

From the Department of Clinical Science, Intervention and
Technology
Karolinska Institutet, Stockholm, Sweden

MICRODIALYSIS

- POTENTIALS AND LIMITATIONS

Anna von Platen



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Microdialysis - Potentials and Limitations

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Anna von Platen

Principal Supervisor:

Doc. Greg Nowak
Karolinska Institutet
Department of Clinical Science, Intervention and
Technology, CLINTEC
Division of Transplantation

Opponent:

Prof. Per Sandström
Linköping University
Department of Clinical and Experimental
Medicine
Division of Surgery, Orthopedics and Oncology

Co-supervisor:

Prof. Bo Göran Ericzon
Karolinska Institutet
Department of Clinical Science, Intervention and
Technology, CLINTEC
Division of Transplantation

Examination Board:

Prof. Gunnar Tufveson
Uppsala University
Department of Surgical Sciences
Division of Transplantation

Doc. Jacob Freedman
Karolinska Institutet
Department of Clinical Sciences, Danderyd
Hospital
Division of Surgery and Urology

Prof. Magnus Nilsson
Karolinska Institutet
Department of Clinical Science, Intervention and
Technology, CLINTEC
Division of Surgery

To my parents, William and Annika

ABSTRACT

Despite improvements in operating technique and post-operative management, many patients undergoing liver transplantation still suffer post-operative complications, which may lead to graft loss or patient death. Patients are monitored closely post-operatively using blood chemistry, diagnostic imaging and liver biopsies to detect these complications including rejection and arterial- and portal vein thrombosis. However, signs and symptoms of these complications may be subtle and nonspecific leading to a delay in diagnosis and intervention. Microdialysis is a sampling technique, which allows for continuous monitoring of metabolites in the tissue in which it is placed. The aim of this thesis was to study intraorgan metabolism using microdialysis early after liver transplantation as well as in tissue subject to compression, to identify metabolic patterns signaling distress so that major complications may be detected before they are reflected in clinical exam or blood chemistry.

In **paper I** the impact of compression and possible hypoperfusion on tissue metabolism was studied using microdialysis in kidney during pneumoperitoneum in a pig model. A significant increase in glycerol in the renal medulla after desufflation was shown.

In **paper II** a pig liver transplantation model was used to study the impact of short and long cold ischemia time on the restoration of glucose metabolism. An increase in intrahepatic glucose following portal reperfusion was seen in both groups and was significantly higher in the group with long cold ischemia. An increase in intrahepatic lactate/pyruvate ratio was also seen in both groups but was significantly higher and prolonged in the group with long cold ischemia time.

In **paper III** a clinical study showed no correlation between episodes of increased intrahepatic lactate/pyruvate and development of ischemic complications after liver transplantation.

In **paper IV** an increase in the lactate/pyruvate ratio in the liver during the initial 12 hours post-transplant was found to be correlated to development of post-transplant rejection in a clinical study.

Paper V investigated whether a microdialysis catheter placed in the middle hepatic vein is comparable to intrahepatic microdialysis for monitoring liver metabolism during clamping of the hepatic artery using a pig model. It was not.

In conclusion, the microdialysis technique has proven to be a promising tool for continuous monitoring of tissue metabolism and pharmacological studies. However, despite encouraging results from animal experiments and a wide range of clinical studies over the past two decades, microdialysis has not gained a foothold in standard clinical practice. It seems that microdialysis is useful for the detection of primary metabolic changes. However, a better understanding of the recovery of metabolic processes after the ischemia reperfusion injury occurring during the transplantation process is needed to use microdialysis for monitoring of secondary events following liver transplantation.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Trots förbättringar av operationsteknik och postoperativ vård drabbas många patienter som genomgår levertransplantation av komplikationer som kan leda till döden eller förlust av det transplanterade organet. Av denna anledning följs levertransplanterade patienter noga postoperativt med blodprover, bilddiagnostik och leverbiopsier. Syftet är att upptäcka komplikationer såsom avstötning och artär- och portaventrombos (blodpropp i leverns blodkärl) så att behandling kan sättas in i ett tidigt skede. Symtombilden för dessa komplikationer varierar och är ibland mycket subtil vilket kan leda till sen diagnos och intervention. Mikrodialys är en diagnostisk metod för att mäta olika ämnen i kroppens kolhydrat- och fettomsättning. Vanligtvis mäts metaboliterna glukos, laktat, pyruvat och glycerol. Tidigare studier har visat att laktat/pyruvat-kvoten (L/Pr) stiger vid syrebrist i vävnaden. Genom att mäta dessa ämnen i den transplanterade levern får man en detaljerad och kontinuerlig övervakning av organet. Målet för de studier som ligger till grund för denna avhandling var att med hjälp av mikrodialys upptäcka komplikationer innan de ger kliniska symtom och påverkade blodprover.

I vår första studie undersöktes hur ökat tryck i bukhålan påverkar njurvävnad i samband med njurdonation. För att studera detta användes en djurförsöksmodell med gris. Resultaten visar ökade nivåer av glycerol i njurmärgen vilket tyder på vävnadsskada.

I samband med levertransplantation utsätts levern för en period med cirkulationsuppehåll då den kyls ned för att begränsa cellskada, så kallad kall ischemitid. I vår andra studie undersökte vi hur lång respektive kort kall ischemitid påverkar kolhydrat- och fettmetabolismen i levern genom att använda en djurförsöksmodell med gris. Vi fann att L/Pr steg efter att levern återfått cirkulation och stegringen var betydligt högre i gruppen med lång kall ischemitid. Resultaten tyder på att de mekanismer som styr kolhydratomsättningen i levern påverkas under ischemitiden och att det tar längre tid för dessa system att återhämta sig vid lång kall ischemitid.

Vår tredje studie är en klinisk studie i vilken patienter som genomgick levertransplantation övervakades med mikrodialys postoperativt. Vi fann inget samband mellan ökad L/Pr och komplikationer kopplade till syrebrist. Resultaten tyder på att L/Pr inte kan användas för att upptäcka dessa komplikationer hos patienter.

Vår fjärde studie är en klinisk studie i vilken patienter som genomgick levertransplantation övervakades med mikrodialys postoperativt. Vi fann att patienter som senare utvecklade avstötning hade högre L/Pr under de första 12 timmarna efter levertransplantation.

I vår femte studie undersöker vi om mikrodialys som placeras i levervenen (intravenöst) kan ersätta mikrodialys placerad i leverparenkymet för övervakning av kolhydrat- och lipidmetabolism i lever som utsätts för syrebrist. Resultaten visar att intravenös mikrodialys inte kan ersätta mikrodialys i levern.

Sammanfattningsvis har mikrodialys varit ett lovande verktyg för att övervaka metabolism i vävnader och i farmakologiska studier. Trots initialt positiva resultat från experimentella och kliniska studier har tekniken inte etablerats som klinisk standard för övervakning av patienter. Mikrodialys är användbart för att följa primära metabola förändringar i vävnaden. För att kunna använda mikrodialys för att upptäcka komplikationer efter levertransplantation behövs dock mer kunskap om hur metabola processer återhämtar sig efter transplantationsprocessen.

LIST OF SCIENTIFIC PAPERS

- I. Microdialysis monitoring for evaluation of the influence exerted by pneumoperitoneum on the kidney: an experimental study
Surgical Endoscopy 2008 Apr;22(4):938-42.
- II. Effect of extended cold ischemia time on glucose metabolism in liver grafts: experimental study in pigs
Journal of Hepatobiliary Pancreatic Surgery 2007;14(2):183-8
- III. Evaluation of intrahepatic lactate/pyruvate ratio as a marker for ischemic complications early after liver transplantation – a clinical study
Transplantation Direct 5(12):e505, December 2019
- IV. Intrahepatic microdialysis for monitoring of metabolic markers to detect rejection early after liver transplantation
Resubmitted
- V. Hepatic vein microdialysis is not equivalent to intrahepatic microdialysis monitoring for the detection of metabolic changes induced by arterial ischemia in a pig liver model
Submitted

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LIST OF ABBREVIATIONS

Acetyl CoA	Acetyl coenzyme A
ACR	Acute cellular rejection
ATP	Adenosine triphosphate
AUC	Area under the curve
CI	Cold ischemia
CMV	Cytomegalo virus
CR	Chronic rejection
CT	Computer tomography
ESRD	End-stage renal disease
HAT	Hepatic artery thrombosis
HCV	Hepatitis C virus
IRI	Ischemia reperfusion injury
L/Pr	Lactate/pyruvate ratio
MELD	Model for end-stage liver disease
MS	Mass spectrometry
MWCO	Molecular weight cut-off
NADH	Nicotinamide adenine dinucleotide
NMR	Nuclear magnetic resonance
PAS	Periodic acid Schiff
RAI	Rejection activity index
UW	University of Wisconsin preservation solution
WI	Warm ischemia

1 INTRODUCTION

1.1.1 Background of the study

Each year approximately 30.000 liver transplants are performed world-wide (1). For patients suffering from terminal liver disease, receiving a liver transplantation is often a lifesaving treatment (2-4). Improvements in operating technique and post-operative management have dramatically increased the safety of the procedure since the first successful liver transplantation performed by Thomas Starzl in 1967 (2, 5, 6). Yet many patients still suffer post-operative complications which may lead to graft loss or death (4, 7, 8). Patients are monitored closely post-operatively using blood chemistry, diagnostic imaging and liver biopsies to detect these life-threatening complications including rejection and arterial- and portal vein thrombosis. However, signs and symptoms of these complications in early stage may be subtle and nonspecific leading to a delay in diagnosis and intervention and ultimately worse outcome (9, 10). As organ shortage has pushed toward the usage of extended criteria donors (previously known as marginal donors), new methods of assessing and monitoring liver grafts intra- and post operatively would be helpful for prediction of graft function and early detection of complications (11-14).

1.1.2 Outcome after liver transplantation

Factors, which impact outcome after liver transplantation depend on recipient-, donor- and peri-operative parameters (3, 13). Recipient characteristics that influence outcome include urgent indication for liver transplantation, age, presence of infection and cancer. End-stage liver disease is assessed by use of a scoring system; Model for End-Stage Liver Disease (MELD) which is used for prioritizing patients for receipt of a liver transplant (15). Donor characteristics such as advanced age, degree of steatosis of the liver, length of hospital stay and infection can have a negative impact on the posttransplant outcome (3). Such donors with high risk of post-transplant complications are called extended criteria donors (13, 14). As for perioperative factors, the organ preservation and length of cold and rewarming ischemia time as well as the experience level of the transplant centre also influence outcomes after liver transplantation (3). An overview of the liver transplantation procedure is outlined in Figure 1.

Post-operative complications may follow any surgical procedure. In this chapter some major complications occurring in the early post-operative period after liver transplantation are discussed.

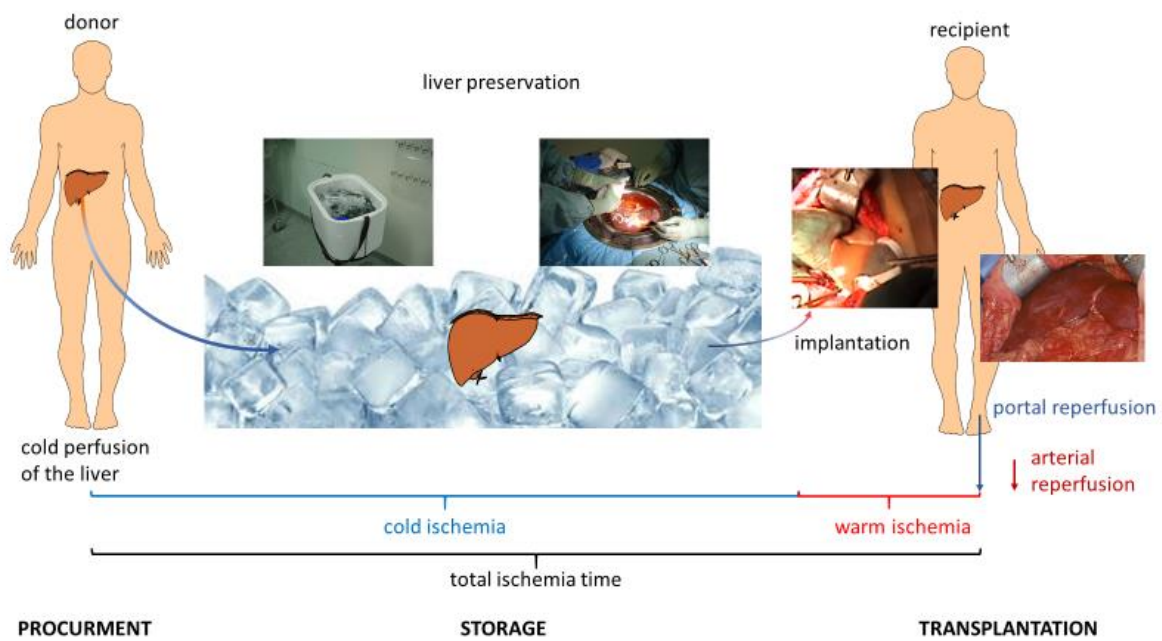


Figure 1. Overview of liver transplantation procedure including procurement, storage and transplantation

1.2 VASCULAR COMPLICATIONS

1.2.1 Hepatic artery thrombosis

Following orthotopic liver transplantation circulation to the liver graft is re-established by anastomosis of the supra- and infra-hepatic inferior vena cava, portal vein and the hepatic artery (16) (Figure 2).

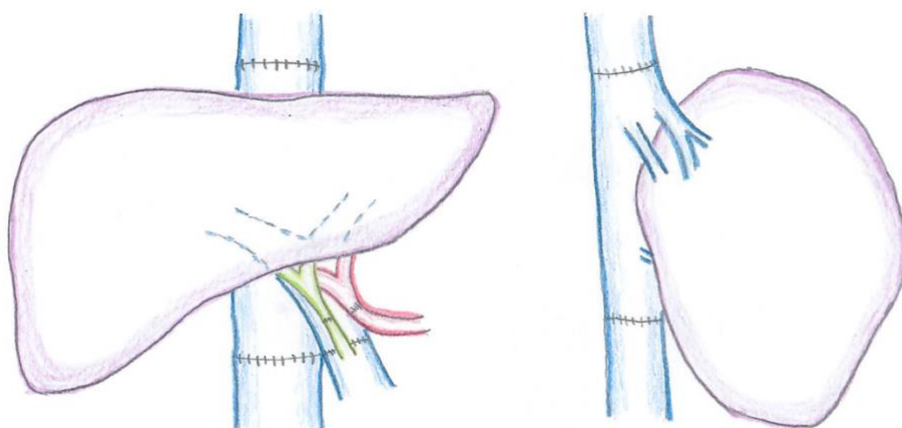


Figure 2. Anatomy of conventional orthotopic liver transplantation in anterior-posterior view and lateral view.

Nowadays, a modification of this classical technique is used with vena cava preservation on the recipient site (piggy-back technique) (Figure 3) (17).

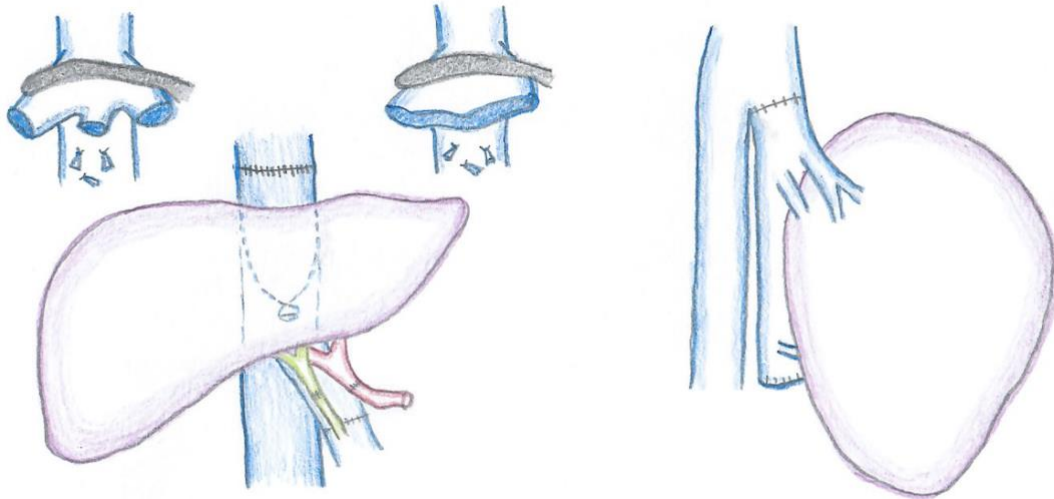


Figure 3. Classical “piggy-back” technique in anterior-posterior view and lateral view.

Hepatic artery thrombosis (HAT) is the most common vascular complication and has an incidence of 1.6-9% in adults and 5-8% in paediatric patients (7, 18-20). It is also the most common complication requiring re-transplantation (7, 21). For this life-threatening complication mortality rates range from 11% to 23% (20, 22, 23).

HAT can occur at any time following liver transplantation. The clinical presentation varies and depends on the time interval between transplant, thrombosis and the time of diagnosis (20). Symptoms range from fulminant hepatic necrosis with hepatic decompensation, sepsis and coagulopathy to less dramatic but progressive symptoms developing because of ischemic bile duct injury. The latter may give rise to non-specific symptoms such as fever, increased transaminases, cholangitis, and sepsis. HAT is diagnosed using radiological imaging or surgical exploration or both. Ultrasonography is used as a screening method in many centres during the initial postoperative period and has been shown to have a high sensitivity for HAT detection (24). Factors associated with the development of HAT include celiac trunk stenosis, technical imperfections of the anastomosis and increased vascular resistance distal to the anastomosis due to rejection or ischemia-reperfusion injury (7).

Depending on the clinical status of the patient HAT is managed differently. Fulminant hepatic failure due to HAT requires re-transplantation. In patients where HAT has been discovered early and symptoms are less severe graft salvage is attempted by expeditious surgical intervention. Following advances in recent years in endovascular intervention, this

has become an acceptable alternative treatment option for revascularization of the graft in some cases (25-27). Asymptomatic HAT cases are treated conservatively.

Considering the persistent shortage of organs and the increased risk of poor outcome associated with vascular complications, diagnostic improvements allowing for earlier detection and treatment of HAT may have profound impact on patient and graft survival.

1.2.2 Portal vein thrombosis

Approximately 75% of the blood flow and 50% of the oxygen supply to the liver is delivered through the portal vein. Portal vein thrombosis is a rare but severe complication following liver transplantation. The incidence is reported at 1-3% in the adult population (28-30). Presenting symptoms include liver failure, ascites and gastrointestinal bleeding (7). If detected and treated expeditiously radiological invasive intervention and anticoagulative therapy may be successful in preventing graft loss. However, re-transplantation rate is reported around 20% and long-term survival rates are significantly reduced (7, 23). Portal vein thrombosis triggers a compensatory vasodilation of the hepatic artery called the arterial buffer response (31). This ensures enough oxygen delivery to the liver to preserve liver function. Patients without pre-transplant portal hypertension develop persistent or de novo signs of portal hypertension such as hepatopulmonary syndrome, ascites, gastrointestinal bleeding and encephalopathy.

1.3 REJECTION

Allograft rejection is classified as acute or chronic depending on onset, reversibility and histological characteristics. The incidence of acute cellular rejection (ACR) has decreased due to improvements in immunosuppressive therapy but still occurs in 15-40% of liver transplanted patients with most cases occurring within the first year after transplantation (10, 32-34). Several risk factors for development of ACR have been identified including but not limited to autoimmune aetiology of liver disease prompting liver transplantation, CMV infection, male donor to female recipient, higher donor age and long ischemia time (10, 33). ACR usually responds well to treatment with steroid boluses or increased basic immunosuppressive regimen (10). Isolated events of ACR do not affect long-term outcome in terms of patient or graft survival, however repeated insults may predispose to chronic rejection (CR) (10, 35, 36). ACR may be asymptomatic or present with malaise, fever or abdominal pain. The condition is often suspected following elevation of transaminases, alkaline phosphatase, gamma-glutamyl transpeptidase and bilirubin (10). However, neither symptoms nor blood chemistry changes are specific for distinguishing ACR from other complications after liver transplantation and a liver biopsy continues to be the gold standard required for final diagnosis. The severity of ACR is graded using the Rejection Activity Index (RAI) which takes into account the degree of portal inflammation, bile duct inflammation damage and venous endothelial inflammation (37).

CR is an immunological reaction to the liver allograft resulting in a progressive destruction of bile ducts and cholestatic liver dysfunction without major inflammation (38). The incidence

rate of CR is reported at 2-17% in liver transplanted patients (39-41). CR is chronic but can present at any time following liver transplantation. While episodes of ACR usually respond well to treatment, CR is associated with a significant risk of graft loss and death despite the use of high doses of immunosuppression (39).

Following the introduction of modern immunosuppressive agents the incidence rate of allograft rejection has dropped markedly and the general outcome after liver transplantation has improved substantially (42). However significant morbidity results from side effects of continuous immunosuppressive treatment with increased risk of cardiovascular disease, nephrotoxicity, infection and a two to four-fold elevated risk of neoplasms (42, 43). In view of this, tolerance induction (total withdrawal of immunosuppressive medication after transplantation without negative effect on the graft in the form of rejection) has become a major goal for the transplant community in recent years. Studies suggest that tolerance may be possible to achieve in 20%-70% of liver transplanted patients (44-47). However, a better understanding of the underlying mechanisms of tolerance induction is warranted and new biochemical- and genetic markers are needed to identify patients who may be candidates for weaning protocols (34, 47, 48).

1.4 ISCHEMIA-REPERFUSION INJURY OF THE LIVER

Procurement, storage and implantation of the donor liver entails a transient deprivation of oxygen followed by restoration of blood circulation and oxygen delivery to the tissue resulting in cellular damage called ischemia-reperfusion injury (IRI) (49, 50). After harvesting, the liver graft is stored in hypothermic conditions to reduce metabolic activity and energy demand during transport, a period referred to as cold ischemia time (CI) (Figure 1). During implantation re-warming of the organ inevitably occurs. Before circulation has been re-established, the liver graft is thus exposed to a period of warm ischemia (WI) (rewarming time). IRI is the result of a continuum of complex inflammatory and immunological processes causing structural cell- and tissue damage in different ways during cold and warm ischemia time and after reperfusion. The length of ischemia time is strongly correlated to the degree of IRI with longer cold ischemia time resulting in more severe injury (51).

IRI is related to a higher incidence of early graft non-function as well as acute and chronic rejection (49, 52, 53). Also, organs from extended criteria donors as well as organs exposed to prolonged ischemia are more susceptible to IRI (50, 52). New diagnostic methods which allow for assessing the degree of ischemic injury followed by reperfusion injury could aid in identifying patients who are at greater risk of developing allograft rejection. Furthermore, a better understanding and prevention of IRI has the potential of significantly expanding the donor pool as more organs from extended criteria donors could be considered for transplantation.

1.5 LIVING DONOR NEPHRECTOMY AND KIDNEY TRANSPLANTATION

1.5.1 The Kidney

The kidney has several functions including regulation of fluid balance, electrolytes, blood pressure and production of various hormones. The smallest functional unit of the kidney is the nephron which produces urine by filtration of fluid from the blood. Filtration occurs from a spool of branching capillaries in the glomerulus. The filtrate being passed into Bowman's capsule, flows through different sections of tubules until finally reaching collecting ducts and the renal hilus as urine. Morphologically, the kidney is made up of an outer renal cortex and the inner renal medulla.

1.5.2 Renal Transplantation

Renal transplantation is the preferred treatment for patients with end-stage renal disease (ESRD) (54-57). Since the first long-term successful kidney transplant performed in 1953 between monozygotic twins, the safety and outcome of the procedure have improved radically. Living donor renal transplantation has become an increasingly important treatment option for ESRD given the growing shortage of donors and superior outcomes for recipients (57, 58). Open surgery for donor nephrectomy was previously the first choice for living kidney transplantation. However, due to improvements in the laparoscopic technique and the associated advantages such as less post-operative pain, shorter hospital stay and better cosmesis than the open procedure, laparoscopic donor nephrectomy has become the standard of care for living donor renal transplantation (59, 60). By reducing donor morbidity, laparoscopic nephrectomy has also lowered the threshold for donation which helps to expand the donor pool (58). Laparoscopic donor nephrectomy can be performed through a transperitoneal- or retroperitoneal approach. The transperitoneal approach is associated with a higher risk of bowel injury and late onset bowel obstruction than the retroperitoneal approach (61, 62). Recently, robotic-assisted techniques for living donor nephrectomy have also been introduced in several centres (63, 64).

Prior to the study presented in Paper I, laparoscopic living donor transplantation was shown to be associated with higher creatinine levels and delayed initial graft function during the first week after transplantation, in comparison to open living donor nephrectomy (61, 65, 66). Presumably this graft dysfunction can be attributed to handling of the graft, longer warm ischemia time and pneumoperitoneum potentially causing renal ischemia. However, the effect of the procurement procedure on graft metabolism is not fully known.

1.6 METABOLOMICS

Metabolomics is the study of small molecule metabolites (<1.5 kDa) in tissues, cells and biofluids. Metabolites are products or substrates of metabolic pathways central to all living cells. Measuring them in the tissue of interest provides a metabolic profile reflecting biochemical activity on a cellular level (67). The field of metabolomics offers new ways of identifying biomarkers as well as describing and understanding biological phenotypes. Mass

spectrometry (MS) and Nuclear Magnetic Resonance (NMR) spectroscopy are the techniques most commonly used for analysing the 30,000 human metabolites which have been identified so far (68). Glucose, lactate and pyruvate are biomarkers commonly used to describe the balance between aerobic and anaerobic metabolism (69, 70) (Figure 4).

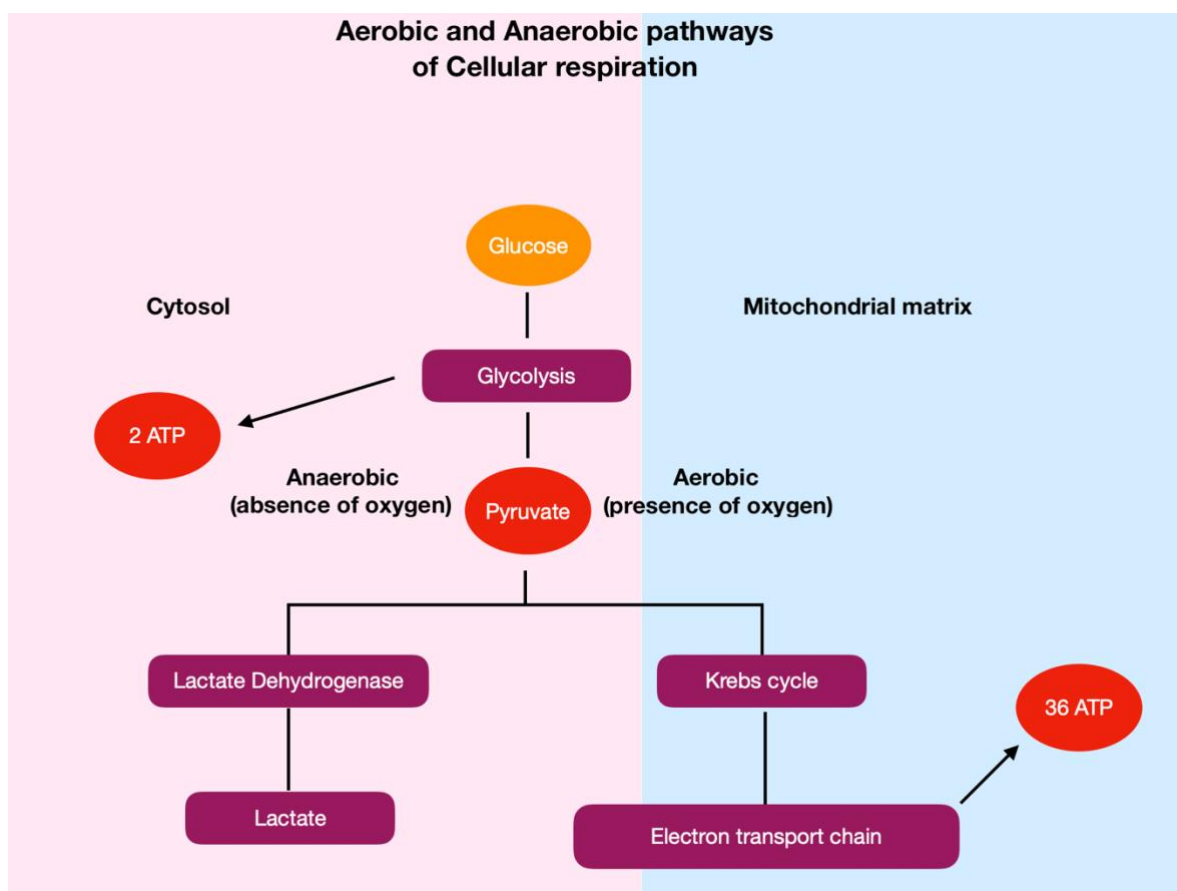


Figure 4. Aerobic and anaerobic pathways of cellular respiration

While MS and NMR describe intracellular metabolism, these techniques do not allow for continuous monitoring over longer time. To better follow the biochemical status of the cells over time microdialysis has been introduced. It offers the potential of direct monitoring of the metabolism in the tissue in which the microdialysis catheter is placed. Microdialysis is a sampling technique which mimics the passive function of a capillary blood vessel and allows for continuous monitoring of metabolites with short time intervals (a few minutes) over up to two-three weeks.

Glucose is the main source of energy in human cells. It is metabolised through the process of glycolysis to yield energy in the form of ATP as well as intermediates essential in other metabolic pathways. Upon entering the cell through a glucose transporter, glucose is phosphorylated and becomes glucose-6-phosphate. This traps glucose within the cell and allows for further metabolization through the glycolysis pathway. Glucose can also be stored in the form of glycogen in skeletal muscle and the liver. When glycogen stores are depleted glucose is synthesised from precursors such as glycerol, pyruvate and lactate by the process of gluconeogenesis (71).

In the presence of oxygen, the end product of glycolysis is two **pyruvate** molecules. Pyruvate is converted to acetyl CoA, -the main substrate of the citric acid cycle. The citric acid cycle is the metabolic pathway by which energy is produced from oxidation of NADH in the mitochondria of the cell. The net production of energy from one glucose molecule is 36 ATP. In the absence of oxygen pyruvate is reduced to **lactate**. This upholds glycolysis and ATP production in the cell albeit with a significantly lower energy yield of 2 ATP and an accumulation of lactate causing acidosis (Figure 4).

Although an increase in serum lactate levels is often seen in late stages of extensive tissue ischemia, lactate has proven to be a poor early marker for tissue ischemia (72, 73). This may be explained by clearance of lactate from serum by the liver causing a delay in lactate increase in the serum. Also, lactate increase can be seen in non-ischemic conditions as a result of hypermetabolism (73). Instead the **lactate/pyruvate ratio** (L/Pr) is widely used as an indicator of ischemia (74-77). During ischemia lactate accumulates and pyruvate is consumed as glycolysis increases, resulting in an increase in the L/Pr.

Measurement of tissue levels of glucose and its derivatives has been described in abdominal surgery, plastic surgery and is a well-established laboratory tool in neurosurgery for detection of ischemic complications in the tissue of interest (74, 78-80).

Glycerol is a three-carbon molecule central to lipid metabolism in the human body. It constitutes the backbone of glycerophospholipids which make up internal and external cellular membranes. Glycerol is also found bound to fatty acids in triacylglycerol in adipose cells. When energy requirements increase during stress or starvation, glycerol is released from fatty acids through lipolysis. The free fatty acids are either used in the energy production within the cell or released to the blood and carried to other tissues. As adipose cells lack the ability to metabolize glycerol it is transported to the liver where it is converted to dihydroxyacetone phosphate which can enter glycolysis or gluconeogenesis (71, 81). During apoptosis or cell injury, phospholipases break down cellular membranes, releasing glycerol and fatty acids to the extra cellular space. Increase in glycerol may thus reflect increased lipolysis or indicate disintegration of cellular membranes (82). It has been considered as a marker for tissue damage and cell death in neurosurgical studies (83).

1.7 LIVER METABOLISM DURING TRANSPLANTATION

During the transplantation process the liver is deprived of oxygen and suffers from periods of cold preservation and warm ischemia before reperfusion. The lack of oxygen halts aerobic energy production and the degradation of glucose through the citric acid cycle. However, several intra-cellular pathways requiring energy are still in operation. In order to uphold energy production pyruvate is converted to lactate. Also, the balance between glycolysis and gluconeogenesis shifts toward gluconeogenesis during warm and cold ischemia (84). While short periods of oxygen deprivation can be compensated for in this way, longer ischemic insults result in deterioration of essential metabolic pathways such as mitochondrial oxidative

phosphorylation (85, 86). Ultimately this leads to hepatocyte dysfunction and poor transplant outcome (84, 87).

The liver plays an essential role in the regulation of carbohydrate-, lipid- and protein metabolism (Figure 5). In the well-fed state the liver stores glucose in the form of glycogen. When blood glucose levels drop, glycogen is broken down to glucose and released into the bloodstream to maintain stable blood glucose levels. Some of the metabolic pathways and enzymes which regulate these functions are unique to hepatocytes and upholding them throughout the procurement, storage and reimplantation process is paramount for graft and patient survival (84, 87).

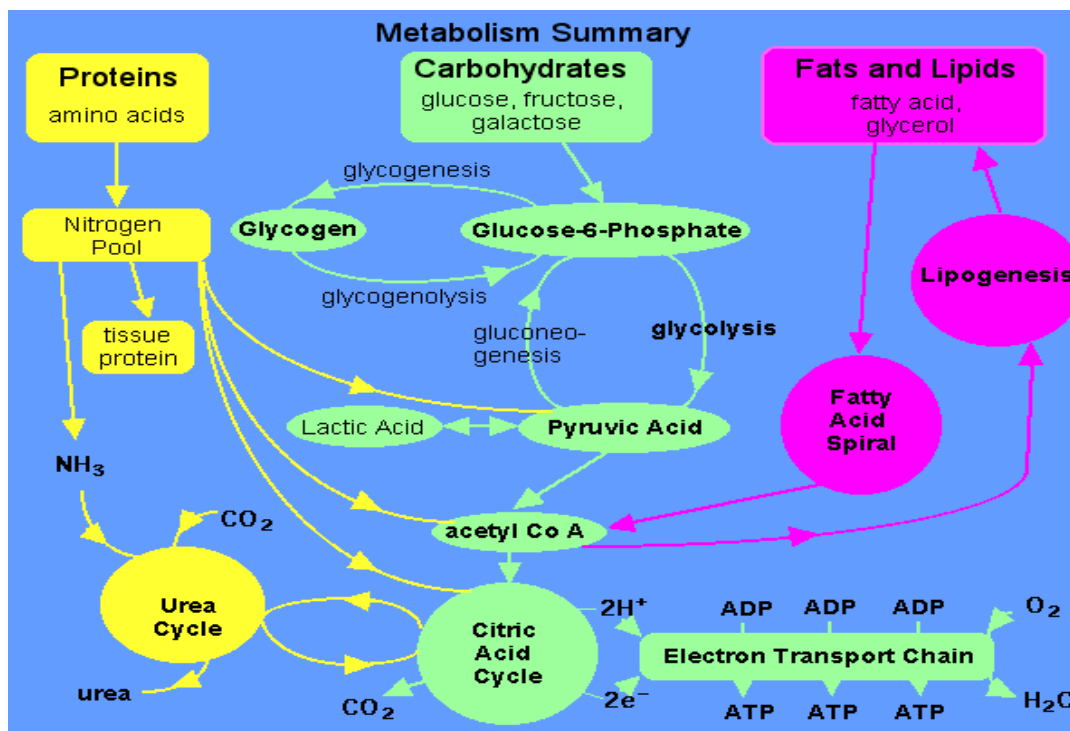


Figure 5. Summary of carbohydrate-, lipid- and protein-metabolism in the liver.

By studying intrahepatic metabolism using microdialysis during and early after liver transplantation as well as metabolism in tissue subject to compression we hope to identify metabolic patterns signalling distress so that major complications may be detected before they are reflected in clinical exam or blood chemistry.

2 AIMS

General

The thesis aims to evaluate monitoring of glucose, lactate, pyruvate and glycerol in the kidney and in the liver using microdialysis, for detection of major complications such as ischemia and rejection early after liver transplantation

Aims of the individual studies

Study I

To study the impact of compression and possible hypoperfusion on tissue metabolism using microdialysis in kidney during pneumoperitoneum in a large animal model.

Study II

To study the impact of short and long CI time on the restoration of glucose metabolism in the liver using a pig experimental model of liver transplantation.

Study III

To evaluate the intrahepatic L/Pr as a clinical marker for ischemic complications such as hepatic artery thrombosis by using microdialysis early after human transplantation.

Study IV

To evaluate intrahepatic glucose, lactate, pyruvate and glycerol as potential markers for rejection early after human liver transplantation using microdialysis.

Study V

To study if results from a microdialysis catheter placed in the middle hepatic vein is comparable with results from intrahepatic microdialysis during monitoring of liver metabolism during arterial ischemia a pig model.

3 METHODS USED IN THE THESIS

3.1.1 Microdialysis: Principles and technique

The notion of analysing samples of interstitial fluid collected by use of dialysis bags was introduced in the 1960's by Bito et al (49). In 1974 Ungerstedt and Pycock made substantial improvements to the concept and catheter design which has given the technique its current form (50).

Microdialysis is a sampling technique for continuous measuring of small-molecular-weight substances in the interstitial space of living tissue. A double lumen catheter is placed in the tissue of interest and perfused with a physiological fluid. The catheter, or microdialysis probe, is fitted with a semipermeable membrane at the tip over which small molecules can cross by passive diffusion (Figure 6).

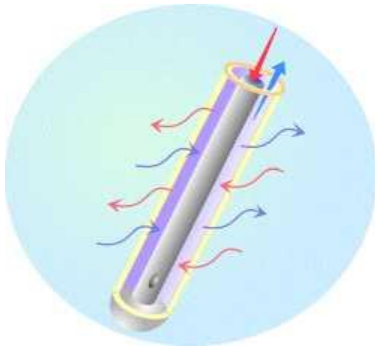


Figure 6. Illustration of semipermeable membrane at the tip of the microdialysis probe. With courtesy of M Dialysis AB.

The perfusate is pumped through the inner lumen of the catheter, to the semipermeable membrane where it equilibrates with the extra-cellular fluid. The perfusate is then collected through the outer lumen, in vials which are analysed at the bedside (Figure 7).

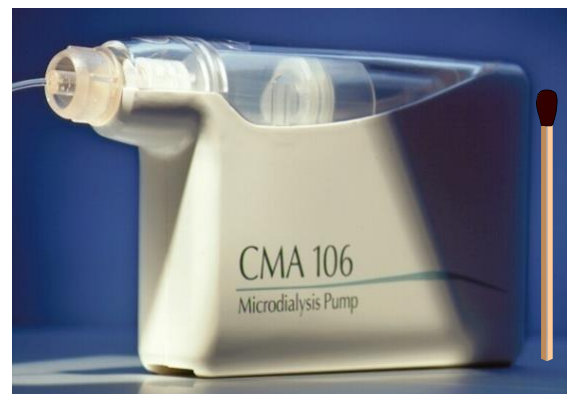


Figure 7. Microdialysis catheter, sampling vial and pump. With courtesy of M Dialysis AB.

The recovery of the substance to be measured is dependent on the perfusion rate of the liquid pumped through catheter, the length and properties of the dialysis membrane and the diffusion of the substance through extracellular fluid (70). A high perfusion rate gives less time for diffusion over the membrane and thus lower recovery rate. A low perfusion rate gives a higher recovery but the collection period may become inappropriately long. A perfusion rate of 0.3 $\mu\text{l}/\text{min}$ is considered to give close to 100% recovery for measurements in biological tissue (70, 88). Usually perfusion rates range from 0.3-1.0 $\mu\text{l}/\text{min}$. The microdialysis probe is available in several different membrane- and shaft lengths and the molecular weight cut-off (MWCO) ranges between approximately 6 to 100 kDa. The speed by which molecules diffuse through the extracellular tissue will affect the amount of substance which will be recovered as well as how quickly any change can be detected (70). Also, molecules with molecular weight close to that of the MWCO have a lower recovery rate. Analytes with molecular size of one third to one fifth of the MWCO are considered to be recovered at a significant rate (89). The catheters used in our studies (CMA 70, with a 60 mm shaft, diameter 0.9 mm and a 30 mm membrane, diameter 0.6mm) has a MWCO of 20 kDa. The analytes studied are glucose (180 Da), lactate (90 Da), pyruvate (88 Da) and glycerol (92 Da). As the analytes all have a molecular weight well below one fifth of the MWCO they can be expected to diffuse freely across the membrane. Enzymes such as lactate dehydrogenase (143 kDa) which might affect the concentration of analytes are too big to pass over the membrane wherefore the concentration of analytes in the sampled fluid is considered stable. Analytes diffuse more easily through an aqueous solution than in tissue (90). This must also be considered when estimating recovery rate. The sampled fluid, collected in vials, is analysed bedside using enzyme reaction specific for each analyte, followed by liquid chromatography. Samples may also be stored for up to 1 month at -20°C .

3.1.2 Strengths and limitations of microdialysis

Microdialysis has proven to be a minimally invasive and easy to use technique for studying metabolic changes in various tissues such as brain, muscle, small intestine and adipose tissue (78, 91-93). The microdialysis catheter brings little or no discomfort to the patient and is easily removed at the end of monitoring. The technique provides an opportunity to monitor metabolic trends in real time with approximately 20-minutes delay owing to sampling. However, there are potential limitations to the microdialysis technique. Measurements obtained show analyte concentrations directly surrounding the catheter and may not be representative of the entire organ. Also, the introduction of the catheter inevitably causes some degree of damage in the tissue leading to inflammatory reactions which could influence test results (69). As scar tissue forms around the catheter the ability for metabolites to diffuse across the membrane changes which may affect recovery rate over time (94). When studying glucose and its metabolites the fact that concentrations may also be influenced by nutrition, intravenous infusions, medication and anaesthesia must be considered when interpreting test results (88).

3.1.3 Microdialysis monitoring of the kidney (Paper I)

In the renal study the microdialysis probes are introduced into the renal cortex and medulla using introduction needles. At the time the study was performed microdialysis monitoring of the kidney had not been previously studied to any extent. However, since then several studies have demonstrated microdialysis to be a safe and reproducible method for studying renal metabolism in the transplantation setting in clinical and experimental models (95-98).

3.1.4 Microdialysis monitoring of the liver (Paper II, III, IV and V)

For intrahepatic microdialysis monitoring, a microdialysis catheter is placed in liver segment IV at the end of surgery, before closure of the abdomen. It can be sutured to the falciform ligament for stability and the pump is fixed to the abdominal wall using dressings (Figure 8). Previous studies have shown the microdialysis monitoring technique to be safe and easy to perform in liver transplantation in the experimental and clinical setting (76, 99). In a clinical study by Håugaa et al., on microdialysis after liver transplantation, performed around the time of the studies described in this thesis, suggested cut-off values of lactate and L/Pr for detection of ischemic complications were presented (100).

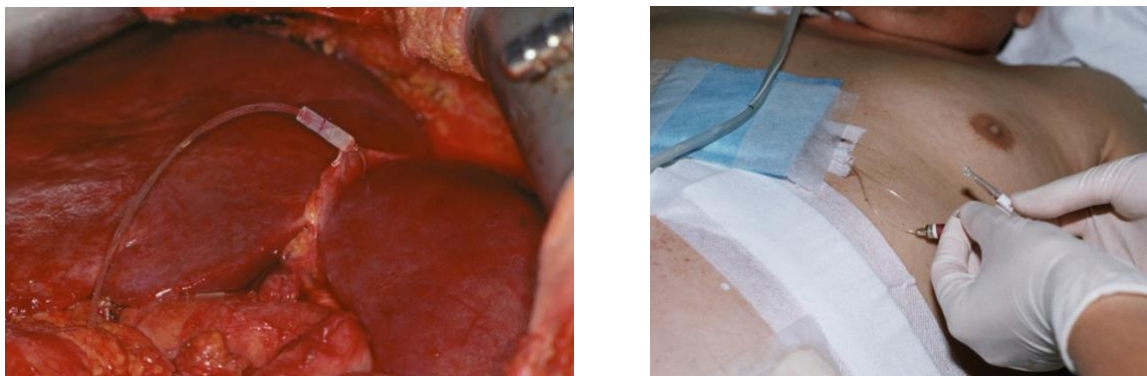


Figure 8. Microdialysis probe placement in the liver and fixation (to the left) with dressings following abdominal closure (to the right)

3.2 EXPERIMENTAL STUDIES (PAPER I, II AND V)

All procedures were conducted in strict accordance with the criteria of the Ethics Committee for Animal Experiments at Karolinska Institutet. A porcine model was used for its similarity to the clinical setting with regards to solid organ transplantation (Paper I, II and V). Cross-bred (Swedish Landrace/Yorkshire/Hampshire) female pigs were used. Before operation, animals were fasted with free access to water for up to 36 hours. Anaesthesia was induced with intramuscular premedication of 12mg/kg ketamine hydrochloride, 5mg/kg azaperone and 0.05mg/kg atropine. 1-4mg/kg Midazolam was given prior to intubation. Anaesthesia was maintained by inhalation of a mixture of oxygen, nitric oxide and halothane. Invasive blood pressure, electrocardiogram, urine production, body temperature and blood gas analysis (study V), was monitored throughout the experiment. Body temperature was kept between

38-39°C using an external heating device. Animals were kept on continuous infusion of Ringer acetate solution at a volume of 6-8 ml/kg. At the end of the experiment animals were sacrificed under deep anaesthesia by intravenous injection of over-dose pentobarbital.

3.3 CLINICAL STUDIES (PAPER III AND IV)

Patients undergoing liver transplantation at the Karolinska University Hospital were included. Liver transplantation was performed with preservation of the recipient vena cava inferior using the piggyback technique as standard (Figure 3). Venovenous bypass was used in selected cases. Basic immunosuppression was achieved with steroids and Tacrolimus. High molecular weight Dextran was used post transplantation for thrombosis prophylaxis followed by acetylsalicylic acid. Throughout post-operative intensive care stay, blood glucose was measured every hour by arterial blood gas. During post-operative days 1 and 2, patients were administered 5% glucose infusions at 30 ml/kg/day. On the third postoperative day, patients were given half a dose of total parenteral nutrition (25 kcal/kg/day). Thereafter full dose of parenteral nutrition was given and patients were allowed to eat. Target blood glucose value was < 8 mmol/L.

3.4 METHODOLOGICAL OVERVIEW

	Population	Methods
Paper I	8 cross-bred (Swedish Landrace/Yorkshire/Hampshire) littermate female pigs, 28-34 kg	Prospective study. Intra-renal microdialysis followed by pneumoperitoneum. Retrospective analysis of microdialysis data.
Paper II	26 cross-bred (Swedish Landrace/Yorkshire/Hampshire) female pigs, 30-34 kg	Prospective study. Group I (n = 6) liver transplantation with CI of 5h. Group II (n = 7) liver transplantation with CI of 15h. Intrahepatic microdialysis. Retrospective analysis of microdialysis data.
Paper III	45 patients undergoing liver transplantation at Karolinska University Hospital Huddinge	Prospective study. Intrahepatic and subcutaneous microdialysis up to 6 days post-op. Bedside and retrospective analysis of medical and microdialysis data.
Paper IV	71 patients undergoing liver transplantation at Karolinska University Hospital Huddinge	Prospective study. Intrahepatic and subcutaneous microdialysis up to 6 days post-op. Retrospective analysis of medical and microdialysis data.
Paper V	8 cross-bred (Swedish Landrace/Yorkshire/Hampshire) female pigs, 30-35 kg.	Prospective study. Intrahepatic, subcutaneous and intravenous (middle hepatic vein) microdialysis. Induction of ischemia by clamping of hepatic artery. Retrospective analysis of microdialysis data.

Table 1. Patients, experimental models and methods used in the studies

In **Paper I** Eight cross-bred littermate female pigs were used in the study. Under anaesthesia and following laparotomy, microdialysis catheters (CMA 70) with a 60 mm shaft (diameter 0.9mm) and a 30-mm membrane (20 kDa cut-off) were inserted in the cortex and the medulla of the left kidney. Samples were collected at 30-min intervals and analysed with respect to glucose, lactate, pyruvate and glycerol. The L/Pr was calculated. Following placement of the catheters the abdomen was closed and pneumoperitoneum was induced by intraperitoneal insufflation of carbon dioxide via a standard laparoscopic port. Intraabdominal pressure was kept at 16 to 18 mmHg for a duration of 4 h. After 4 h, desufflation was performed and the kidney was monitored for another 2 hours. Microdialysis data was analysed for differences during and after insufflation as well as differences between cortex- and medullar placement.

In **Paper II** 26 cross-bred female pigs were used for 13 orthotopic liver transplantations. Animals were divided into 2 groups: group I (n = 6) with liver graft exposed to 4 h of CI and group II (n = 7) with liver grafts exposed to 14 h of CI. In both groups cold ischemia was followed by 1 h of rewarming ischemia after which time the liver was reperfused. Liver grafts were perfused with 4°C University of Wisconsin (UW) solution via the portal vein and hepatic artery following hepatectomy. They were then statically stored in UW solution on ice throughout the cold ischemia period. Following abdominal incision microdialysis catheters (described above) were inserted into the liver and subcutaneous tissue which was used for reference. Microdialysis samples were collected at 20-minute intervals throughout the experiment and analysed as described for paper I. Liver graft monitoring was continued 2 hours after reperfusion was achieved. Microdialysis data was compared between the groups.

In **Paper III** 45 patients undergoing liver transplantation were included in the study. Familial amyloidotic polyneuropathy was the most common indication for transplantation (20%) followed by HCV (18%), hepatocellular carcinoma (16%) and primary sclerosing cholangitis (11%). Liver transplantation was carried out using piggyback technique as the standard. At the end of the operation microdialysis catheters were placed in the liver graft and in subcutaneous tissue for reference. Microdialysis samples were collected once every hour up for up to 6 days post-operatively and analysed as previously described. Suspicion of ischemic complications were investigated by contrast enhanced ultrasound of the liver and CT angiography scan. An ischemic complication was defined as vascular occlusion or infarction confirmed by radiology. Based on our previous studies episodes with increasing L/Pr in three consecutive samples and an increase of at least 30% in total, were regarded as related to tissue ischemia (76, 101). Also suggested cut-off values were used (100). Microdialysis data was compared to clinical data.

In **Paper IV** 71 patients undergoing liver transplantation were included in the study. Hepatocellular carcinoma was the most common indication for transplantation (18%), followed by primary sclerosing cholangitis (15%), familial amyloidotic polyneuropathy (14%) and HCV (14%). At the end of liver transplantation microdialysis catheters were placed in the liver and in subcutaneous tissue for reference. Samples were collected every hour and analysed as previously described. Time-zero biopsies were taken and graded for

IRI. Rejection was classified according to RAI (37). Microdialysis data was compared for groups with and without rejection.

In **Paper V** Eight cross-bred littermate female pigs were used in the experiment. Following laparotomy, microdialysis catheters were placed in the liver and in subcutaneous tissue for reference. A third catheter was placed in the middle hepatic vein by introduction through the internal jugular vein. Flow rate was 0.3µl/min in the intrahepatic and reference catheters and 1µl/min in the intravenous catheter. Following a period of equilibration the hepatic artery was clamped for four hours to induce ischemia. Microdialysis samples were collected at 15-minute intervals from the intrahepatic and intravenous catheters and at 30-minute intervals from the reference catheter. Microdialysis data from the three different catheters was compared.

3.5 ETHICAL CONSIDERATIONS

Experimental studies were approved by the Ethics Committee for Animal Experiments at the Karolinska Institute. Clinical studies were approved by the Ethics Committee for Clinical Studies at the Karolinska Institute in accordance with guidelines of the Helsinki Declaration. Written and oral consent was obtained from all patients included in the clinical studies.

3.6 STATISTICAL METHODS

ANOVA test for repeated measurements was used in **paper II, VI and V** for analysing differences between groups. Mann-Whitney *U*-test was applied in **paper II and IV** for analysing differences between groups. Area under the curve (AUC) was calculated for entire time points and analysed using a two-tailed t-test for dependent samples in **paper I, II and IV**. To analyse the difference between values during pneumoperitoneum and after desufflation nonparametric Wilcoxon's matched-pairs tests was used in **paper I**. Bonferroni post-hoc test was used to analyse differences between groups over time in **paper V**. For **paper III** descriptive statistics were applied. Differences were considered significant in tests with a *p*-value less than 0.05.

4 RESULTS

	Main findings
Study I	There was a significant increase in glycerol in the renal medulla after desufflation.
Study II	An increase in intrahepatic glucose following portal reperfusion was seen in both groups and was significantly higher in the group with longer CI time. An increase in L/Pr was seen in both groups during rewarming ischemia. This increase was significantly higher and persisted 20 min after portal reperfusion in the group with long CI.
Study III	There was no correlation between episodes of increased L/Pr and ischemic events after transplantation.
Study IV	A significant increase in L/Pr during the initial 12 hours post-transplant was correlated to development of post-transplant rejection .
Study V	There were no changes in glucose, lactate, pyruvate or L/Pr measured i.v. in the hepatic vein during or after clamping of the hepatic artery.

Paper I

Glucose remained stable in both renal cortex and medulla throughout the experiment. Lactate levels showed a tendency to decrease in the cortex and medulla after induction of pneumoperitoneum. Following decompensation, lactate levels increased and showed a plateau. There were no significant changes in pyruvate or L/Pr levels in the cortex or medulla throughout the experiment. Glycerol levels were stable in the cortex and medulla during pneumoperitoneum (Figure 9). Following desufflation, there was a significant increase in glycerol in the medulla which was persistent over time. Glycerol levels remained stable in the cortex.

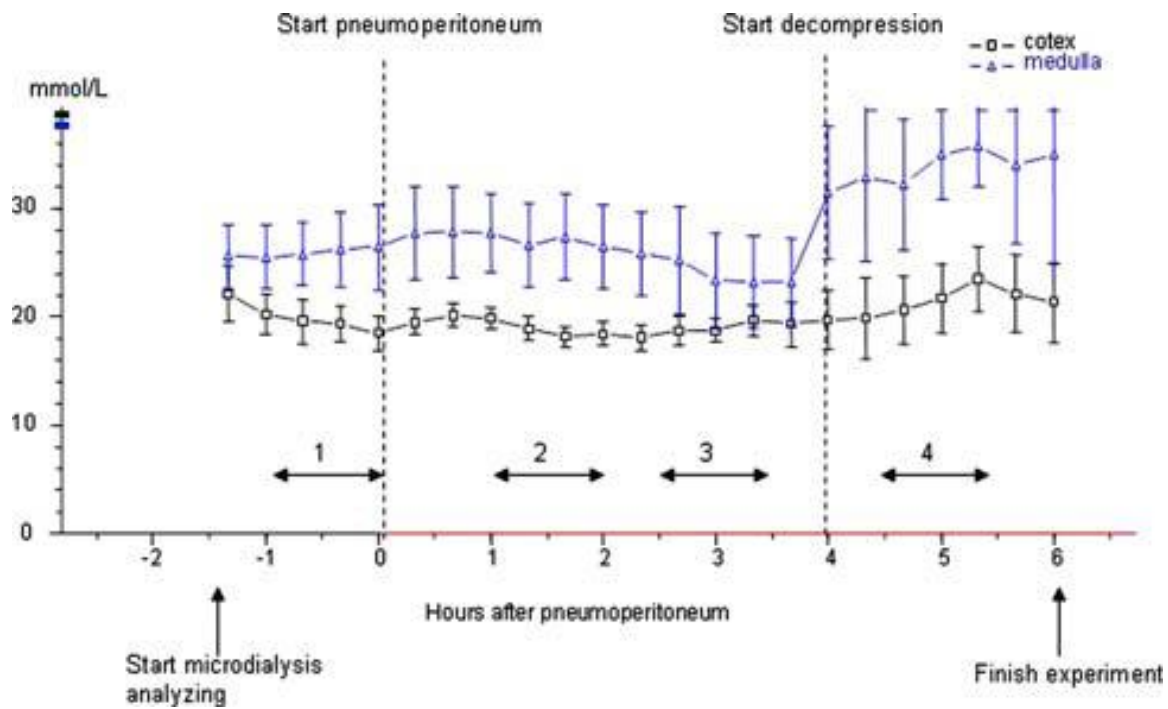


Figure 9. Glycerol concentrations in the renal cortex and medulla. There was no change before (area 1) or during (area 2 and 3) pneumoperitoneum in the cortex or the medulla. After decompensation (area 4), tissue glycerol in the medulla increased. Paper I

Paper II

Significantly higher levels of glucose were observed after portal reperfusion in livers exposed to a longer CI time. Also, in this group peak levels were reached with a delay compared to livers with short CI time (Figure 10).

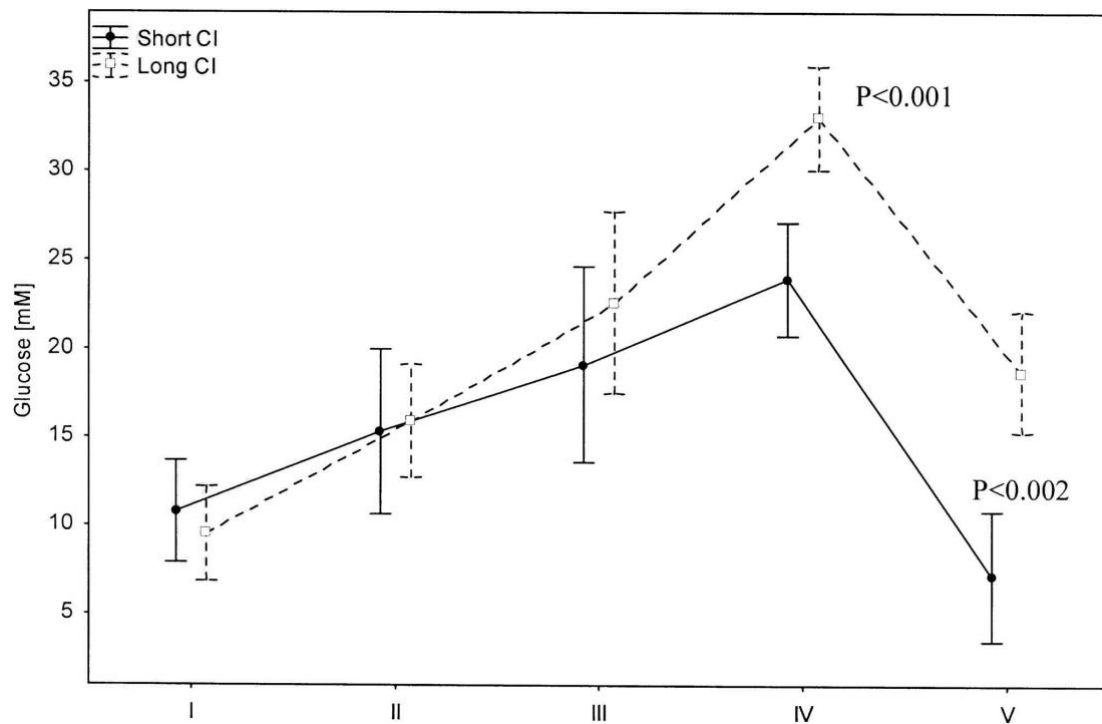


Figure 10. Intrahepatic levels of glucose at the time of: (I) before donation, (II) the end of cold ischemia, (III) portal reperfusion, (VI) peak levels after portal reperfusion, and (V) 2 h after portal reperfusion: in transplanted liver grafts with short (4h) and long (14h) periods of cold ischemia (CI). Paper II

Monitored increases in glucose observed in both groups correspond to a decrease in glycogen in histological exam using Periodic acid Schiff (PAS) staining (Figure 11).

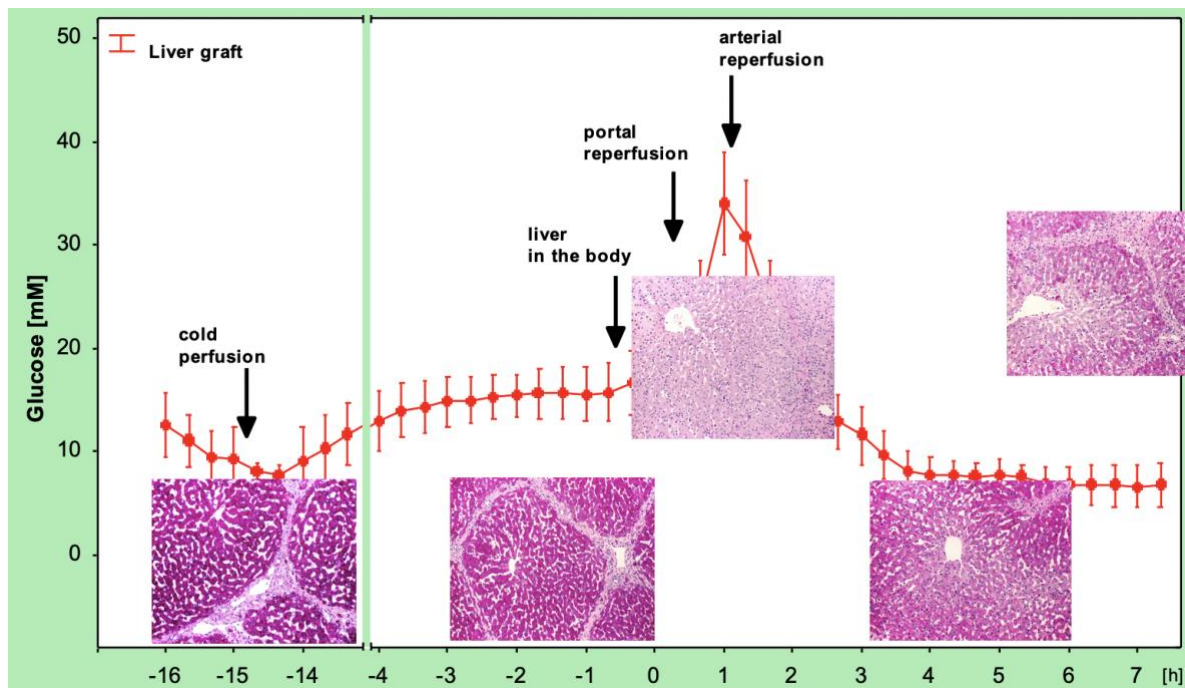


Figure 11. Intrahepatic glucose levels measured with microdialysis and glycogen storage in liver biopsy (amount of glycogen corresponding to the intensity of the color in histopathological staining (PAS)).

Lactate levels remained stable in both groups after cold perfusion and during CI. During WI, lactate levels increased in both groups (up to $4.0 \pm 1.6\text{mM}$ and $5.1 \pm 1.2\text{mM}$, respectively). The increase continued after portal reperfusion and reached peak levels of $9.9 \pm 1.8\text{mM}$ and $11.7 \pm 2.6\text{mM}$ respectively. Thereafter lactate levels decreased. No difference between groups was observed despite significant changes over time. **Pyruvate** decreased rapidly following cold perfusion in both groups (from $163.1 \pm 93.8\mu\text{M}$ to $14.9 \pm 7.6\mu\text{M}$ in the short CI group and from $179.4 \pm 56.0\mu\text{M}$ to $< 10\mu\text{M}$ in the long CI group). An increase in pyruvate was observed after portal reperfusion in both groups. Despite significant changes in pyruvate levels over time, there was no difference between the groups. **L/Pr** increased in both groups during CI (Figure 12). Following this increase a stabilization was seen. During WI **L/Pr** increased further in both groups. After portal reperfusion **L/Pr** continued to increase in livers with long CI whereas a rapid decrease was seen in the group with short CI. This difference between groups was significant ($p < 0.01$).

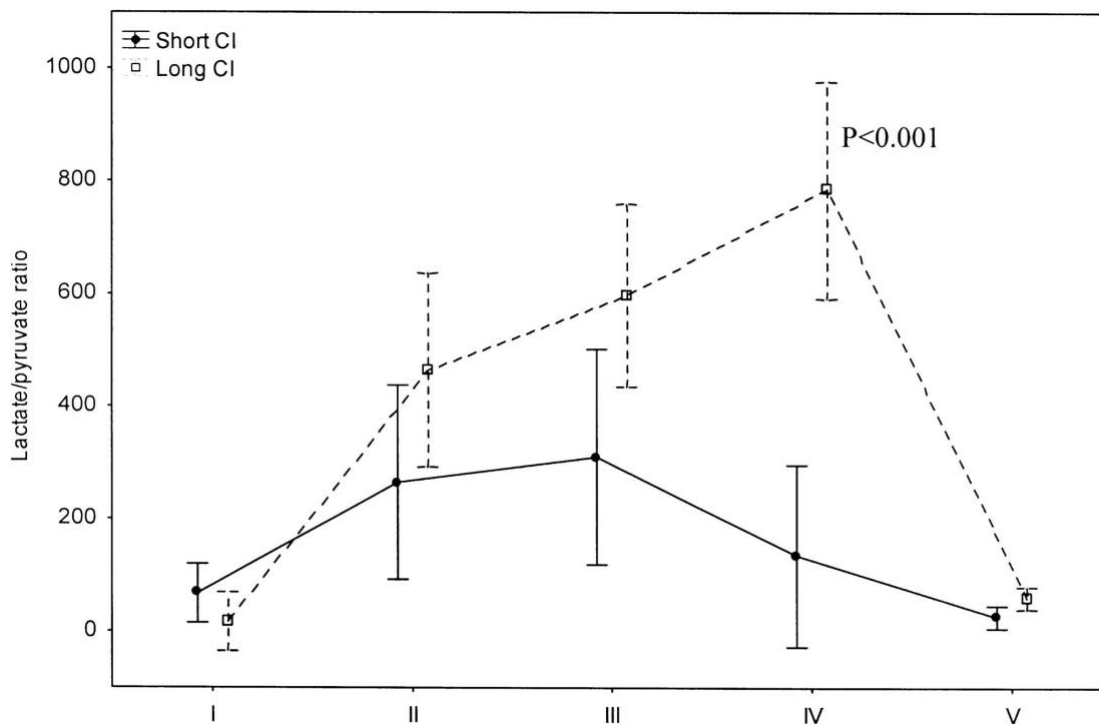


Figure 12. Intrahepatic L/Pr at the time of: (I) before donation, (II) the end of cold ischemia, (III) portal reperfusion, (IV) peak levels after portal reperfusion and (V) 2h after portal reperfusion in transplanted liver grafts with short (4h) and long (14 h) periods of cold ischemia. Paper II

Paper III

No adverse events related to the microdialysis monitoring were observed in the patient cohort. One patient developed HAT. Two patients were of particular interest and are described below:

Patient 1

A 22-year old male with cryptogenic liver cirrhosis underwent liver transplantation with a split liver from a deceased donor. The initial post-operative period was uneventful. One week after liver transplantation bilirubin increased and the patient was diagnosed with HAT following radiological investigation. Retrospective analysis of the microdialysis data showed that at 22 hours (Figure 13). Arterial thrombosis was expected to result in a higher and prolonged increase in L/Pr and therefore, unfortunately, the observed changes did not lead to any further investigation at the time.

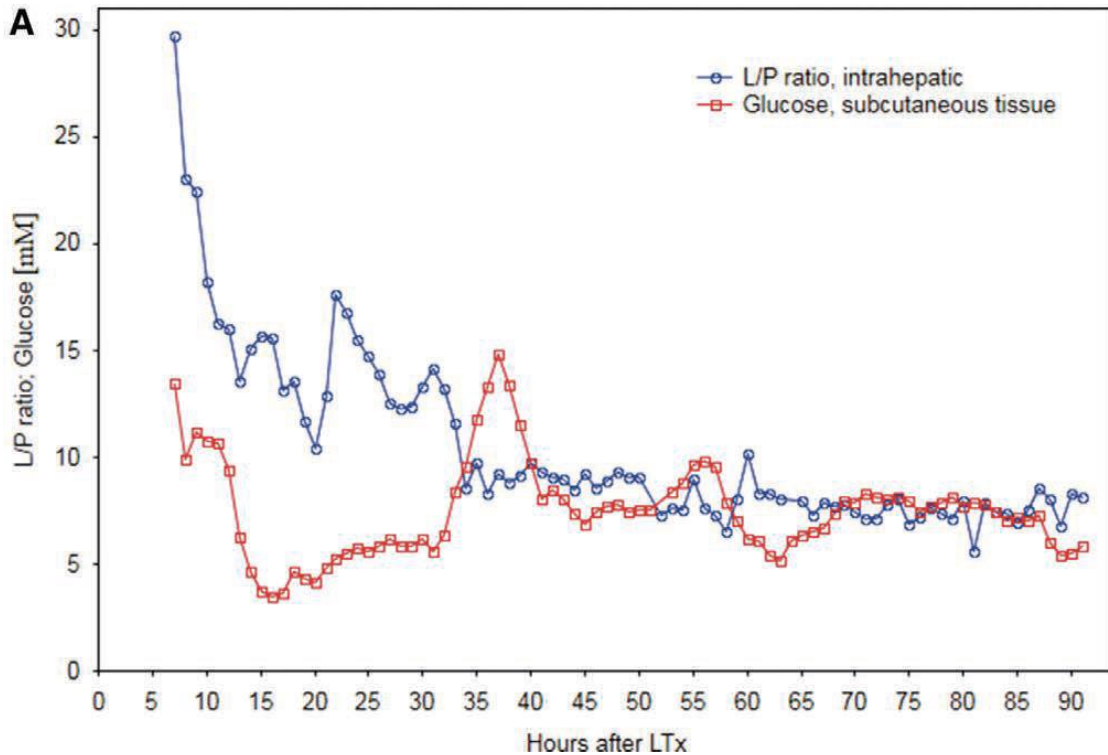


Figure 13. Intrahepatic L/Pr and subcutaneous glucose measured by microdialysis, over time after liver transplantation in patient 1. Paper III

Patient 2

A 55-year old male underwent liver transplantation due to HCV cirrhosis. Initial post-operative recovery was uneventful. Sixty-five hours after liver transplantation L/Pr increased by 50% over a period of three hours (Figure 14). The patient was investigated under suspicion of HAT but was found to have normal arterial and portal circulation.

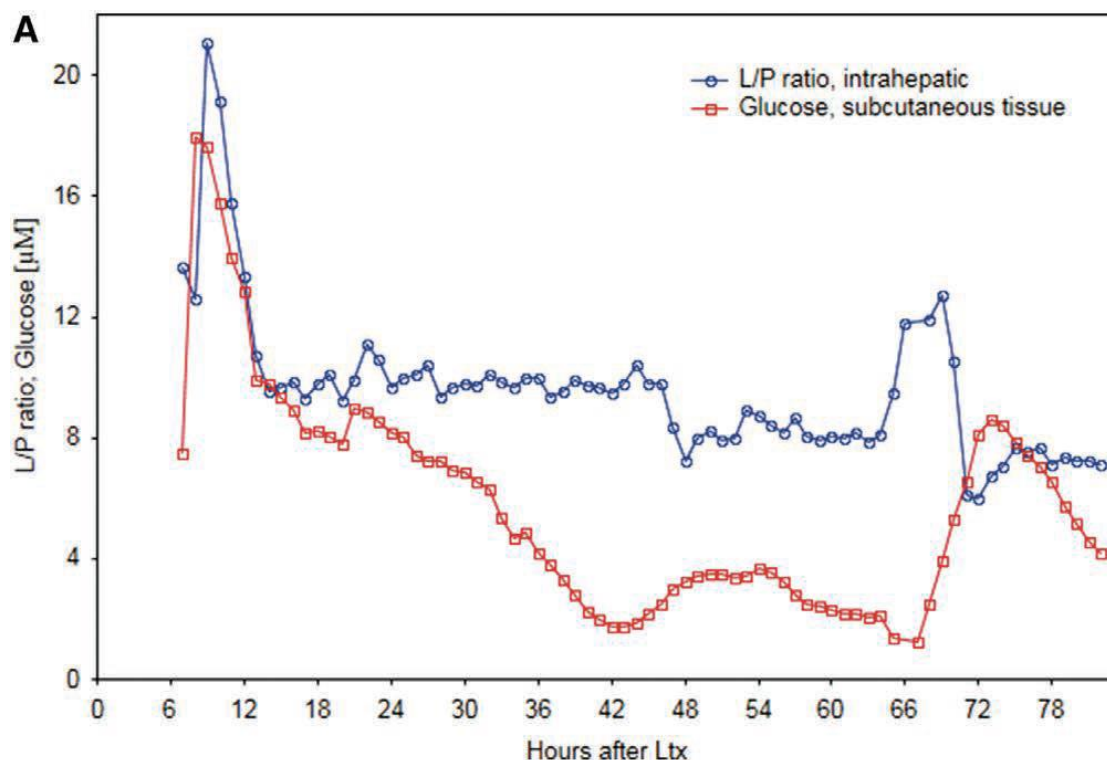


Figure 14. Intrahepatic L/Pr and subcutaneous glucose measured by microdialysis over time after liver transplantation in patient 2. Paper III

Overall results

Forty-four events of increased L/Pr (defined in the study protocol) were identified in 22 patients (Table 2). Eight patients had simultaneous L/Pr and lactate values above cut-off values suggested by Håugaa et al (100). None of these 22 or eight patients experienced ischemic events. Detailed analysis of the events of increased L/Pr was done to study lactate and pyruvate levels at the time of L/Pr increase (Table 3). Only 18% of events of increased L/Pr was the result of increase in lactate and decrease in pyruvate, changes which might indicate ischemia.

Metabolic events and ischemic complications

	1 patient with ischemic complication ^a	44 patients without ischemic complications
Events of increase in L/Pr	0	44 (in 24 patients)
Events of increase in systemic glucose	0	116 (in 39 patients)
Events of increase in systemic glucose with simultaneous increase in L/Pr	0	15
Number of simultaneous L/Pr and lactate values above cutoff	0	8

^aHepatic artery thrombosis.
L/Pr, lactate/pyruvate ratio.

Table 2. Metabolic events and ischemic complications. Paper III

Number of cases of increased intrahepatic L/Pr explained by simultaneous increase, simultaneous decrease or increase and decrease of lactate and pyruvate respectively early after LT

	Lactate ↑	Lactate ↓
Pyruvate ↑	n = 10 (23%)	n = 0 (0%)
Pyruvate ↓	n = 8 (18%)	n = 26 (59%)

L/Pr, lactate/pyruvate ratio; LT, liver transplantation.

Table 3. Detailed analysis of events of increased L/Pr in liver transplanted patients

Paper IV

Of the 71 patients included in the study, 33 (46%) developed ACR within the first month after liver transplantation. The microdialysis catheter was removed to increase mobility for most patients on day 3 following liver transplantation. For this, only the first 72 hours of data are presented. The L/Pr was higher in the rejection group at 1-12 hours after liver transplantation (Figure 15). In the group without rejection glucose was higher at 24-48 hours. Intrahepatic lactate levels were higher at 48-72 hours and pyruvate levels were higher at 60-72 hours after liver transplantation in the group with rejection as compared to non-rejectors. There was no significant difference in glycerol levels between groups. Forward stepwise logistic regression analysis showed L/Pr at 1-12 hours after transplantation to be the only independent predictor of early post-transplant rejection ($p = 0.05$).

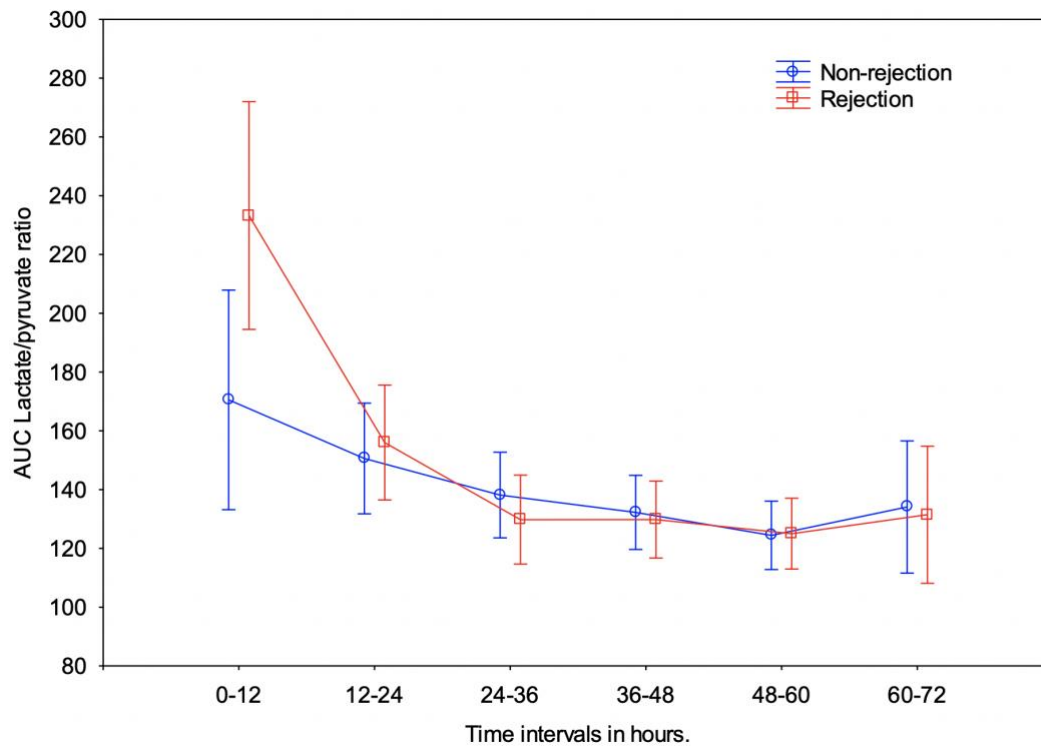


Figure 15. L/Pr in the liver after transplantation. Data presented as mean \pm 95% confidence intervals. Paper IV

Paper V

There were no complications associated with the insertion or removal of the microdialysis catheters. In the intrahepatic catheter a significant increase in lactate and decrease in pyruvate levels was seen during clamping of the hepatic artery. However, the resulting increase in L/Pr was not significant. In the subcutaneous catheter both lactate and pyruvate levels increased during clamping. There were no significant changes in glycerol in any of the catheters. The catheter placed in the hepatic vein showed no significant changes for any of the measured metabolites throughout the experiment. This indicates that intravenous microdialysis sampling in the hepatic vein cannot mimic the metabolic changes seen in intrahepatic microdialysis.

5 GENERAL DISCUSSION

5.1 ISCHEMIA AND ISCHEMIC COMPLICATIONS

The liver plays a central metabolic role in the human body. When exposed to ischemic conditions during the transplantation process, significant changes in liver metabolism occur. As shown in **paper II**, glucose levels in the liver remain stable throughout cold ischemia. Also, despite obvious ischemic conditions, lactate levels are low and stable in the same period. This indicates that glucose metabolism is effectively suppressed during cold ischemia. During warm ischemia glucose levels start to increase. Histopathological evaluation of the liver grafts prior to transplantation showed normal glycogen storage. The same examination performed at the time of reperfusion showed depleted glycogen storage in both groups, suggesting that the glucose increase in liver tissue seen during warm ischemia is the result of glycogenolysis. Following portal and arterial reperfusion, glucose released from the liver during warm ischemia, is flushed into the systemic circulation and results in post-reperfusion hyperglycemia (102, 103). The lactate/pyruvate ratio which is frequently used as an indicator of ischemia, was seen to decrease after reperfusion as expected. Interestingly, in the group with liver grafts exposed to longer cold ischemia, the decrease in lactate/pyruvate ratio occurred later than in the group with short cold ischemia, indicating a delay in the recovery of aerobic metabolism despite restoration of aerobic conditions. Detailed analysis shows that the increase in lactate/pyruvate ratio during this time is a result of delayed pyruvate production, rather than an increase in lactate production. Thus, the prolonged effect of ischemia seems to be related to a delayed start of glucose utilization rather than the rate of nonoxidative glycolysis. Our results suggest that glycolysis does not take place during cold ischemia. However, an injury to the mechanism controlling glycolysis, most likely enzymes, seems to take place during this time and the degree of metabolic dysfunction seems to increase significantly with the length of cold ischemia.

Similar metabolic changes can be expected to be seen during ischemic insult to the transplanted liver caused by hepatic artery thrombosis or portal vein thrombosis. A previous study by our group confirms this in the experimental setting (101). Vascular complications following liver transplantation though relatively rare, are life- and graft threatening. Methods for detection should therefore ideally have a high sensitivity. In **paper III**, only 1 patient (2%) suffered from hepatic artery thrombosis which makes drawing statistically valid conclusions regarding metabolic patterns during ischemia difficult. However, this patient did not experience an increase in lactate/pyruvate ratio high enough to indicate ischemia as defined by the study protocol. Nor was the increase in lactate and lactate/pyruvate ratio high enough to reach the cutoff values suggested by Håugaa et al (100). Furthermore, analysis of the microdialysis data from the study cohort identified 44 events of increased lactate/pyruvate ratio suggesting hepatic ischemia in 24 patients, none of whom suffered vascular complications. Detailed analysis of the events of increased lactate/pyruvate showed that 59%

of cases occur during decreasing levels of lactate and pyruvate, with pyruvate decreasing at a faster rate. A decrease in metabolites suggest metabolic recovery rather than ischemia.

It is not fully understood how the metabolic pathways involving glucose are affected or injured by the ischemia which the transplantation process entails (104). While microdialysis is useful for detecting primary metabolic changes, the lactate/pyruvate ratio is not a reliable or specific marker for secondary events such as ischemic complications, early after liver transplantation.

5.2 REJECTION

When using microdialysis, the recovery rate of metabolites is highly dependent on previously mentioned factors and absolute values of metabolite concentrations should not be used for interpretation. Instead relative changes over time more accurately reflect changes in metabolite concentration in the tissue. As acute cellular rejection is not an instantaneous event like a vascular complication, evaluating trends over time is a more appropriate approach for studying rejection. Results from **paper IV** show that intrahepatic lactate/pyruvate ratio at 0-12 h after transplantation is higher in patients who later develop rejection. Also, the lactate/pyruvate ratio was the only parameter associated with rejection during this time. The difference in lactate/pyruvate ratio between the groups in the early post-transplant period is a likely result of differences in the degree of perioperative ischemia-reperfusion injury which has previously been linked to the development of acute cellular rejection (52, 105, 106). However, this correlation was not seen in the zero-time biopsy or in other donor related parameters.

Patients who went on to develop acute cellular rejection had higher intrahepatic lactate and pyruvate levels day 3 after transplantation than patients who did not. This could be the result of post-ischemic hypermetabolism which has been shown to occur as a response to ischemia in other tissues (107, 108). The results from **paper IV** indicate that microdialysis monitoring following liver transplantation may be useful in the detection of metabolic events that precede acute cellular rejection. As such, microdialysis may be helpful in selecting patients who would benefit from higher levels of immunosuppression within the recommended concentration ranges. However, it is questionable whether microdialysis can detect rejection by itself. The observed association between ischemia-reperfusion injury and development of acute cellular rejection stresses the importance of reducing the duration and severity of ischemia and limiting the effects of ischemia-reperfusion injury after liver transplantation.

5.3 TISSUE DAMAGE

Cellular membrane disintegration causes a release of glycerol which is used as an indicator of cellular damage (109). In **paper I** an increase in glycerol levels is seen in the renal medulla following release of pneumoperitoneum. Glucose levels, reflecting blood flow, and the parameters of anaerobic metabolism including the lactate/pyruvate ratio, remain unchanged throughout the experiment. This indicates that pneumoperitoneum does not induce reduced blood flow or oxygen supply to the kidney. The observed increase in glycerol levels in the

renal medulla is most likely related to some type of cell membrane injury which does not seem to be related to ischemia. Laparoscopic procedures may cause ischemia-reperfusion-like injury to the abdominal organs and tissues with release of free radicals during a reperfusion period (110, 111). Possibly, rapidly increasing glycerol levels in the renal medulla after desufflation may reflect cell damage due to this phenomenon. Tissue biopsies were not taken as previous studies have shown there to be no histological difference between kidneys harvested by open versus laparoscopic technique (112, 113).

Rejection is expected to result in an increase in glycerol levels due to the break-down of cellular membranes mediated by T-cells during rejection. Most likely, the microdialysis monitoring time in **paper IV** was too short for any changes in glycerol levels to be detected as only 3 patients remained with monitoring between day 5 and 6. This highlights a problem of long-term monitoring as many patients chose to have the catheter removed for improved mobility and comfort.

The ischemia-related tissue damage caused by clamping of the hepatic artery in pigs in **paper V** could also be expected to result in increase in glycerol levels. Although such an increase was seen in several of the individuals in our study, the increase was not significant due to a high level of variation between individuals.

5.4 METHODOLOGICAL CONSIDERATIONS

Microdialysis has proven to be a promising tool for continuous monitoring of tissue metabolism and pharmacological studies. However, despite encouraging results from animal experiments and a wide range of clinical studies over the past two decades, microdialysis has not gained a foothold in standard clinical practice. Several factors contribute to difficulties with applicability of the microdialysis technique in the surgical field and especially in transplantation. Despite the large number of studies on microdialysis in surgical settings no consensus has been established on reference values for normal versus complicated outcomes. Glucose, lactate, pyruvate and glycerol are metabolites which in theory may be correlated to ischemia and cellular damage. However, the metabolic pathways at play during inflammation and ischemia are highly complex and not fully understood. For example as shown in **paper III**, increase in lactate/pyruvate ratio does not exclusively result from increase in lactate and decrease in pyruvate. As such it does not solely describe the balance between aerobic and anaerobic metabolism. It seems that microdialysis is useful for detection of primary metabolic changes. However, a better understanding of the recovery of metabolic processes after ischemia-reperfusion injury is needed to enable the use of microdialysis for monitoring of secondary events following ischemia-reperfusion injury and liver transplantation using more specific biomarkers.

6 CONCLUSIONS

Microdialysis seems to be a safe and useful tool for detection of metabolic changes in tissues such as kidney and the transplanted liver. However, interpretation of these changes is difficult in tissue exposed to ischemia-reperfusion injury since the recovery of the metabolic processes involved is not fully understood.

1. Pneumoperitoneum induces increased glycerol levels in renal medulla after desufflation. This is likely the result of cell damage caused by reperfusion-like injury in the medulla.
2. In liver tissue exposed to cold ischemia, glycolysis does not take place. However, during this time, an injury to the mechanisms controlling glycolysis likely takes place resulting in a slower start in glucose utilisation in liver tissue exposed to longer cold ischemia time.
3. The lactate/pyruvate ratio is a product of the complex interplay of changes in liver metabolism which makes interpretation of changes in intrahepatic lactate/pyruvate ratio early after liver transplantation difficult. The lactate/pyruvate ratio is thus a poor marker of clinically relevant ischemia in the liver early after transplantation.
4. Microdialysis monitoring following liver transplantation may be useful for detection of metabolic events that precede acute cellular rejection. However, it is questionable whether it can detect rejection by itself. The metabolic changes seen in patients who go on to develop acute cellular rejection are possibly linked to ischemia-reperfusion injury during transplantation. Finding ways to reduce the duration of ischemia and the effects of ischemia-reperfusion injury after liver transplantation would be beneficial.
5. Hepatic vein microdialysis could not be used to represent direct intrahepatic microdialysis for detection of metabolic changes induced by hepatic arterial ischemia in the study presented in this thesis.

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