmol/l, and SC-CGM: -2.41 mmol/l). Dotted lines represent the limits of agreement (mean difference ±1.96SD).
Art-BG – arterial blood gas, MD-CGM – microdialysis continuous glucose monitoring, SC-CGM – subcutaneous continuous glucose monitoring.

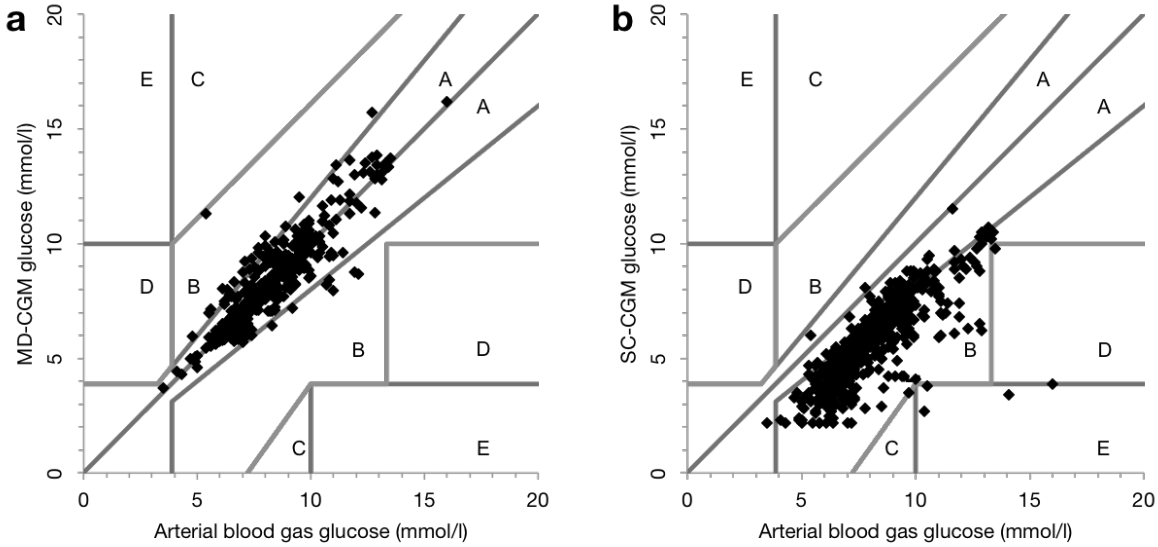

Figure 20. Clarke error grid analysis of a) MD-CGM vs. reference glucose values and b) SC-CGM vs. reference glucose values.
MD-CGM – microdialysis continuous glucose monitoring, SC-CGM – subcutaneous continuous glucose monitoring.

The MD-CGM system met the ISO criteria of 2003. The updated criteria of 2013 were not met, as only 91% of the paired values satisfied the standard (the requirement is 95%). The SC-CGM system did not meet any ISO criteria, nor did the POC function of the FreeStyle Libre sensor reader. The POC function of the FreeStyle Libre sensor reader was used to measure blood glucose on 157 occasions. The mean (SD, range) of the POC glucose values was 6.92 (1.7, 2.9-13.4) mmol/l, and the mean (SD, range) of the paired reference glucose

values was 7.74 (1.79, 3.5-12.9) mmol/l. The MARD was 12.3%. The mean difference (SD) was -0.82 (0.8) mmol/l, with upper and lower limits of agreement of 0.75 and -2.39 mmol/l.

5.6 MICRODIALYSIS FUNCTION, RELIABILITY, AND COMPLICATIONS – RESULTS FROM ALL STUDIES

The Eirus intravascular microdialysis system monitored glucose and lactate without any complications or incidents in the 10 patients included in Study I, as well as in the animals in Study V. In Study II, the system malfunctioned in two patients, resulting in lack of microdialysis data. The reasons for malfunctions were catheter damage in the process of mitral valve surgery in one patient and sensor failure in another patient. In Study III, the microdialysis catheter was accidentally dislocated in one patient, resulting in premature ending of the measurements. This event was detected based on a warning signal for uncertain values due to high fluctuations in glucose concentration issued by the microdialysis system. Furthermore, data collection was paused in three patients when the system indicated sensor malfunction, but was successfully restarted after replacement of the sensor. No problems with the calibration procedure occurred, except in one patient in Study VI. As a result, reliable glucose values were unavailable for two hours for this patient. When calibration could be performed, the system functioned as usual. The reason for the difficulty with calibration remains unknown. All microdialysis catheters were easy to insert, but it is likely that the microdialysis membrane was damaged during insertion in one patient in Study VI, resulting in no microdialysis monitoring.

In Study VI, the microdialysis system performed CGM without interruptions for 2 patients, whereas for the remaining 22 patients there were data gaps, the median number (range) of which was 3 (1-8). The data gaps were mostly short, with a mean duration (SD) of 13 (19) minutes and a median duration (range) of 10 (1-141) minutes. The 141-min data gap occurred when calibration problems were encountered in the patient described above.

No complications, including no blood clotting of the microdialysis membrane, were observed for any of the microdialysis catheters used. In Studies I and II, the separate microdialysis catheter was removed after the end of the study period. In the remaining studies that used the Eirus TLC, the microdialysis catheter was removed when a central venous access was no longer necessary. As a result, some patients in Study III had the microdialysis catheter in place for a longer period of time (up to 10 days) than the planned follow-up period of 48 hours. In 4 patients, microdialysis glucose values were obtained after 48 hours and paired with arterial blood gas glucose values, resulting in a total of 6 paired MD/Art-BG glucose values that were included in the accuracy analyses.

Systematic drift with increased mean difference between calibrations was not detected in Study II and Study III. Moreover, longer monitoring times id not increase the mean difference with longer monitoring times (see figure 21 and 22).

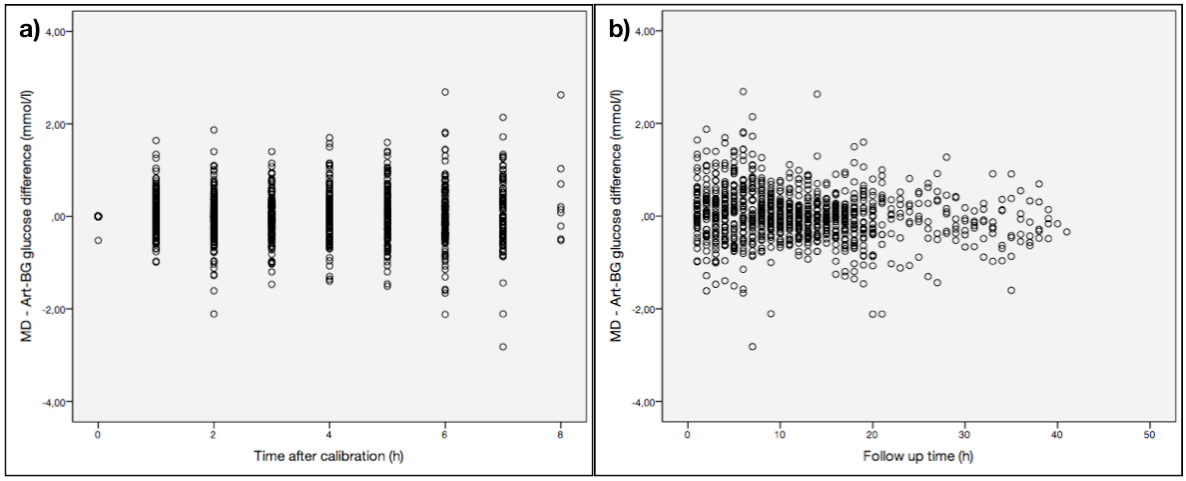

Figure 21. Plots of the difference between paired microdialysis and arterial blood gas glucose values a) over time after calibration and b) during the entire follow-up period for Study II.
Art-BG – arterial blood gas, MD – microdialysis.

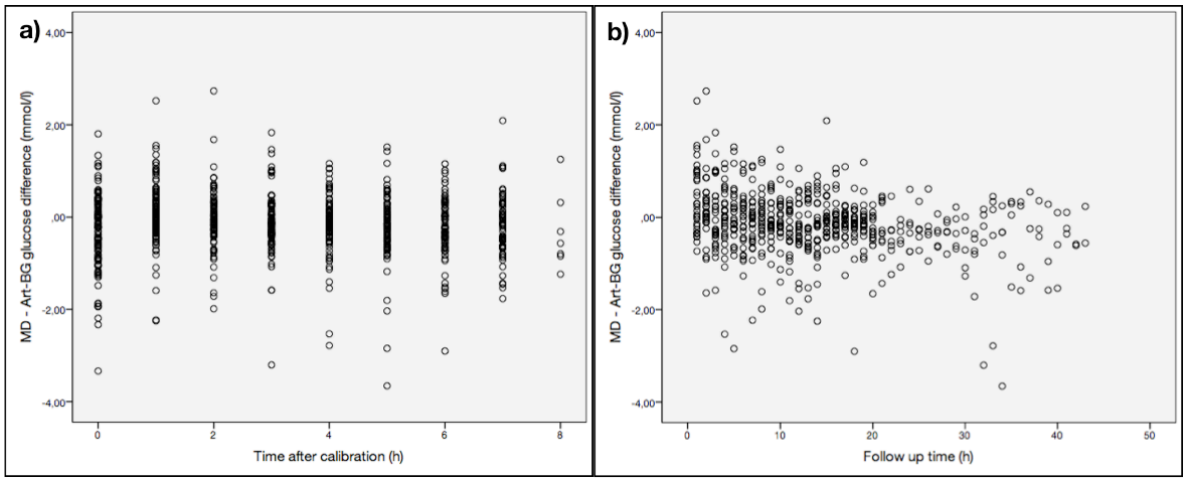

Figure 22. Plots of the difference between paired microdialysis and arterial blood gas glucose values a) over time after calibration and b) during entire follow-up period for Study III.
Art-BG – arterial blood gas, MD – microdialysis.

6 DISCUSSION

This thesis has developed and evaluated the method of intravascular microdialysis for continuous monitoring of glucose and lactate in critically ill patients undergoing cardiac surgery. The main finding is that the use of intravascular microdialysis for this purpose is safe and efficient. The monitoring of glucose is accurate in both hyper- and hypoglycemic conditions, the system is responsive to rapid oscillations in blood glucose concentrations, and the accuracy is not affected by glucose administration. Intravascular microdialysis was found to be more accurate than a less invasive subcutaneous CGM system. The use of intravascular microdialysis may improve metabolic control in critically ill patients.

6.1 PERFORMANCE AND RELIABILITY OF INTRAVASCULAR MICRODIALYSIS

Overall, the results from Studies I-VI demonstrate that the Eirus intravascular microdialysis system can be used for safe and accurate continuous glucose and lactate monitoring, which may improve GC in the ICU and allow early detection of elevated lactate levels. Compared to reference values obtained by arterial blood gas analysis (venous blood gas analysis in Study V), microdialysis glucose and lactate values had low mean differences and acceptable limits of agreement as determined by Bland-Altman analysis. The MARD was low in all studies (below the suggested <14%). The microdialysis system has also been evaluated by a different group with similar results.[142] Furthermore, the accuracy of glucose monitoring was similar to that of other intravascular devices, such as the GluCath system[39, 40] and the GlucoClear system.[41]

All paired glucose values were in zones A and B of the Clarke EGA, indicating that no incorrect clinical action would have been taken if decisions had been based on the microdialysis values. The ISO criteria of 2003 were met in all the studies, except for Study I. The results of Studies II, III, V and VI were not accurate enough to satisfy the new criteria for glucose monitoring devices suggested by the 2013 consensus meeting, according to which 98% of paired glucose values should be within 12.5% of the reference method (or 0.55 mmol if reference value <5.5 mmol/l). Only 94%, 90%, 85% and 86% of paired values met this condition respectively.

All the microdialysis catheters used in the studies included in this thesis were placed in a central vein. In Studies I-II, the microdialysis catheter was located close to an additional CVC used for drug administration and blood sampling. In the remaining studies, the CVC function was integrated in the microdialysis catheter. No disturbances from the use of the CVC were noticed, probably owing to the high blood flow. This was further confirmed in Study V, in which glucose administration did not lead to increased differences between microdialysis glucose values and reference values. On the contrary, the microdialysis system was found to be very responsive to rapidly fluctuating blood glucose concentrations and unaffected by glucose administration. An important technical feature of the Eirus TLC to avoid this interference, is that the microdialysis membrane is located proximally to the infusion

openings of the CVC (through which drugs are administered), which are thus situated "downstreams".

Overall, the microdialysis system functioned well, with few failures. Microdialysis catheter malfunctions occurred in three patients. The catheter was accidentally damaged during mitral valve surgery in one patient in Study II, unintentionally dislocated after surgery in one patient in Study III, and was likely damaged during insertion in one patient in Study VI. The sensor malfunctioned in one patient in Study II and in three patients in Study III, but monitoring could be resumed after sensor replacement. Problems with calibration occurred in one patient in Study VI, resulting in lack of glucose/lactate monitoring for approximately 2 hours. When calibration was finally performed, the system resumed normal function. The intravascular microdialysis system is programmed to issue warnings when rapid and high variations in glucose levels are detected. Such warning signals for uncertain values were issued in all the above cases with complications. The resulting unreliable paired values were excluded from further analysis. We believe this was a well-justified decision, as the ICU staff would not rely on such microdialysis values. When the Eirus TLC is used, the time lag for the intravascular microdialysis system is approximately 5 minutes. The time lag depends on the length of the catheter and perfusion velocity. All the reference glucose and lactate values in Studies I-VI were corrected for this time lag to describe the technical accuracy of the microdialysis system more precisely.

In Study V, we found that arterial blood gas glucose measurements were consistently higher than venous blood gas glucose and microdialysis glucose values when glucose was administered. This may be explained by the fact that the glucose (high concentration) was administered in a central vein and had not been processed by the peripheral cells, which resulted in a higher arterial glucose concentration. The extent of clinical implications of this finding in critically ill patients with ongoing glucose infusions in whom blood glucose concentration is only monitored using arterial blood is currently uncertain. However, in Study II and Study III, there was good agreement between venous blood glucose values (as determined by microdialysis) and arterial blood glucose values (as determined by blood gas analysis), and these values were not affected by glucose or other drug administration. Furthermore, a good agreement between arterial and venous blood glucose concentrations determined by blood gas analysis was demonstrated in Study I. Therefore, it is possible that the concentration of administered glucose in Study V exceeded the higher limit of the range normally used in a clinical setting.

One of the advantages of intravascular microdialysis is that no blood sampling is required for continuous monitoring of glucose and lactate. Furthermore, the technical development of the Eirus TLC eliminates the need to use an additional CVC for central venous access, minimizing the number of catheters required and reducing the risk of infection. Thus, the use of the Eirus TLC, which includes a combination of a CVC and a microdialysis system, may be especially beneficial in critically ill patients requiring a CVC. Although invasiveness is one of the disadvantages of intravascular microdialysis, most critically ill patients require

central venous access. The disadvantage of invasiveness is irrelevant in such patients, as insertion of an intravascular microdialysis catheter provides both central venous access and the microdialysis function. In fact, the use of a microdialysis system may even be a better choice in this setting, since critically ill patients are often sedated, on mechanical ventilation, or otherwise incapacitated such that they are unable to convey signs of hypoglycemia, justifying the use of a more accurate although invasive glucose monitoring system. In contrast, the feasibility of intravascular microdialysis in more stable patients admitted to general wards, who are not in need of a CVC, may be debatable.

It is noteworthy that no blood clotting of the microdialysis membrane was observed in any of the patients. This may be a consequence of the fact that all patients included in the studies were cardiac surgical patients, who often are prone to bleeding. Whether the microdialysis catheters are safe to use in other patient categories has not been studied. Furthermore, intravascular microdialysis system was tested under special circumstances (cardiothoracic surgery and the ICU) and with highly skilled nursing staff available. Therefore, it is possible that our findings are not generalizable to other centers. It should also be emphasized that this thesis has focused on the performance and safety of the intravascular microdialysis system, whereas the clinical outcomes of its application remain to be studied.

Glucose levels determined with microdialysis were not used to guide treatment in the studies included in this thesis. As routine approach in the cardiothoracic ICU, arterial blood gas analysis was used to obtain blood glucose concentrations for guiding insulin treatment. In the patients included in Studies II, III and VI, arterial blood gas analysis was performed once every hour according to the study protocol, resulting in more frequent blood gas analysis than in usual clinical practice. The target blood glucose concentration range in the ICU is 5-10 mmol/l, and patients' glucose levels were within this range 88% (Study II), 85% (Study III) and 88% (Study VI) of the time. It can be speculated that the target blood glucose concentration would be maintained more accurately with CGM by intravascular microdialysis compared to less frequent blood gas analysis than once every hour, but this has not been studied.

6.2 ASPECTS OF GLUCOSE MONITORING

When monitoring blood glucose, it should be specified whether arterial, venous, or capillary blood is used.[17] The plasma glucose level was measured in the hospital's laboratory in Study I (the gold standard of blood glucose analysis) and compared to the glucose values obtained with microdialysis as well as to the values determined with arterial and venous blood gas analysis. Accuracy analysis revealed a good agreement and low mean difference for all comparisons (although the ISO criteria of 2003 were not met for the comparison of microdialysis and plasma glucose values). This indicated that analysis of arterial and venous blood in a blood gas analyzer is an adequate reference method for evaluation of the accuracy of glucose measurements by microdialysis. Additionally, arterial blood gas analysis has previously been validated against analysis in a hospital laboratory,[13] and it is the recommended reference method when evaluating various glucose monitoring systems, if

plasma glucose analysis cannot be performed.[44] This is often the case for ICUs, given that laboratory analysis is time-consuming and impractical.

CGM is considered by many to be the future in glucose monitoring in critically ill patients. The devices providing CGM use various technologies and have different advantages and disadvantages. Generally, SC-CGM systems are minimally invasive but not as accurate as more invasive techniques.[139] The results of Study VI suggest that the intravascular microdialysis system is superior to a SC-CGM system in terms of accuracy.

6.2.1 How to evaluate and compare glucose monitoring devices

Different CGM devices need to be validated against and compared to each other. It is important that the validation is standardized, as studies now use different methods, resulting in difficulties with comparing their results. Commonly used methods for evaluating glucose monitoring accuracy are calculating the MARD, using Bland-Altman analysis, Clarke EGA, and evaluation according to the ISO criteria. These approaches have all been used throughout the studies included in this thesis. It should be stated that the Clarke EGA was not developed for continuous glucose data analysis or for application in critically ill patients, and it has been criticized for inadequately reflecting rapid changes in blood glucose concentrations. An error grid for CGM data has been developed, but it is difficult to use and not readily available, as specific software is required.[140] Additionally, it may be argued that error grids are generally too forgiving.

There is no clear agreement on what is the acceptable accuracy for a glucose monitoring device used in critically ill patients. Not only point accuracy (reflecting differences between test glucose values and true reference glucose values) but also trend accuracy (characterizing rates of change in blood glucose concentration) is important. There are conventional methods that evaluate the point accuracy, but the best approaches to determine and evaluate trend accuracy still remain to be established. Point accuracy can be assessed by calculating and comparing the mean difference and the MARD, which are easy to perform and comprehend, and therefore are often used when comparing glucose monitoring devices.[138] Calculating the median absolute relative difference, rather than the MARD, is probably more correct, since data are usually not normally distributed. Nevertheless, MARD is more often used in the literature, and therefore it is perhaps easier to interpret the results and to compare them between studies. In this thesis, we utilized the MARD for this reason. However, in Study VI we also employed the median absolute relative difference. It has been suggested that MARD should be <14%, and that MARD >18% indicates poor accuracy.[139]

6.2.2 A comparison of intravascular microdialysis and a subcutaneous CGM system

In Study VI, two different CGM systems were compared in terms of reliability and accuracy in patients undergoing cardiac surgery: the Eirus intravascular microdialysis system vs. the FreeStyle Libre subcutaneous system. Analysis of arterial blood in a blood gas analyzer was used as the reference method for accuracy analysis. The MD-CGM system was shown to be

more accurate in glucose monitoring than the SC-CGM system. The impact on glycemic control was not assessed, as this was not an aim in this study.

Both systems were reliable and effective, and no instances of interference with clinical care were observed. The SC-CGM system measured the blood glucose concentration every 15 minutes entirely without interruptions, and consequently there were no gaps in data. The MD-CGM system measured the blood glucose concentration every minute, although occasionally interruptions occurred in 22 patients, resulting in data gaps. These data gaps were commonly very short, lasting only a few minutes.

The MD-CGM system performed CGM more accurately than the SC-CGM system. The MD-CGM system had a lower MARD, lower mean difference, and more acceptable limits of agreement as assessed with a Bland-Altman plot, than the SC-CGM system. Additionally, the MD-CGM system met the ISO criteria of 2003, whereas the SC-CGM system did not. If the MD-CGM system had been used for clinical guidance, no improper clinical actions would have been taken, as all the paired values were in the zones A and B on the Clarke error grid. This would not have been true for the SC-CGM system.

The glucose monitoring accuracy of the SC-CGM system was worse than that in other studies using subcutaneous CGM systems in critically ill patients.[143] The FreeStyle Libre system consistently measured lower blood glucose values than the reference method. After noticing the persistent underestimation of blood glucose concentration, we reached out to Abbott Diabetes Care Inc. to insure that the sensors were correctly pre-calibrated, which turned out to be the case. It is conceivable that the accuracy of the FreeStyle Libre system could be improved if onsite calibration (instead of factory pre-calibration) was possible.

Impaired microcirculation has often been implied to be the reason for poor performance when using subcutaneous tissue monitoring devices in critically ill patients. However, no relation between reduced microcirculation and sensor function could be demonstrated in a recent study using a SC-CGM system in patients undergoing cardiac surgery.[34] Furthermore, when the time course of glucose concentrations determined by all the glucose monitoring methods used in Study VI (i.e. arterial blood gas analysis, intravascular microdialysis and the subcutaneous FreeStyle Libre system) was plotted, it became apparent that the SC-CGM system followed the trend of the blood glucose concentration measured by the reference method. If impaired microcirculation did in fact explain why the SC-CGM system often reported a lower glucose value than the reference method, the glucose curve would probably be more static.

The Eirus intravascular microdialysis system differs from the FreeStyle Libre system in some important technical details. While both enable CGM without the need of blood sampling, the FreeStyle Libre system is minimally invasive, and the sensor is easily inserted subcutaneously. The microdialysis system is more invasive and difficult to insert, as it requires central venous access and thus can only be inserted by someone capable of placing a CVC, e.g. an anesthesiologist. As a result, setting up the microdialysis system requires more

time and is more difficult. Another principal difference is in the rate of continuous glucose monitoring. The FreeStyle Libre system determines the glucose concentration every 15 minutes. The CLSI (Clinical Laboratory Standards Institute) uses a 15-minute cut-off to define continuous glucose monitoring. Although, the FreeStyle Libre system satisfies this definition, it performs measurements less frequently than the microdialysis system (every minute). Additionally, the glucose value determined by the FreeStyle Libre system is only displayed on the sensor reader when it is turned on, while the microdialysis system continuously displays the glucose concentration as a numerical value and a trend graph on the bedside monitor, making this information more accessible. Moreover, the FreeStyle Libre system requires that the sensor is scanned every time the glucose concentration needs to be obtained, while the microdialysis system provides the glucose value/trend graph without any interventions from the attending staff after the system has been set up (with the exception of the calibration procedure every 8 hours). We believe that these technical features of the intravascular microdialysis system makes it more clinically useful and perhaps more intuitive to use as the glucose trend is always visible, facilitating the interpretation of the data. Additionally, following the glucose trend diminishes the need for absolute point accuracy, as each individual glucose value becomes less important if put in context of a trend.

6.2.3 Which patients benefit from glycemic control?

GC has been shown to be beneficial in surgical critically ill patients[71] and patients undergoing cardiac surgery in particular.[83, 84, 96] In the medical ICU, the benefit of GC is less clearly defined, as an improvement in morbidity has only been demonstrated in patients in need of intensive care for more than three days, and such patients are difficult to identify beforehand.[65]

Why has the effect of GC been found to be more beneficial in surgical critically ill patients? One answer may be that when surgery is the cause of hyperglycemia, the time between the onset of increased blood glucose concentration and treatment (start of GC) is often short. This may be different in a medical setting, as hyperglycemia may have been present for a longer period of time, making the benefit from improved GC during the relatively short monitoring period less noticeable.[144] It is also possible that surgical patients are more suitable and have better prerequisites for glucose monitoring, as they more frequently are in need of an arterial and/or central venous access line, which justifies the use of an accurate (but invasive) CGM system such as the intravascular microdialysis system.

The diabetic status may also affect the outcome of GC. Hyperglycemia has been found to be more associated to increased mortality in non-diabetic critically ill patients, suggesting that patients with known DM tolerate a wider range of blood glucose.[54-56] The same has been demonstrated regarding increased GV.[55] This implies that it may be justified to individualize the target of GC in the ICU depending on the diabetic status.

The Society of Thoracic Surgeons recommends that GC is aimed at achieving a blood glucose concentration <10 mmol/l in cardiac surgery patients.[145] This recommendation is

based on studies demonstrating that less stringent blood glucose range (6.7-10 mmol/l) is not inferior to a strict range (5-6.7 mmol/l) in patients undergoing CABG.[102, 146] A more moderate approach to GC is especially preferred in patients with known DM.[101] However, the benefit of GC aimed at a glucose concentration <10 mmol/l is no longer questionable.[147]

6.2.3.1 Why is glycemic control beneficial during and after cardiac surgery?

The benefits of GC in patients undergoing cardiac surgery may be explained by the alleviation of the negative effects of hyperglycemia on the cardiovascular system. The myocardium can use several substrates in order to produce energy (ATP) to power the contraction of the heart. During normal conditions, ATP is mostly obtained from lipolysis and β-oxidation of FFAs.[148] In the presence of β-oxidation, the acetyl-CoA produced from FFAs inhibit PDH, thereby inhibiting glycolysis.[149]

If ischemia (a state with decreased oxygen concentration) occurs in a non-diabetic myocardium, the metabolism will shift from using FFAs to using glucose. This shift is intended to increase the efficiency of oxygen consumption by the ischemic myocardium, since glucose metabolism is more oxygen-efficient than β-oxidation. Accordingly, glycolysis is accelerated to maintain the production of ATP, stabilizing cell integrity and function. However, the glucose metabolism is impaired in a diabetic patient, and there is a lack of insulin and/or insulin resistance. The myocardium is thus forced to continue using FFAs, at a cost of increasing myocardial oxygen consumption, which may worsen the damage from ischemia. Additionally, FFAs cannot be completely metabolized during ischemia, which results in their accumulation. This has been shown reduce contractility and increase incidence of ventricular arrhythmias.[150] Insulin stimulates PDH in the mitochondria, leading to the conversion of pyruvate to acetyl-CoA. In the absence of insulin, pyruvate accumulates and is instead converted into lactate. Administration of insulin to a hyperglycemic patient may compensate for these metabolic events. Insulin increases cell glucose uptake and stimulates PDH, resulting in accelerated glycolysis[151] and lowered blood glucose concentration. Furthermore, myocardial oxygen consumption is decreased as β-oxidation of FFAs is inhibited by stimulation of glycolysis.[152]

6.2.4 Future directions of glucose monitoring and glycemic control

CGM systems are now standard in many ICUs worldwide, but their impact on GC remains to be determined. To evaluate the clinical effect, it would be interesting to randomize patients to either glucose monitoring using intravascular microdialysis or to the present standard routine with intermittent blood gas analysis. This would allow establishing whether the quality of GC and time within the target blood glucose range differ between the groups.

Many studies have focused on establishing the optimal blood glucose target range in critically ill patients, but this still remains a matter of controversy. For patients undergoing cardiac surgery, there are results indicating that a more moderate approach to GC is superior to a stricter control.[146] What is unknown is if there is any specific time window when GC is

especially important, and whether GC is equally important during the perioperative and postoperative periods? As results from studies investigating GC in cardiac surgery patients have shown, GC seems to be of importance during surgery and the first 3 postoperative days.[89]

Measuring glycated hemoglobin (HbA1c) preoperatively in patients undergoing cardiac surgery may aid in determining the metabolic state. Elevated HbA1c level before surgery is a strong predictor of mortality and morbidity in patients undergoing CABG regardless of the presence of DM.[153] HbA1c is a form of hemoglobin used as a marker of average blood glucose concentration over a longer period of time (approximately 3 months). It is formed when hemoglobin is exposed to plasma glucose, and therefore increased if the plasma glucose concentration is high. Of note, a high HbA1c of 70 mmol/mol (8.6%) has been associated with a four-fold increase in mortality after CABG.[154] It would be interesting to study whether elective patients with elevated HbA1c can benefit from delaying surgery until adequate preoperative GC is achieved. Additionally, an elevated preoperative HbA1c may serve as a predictor of increased perioperative insulin resistance and increased GV in the postoperative period, which has been associated with adverse patient outcome.[92, 155] Analyzing HbA1c may also be of value in critically ill patients, as hyperglycemia has been associated with increased mortality in primarily non-diabetics and diabetic patients with good metabolic control. Critically ill patients with insufficiently controlled diabetes seem to tolerate hyperglycemia to a greater extent, and the association with adverse outcomes is not as definite. Thus, GC in critically ill patients may need to be individualized based on the pre-critical illness metabolic state, and measuring the HbA1c fraction may be useful in this assessment.[57]

Insulin algorithms are an interesting development that may simplify GC in the ICU. These algorithms aim to standardize clinical practice and to aid in decisions regarding insulin administration. The majority of these systems rely on intermittent blood glucose monitoring, and are now often controlled using computers rather than manually.[156] Further development of a closed-loop system that automatically administers inulin based on readings of a subcutaneous CGM system is an interesting improvement, which has been shown to facilitate GC in critically ill patients.[157] In the future, it would be interesting to use the intravascular microdialysis system together with a computer-based insulin algorithm to determine whether better GC can be achieved, and if this approach has any clinical benefits.

6.3 ASPECTS OF LACTATE MONITORING

Intravascular microdialysis was demonstrated to be safe and accurate for continuous lactate monitoring in Study IV. Monitoring of blood lactate may be beneficial in critically ill patients since it can be of prognostic value and can be used to enable lactate-guided treatment, i.e. treatment aiming at lowering the lactate concentration in the blood. The microdialysis lactate values correlated well with the reference arterial blood gas lactate values, and the mean difference and the MARD were low.

POC testing has made monitoring of lactate easier.[158] There seems to be no significant

difference between lactate values measured in arterial and venous blood,[159, 160] and this observation was further confirmed in Study I. It is preferable to perform lactate monitoring in critically ill patients by assessing the trend in lactate levels based on repeated lactate sampling. This may be used for predicting in-hospital mortality.[121, 122]

A rise in blood lactate concentration was detected in every patient that suffered from an adverse event. Furthermore, there was an association between a higher rate of complications and a postoperative blood lactate concentration >3 mmol/l, although this was merely an observational finding, since Study IV was not aimed at assessing this association and the sample size was definitely not sufficient to correctly analyze this potential association. We based the theoretical cut-off value of blood lactate concentration >3 mmol/l on the results of a study investigating high lactate values and postoperative complications in adult cardiac surgery patients.[125] A higher blood lactate cut-off value (3.0-3.5 mmol/l) has been shown to predict adverse outcomes better,[115] but also a lower blood lactate cut off value (>1.5 mmol/l) has been demonstrated to be of prognostic use in critically ill patients.[161]

Continuous lactate monitoring may be used for lactate-guided treatment in critically ill patients. Reducing blood lactate levels in treatment of sepsis has been shown to be as effective as targeting central venous oxygen saturation (SvO_2).[131] Achieving successful lactate clearance could even be more important in critically ill patients than improving SvO_2, as improving SvO_2 alone has been demonstrated to result in higher mortality than increasing lactate clearance alone.[162]

Lactate-guided treatment in critically ill patients with hyperlactatemia has been demonstrated to result in improved outcome.[132] In that study, lactate-guided treatment was aimed at reducing the blood lactate concentration by 20% per 2 hours and achieving SvO_2 >70%, whereas in the control group blood lactate was not analyzed during the first 8 hours after randomization. Lactate-guided treatment resulted in administration of larger amounts of fluids during the intervention period (and smaller amounts during the following observation period), increased use of vasodilators, reduced mortality and a significantly reduced morbidity.[132] In addition, in patients undergoing cardiac surgery, specific treatment aimed at reducing blood lactate levels has been shown to be beneficial.[133]

In conclusion, adequate lactate monitoring used for lactate-guided treatment in critically ill patients can potentially reduce morbidity and mortality, although the exact underlying mechanism remains uncertain. It remains to be determined whether continuous lactate monitoring is superior to intermittent monitoring. The studies evaluating lactate-guided treatment have all been performed early in critical illness implying that lactate monitoring should be initiated as early as possible in critically ill patients. This further means that eligible patients should receive the Eirus TLC providing both central venous access and continuous lactate monitoring (if intravascular microdialysis is the method of choice for lactate monitoring). The invasiveness of the method is unlikely to be a major clinical problem, as critically ill patients usually require a central venous access anyway. The use of an intravascular microdialysis system in these patients will provide continuous monitoring and

consequently improved control of both glucose and lactate concentrations, with a possibility of early detection of metabolic derangement, which may result in a clinical benefit.

7 CONCLUSIONS

I. Intravascular microdialysis can be used in critically ill patients, with good agreement between blood glucose and lactate concentrations determined by arterial and venous blood gas analyses, and dialysate glucose and lactate concentrations.

II. The intravascular microdialysis system can be connected to a sensor that continously analyzes the glucose concentration in the dialysate fluid, which correlates well with the glucose concentration in the blood.

III. The intravascular microdialysis system can be integrated in a central venous catheter, without changes in glucose monitoring accuracy.

IV. Intravascular microdialysis is accurate for continuous lactate monitoring in critically ill patients.

V. In an experimental animal model, the intravascular microdialysis system was accurate for continuous glucose monitoring during hypoglycemia, and the system was responsive to rapid fluctuations in blood glucose concentrations. The accuracy was not affected by glucose administration via the microdialysis catheter.

VI. The Eirus intravascular microdialysis system is more accurate than the FreeStyle subcutaneous system for continuous glucose monitoring in patients undergoing cardiac surgery.

The results of this thesis support the use of intravascular microdialysis as the preferred method for continuous glucose monitoring in critically ill patients admitted to intensive care units, in light of the importance of accurate glucose monitoring for adequate glycemic control. The intravascular microdialysis system also provides continuous lactate monitoring, which may further enhance the positive clinical effect.

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9 CONFLICTS OF INTEREST

Main supervisor Jan Liska and co-supervisor Anders Franco-Cereceda were shareholders in CMA Microdialysis AB, Solna, Sweden, and have a royalty agreement with Maquet Critical Care, Solna, Sweden.

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