MYOCARDIAL METABOLISM AND ISCHEMIA ASSESSED BY MICRODIALYSIS

Clinical and experimental studies in cardiac surgery

VITTORIO MANTOVANI

The Sahlgrenska Academy at Göteborg University

Göteborg 2006

From the Institution of Medicine, Department of Metabolic and Cardiovascular Research/

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To my family

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Abstract

Background: The available methods to study myocardial metabolism and ischemia show considerable limitations when employed during and after cardiac surgery. Microdialysis is a technique for continuous sampling of substances from the interstitium. It has been extensively used in experimental settings in the heart but seldom in clinical studies, due to technical difficulties. The aim of these studies was to test whether microdialysis could be used to study cardiac metabolism and ischemia during and after cardiac surgery.

Method: A microdialysis probe, developed specifically for myocardial implantation in our laboratory, was used in the first two, clinical, studies in order to assess the implantation trauma and to measure the interstitial levels of glucose and lactate during and after cardiac surgery. In a third, experimental, study a commercially available CE-marked probe was adapted for cardiac use. In the fourth, clinical, study this probe was used to assess differences in myocardial metabolism in two randomized groups of patients undergoing coronary artery bypass surgery, with or without cardio-pulmonary bypass, respectively.

Results: In the first study an implantation reaction was indicated by a local release of troponin-T as demonstrated by means of microdialysis. This could be differentiated from the subsequent release of troponin-T due to the surgical trauma. The second study showed that cardioplegic arrest caused a significant decrease of interstitial glucose, but not a total depletion, while lactate accumulated in the interstitium without reaching critically high levels. In the third study, a new implantation method was developed to ensure a quick and easy positioning of the probe in the desired place and to give a stable function. In the fourth study, microdialysis showed that off-pump bypass surgery caused less metabolic derangements compared to on-pump surgery. Microdialysis was also able to correctly detect episodes of cardiac ischemia.

Conclusions: Microdialysis can be used to monitor myocardial metabolism and ischemia without delay and with high precision. The behavior of several interstitial markers during and after cardiac surgery has been described for the first time.

Key words: microdialysis, cardiac metabolism, myocardial ischemia

ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals:

I. Mantovani V, Kennergren C, Berglin E, Moratti R, Lönnroth P, Hamberger A, Viganò M. Intramyocardial troponin-T monitoring with microdialysis in coronary artery bypass surgery. Scand Cardiovasc J. 2002 Sep;36(5):308-12.

II. Kennergren C, Mantovani V, Strindberg L, Berglin E, Hamberger A, Lönnroth P. Myocardial interstitial glucose and lactate before, during, and after cardioplegic heart arrest. Am J Physiol Endocrinol Metab. 2003 Apr;284(4):E788-94.

III. Mantovani V, Kennergren C, Goiny M, Ungerstedt U, Lönnroth P, Sala A, Berglin E. Microdialysis for myocardial metabolic surveillance: developing a clinical technique. Clin Physiol Funct Imaging. 2006 Jul;26(4):224-31.

IV. Mantovani V, Kennergren C, Bugge M, Sala A, Lönnroth P, Berglin E. Myocardial metabolism assessed by microdialysis: A prospective randomized comparison in off-pump and on-pump coronary artery bypass surgery. Submitted

CONTENTS

ABBREVIATIONS

ACC	aortic cross clamp
ANOVA	analysis of variance test
ASAT	aspartate amino-transferase
ATP	adenosine triphosphate
CABG	coronary artery bypass graft
CK	creatine kinase
CK-MB	creatine kinase, muscle and brain
CoA	coenzyme A
CPB	cardio-pulmonary bypass
ECG	electrocardiogram
ELISA	enzyme-linked immunosorbent assay
IHD	ischemic heart disease
kDa	kilo Dalton
LAD	left anterior descending coronary artery
LDH	lactate dehydrogenase
LIMA	left internal mammary artery
LVEF	left ventricular ejection fraction
NaCl	sodium chloride, “saline”
NADH	nicotinamide adenine dinucleotide plus hydrogen
N-IHD	non-ischemic heart disease
OPCAB	off-pump coronary artery bypass
RIMA	right internal mammary artery
SVG	saphenous vein graft

1. INTRODUCTION

1.1 Background

The early detection of myocardial ischemia during and after cardiac surgery and the study of myocardial metabolism in relation to cardiac surgery are important issues for the safety of the patients and for the development of more effective techniques. The available methods to pursue these two aims clearly have limitations and drawbacks as to the specific setting of cardiac surgery.

Microdialysis is a technique for the continuous sampling of chemical substances from the interstitial space of various organs and tissues. The basic principle is the implantation of an artificial blood capillary in the target tissue. It consists of a double lumen catheter with a dialysis membrane at the tip. The inflow lumen is perfused with an isotonic solution by a high-precision pump. The chemical substances present in the interstitial fluid enter the probe through the pores of the membrane, following a concentration gradient. The dialysate is then collected at the end of the outflow lumen.

1.2 Advantages of microdialysis

The microdialysis sampling is performed directly in the organ or tissue of interest, ruling out the possibility that the derived substances are released from other organs. This potential misinterpretation is a concern when the function of an organ is studied by peripheral blood sampling. The endothelium of blood vessels is not a passive membrane but a metabolically active organ. The sampling of substances from the interstitium minimizes possible interference from the endothelium. Different areas of the same organ can be selectively studied by implanting several probes. The perfusion of the microdialysis probe is continuous, the time resolution of the method can subsequently be decided by the user by collecting the dialysate when desired. The dialysis membrane has pores of a known size, which determine the maximum molecular weight of the substances that can enter through the membrane. In this way, undesired molecules, such as catabolic enzymes, can be excluded from sampling.

1.3 Clinical development of microdialysis

The importance of obtaining chemical samples directly from the interstitial fluid has long been realized. During the 1960's the push-pull cannula system was developed and reports date back to the early 1970's about studies of the central nervous system using this approach (1-3). During the same period the concept of recovering substances through a dialysis membrane was employed by Bito et al. (4). Delgado et al. in 1972 described the first combination of a push-pull cannula and a dialysis membrane (5). Ungerstedt pioneered the use of a true microdialysis system in the central nervous system (6), followed by Hamberger (7, 8). Lönnroth introduced clinical microdialysis for studies of glucose metabolism in human adipose tissue (9-11). The potential of microdialysis to circumvent the blood-brain barrier makes it a useful clinical tool for monitoring cerebral metabolism after neurosurgery, after head trauma or cerebral vascular incidents (12-14). In plastic surgery microdialysis offers the possibility to monitor in real time the viability of musculo-cutaneous flaps (15-17). Other clinical applications are abdominal surgery (18) and liver transplantation (19).
Cardiac metabolism has been extensively studied with microdialysis in animal experiments. However, cardiac microdialysis has seldom been used in humans and, in addition to our own experience, very few papers have been published describing this application (20-23).

1.4 Other methods for the study and surveillance of myocardial function

A number of physical and chemical methods are available to study cardiac metabolism and to detect the occurrence of myocardial ischemia. However, during and after cardiac surgery, these methods have limitations and drawbacks, which reduce their specificity and usefulness. In particular, a clear diagnosis of ischemia may be delayed beyond the time limit for effective therapy.

1.4.1 Electrocardiography

The interpretation of electrocardiograms after cardiac surgery is often confusing, unspecific and even misleading. In a multicenter study by Jain et al. (24) 566 patients from 20 clinical sites underwent continuous Holter monitoring after cardiac surgery. Episodes of ST-segment deviation and/or major cardiac conduction changes lasting ≥ 30 minutes and/or use of ventricular pacing lasting ≥ 30 minutes were registered in 58% of cases. Combined Q-wave

and CK-MB criteria or autopsy criteria for myocardial infarction were only met in 4% of cases.

1.4.2 Blood samples

Markers of ischemia in peripheral venous blood samples are essential for diagnosis in patients not undergoing cardiac surgery. Some of these markers are routinely tested for after cardiac surgery. In general their specificity is reduced after cardiac surgery and a reliable diagnosis of perioperative myocardial infarction cannot be done earlier than 24 to 48 hours after the operation. The two main groups of markers in clinical use are myocardial enzymes and myocardial structural proteins.

Enzymes:

Aspartate aminotransferase (ASAT) exists in two isoforms, mitochondrial ASAT and cytoplasmatic ASAT, with molecular weights of 47 and 46 kDa, respectively. They catalyze the reaction between aspartate and alfa-ketoglutarate to form oxaloacetate and glutamate. ASAT increases after myocardial infarction, reaching a peak in blood after 24 to 36 hours, the delay decreasing its usefulness. Both forms of ASAT are present in other tissues than the heart, notably liver and skeletal muscles. As a consequence, the specificity of both markers is low after cardiac surgery.

Lactate dehydrogenase (LDH) is a group of enzymes that interconvert lactate and pyruvate. It is present in plasma in five isoforms. The plasma peak is reached 2 to 3 days after a myocardial infarction; in addition, the specificity after cardiac surgery is low.

Creatine kinase (CK) catalyzes the formation of phosphocreatine from ATP and creatine. The heterodimer MB is more common in cardiac muscle (30% of total) than in skeletal muscle (1%). CK-MB begins to increase in blood 3 to 9 hours after a myocardial infarction, peaking after 12 to 36 hours. Its specificity is higher than that of ASAT and LDH, however unspecific release of CK-MB has been described after cardiac surgery, which was not related to myocardial damage (25).

Structural proteins:

Myoglobin is a protein with a molecular weight of 17.5 kDa. It is found both in cardiac and skeletal muscle and is normally present in blood: its concentration is influenced by gender, body weight, muscle mass and glomerular filtration. The specificity of myoglobin is low even in non-surgical patients.

Troponin-I and -T are proteins with molecular weights of 24 and 38 kDa, respectively. They are involved in the regulation of muscular contraction. They begin to increase in blood 3 to 9 hours after a myocardial infarction and remain high for up to 14 days. Their specificity is high and the extended presence in blood makes them useful in patients who are seen late after the onset of symptoms.

There is no consensus in the literature regarding the specificity of troponin measurements after cardiac surgery. Many reports conclude that troponin measurements may be sufficient to confirm a diagnosis of perioperative myocardial infarction (26, 27), even after coronary surgery without heart-lung machine (28). On the contrary, it has also been reported that cardiac surgery may cause unspecific troponin elevations (25) and that troponin release after cardiac surgery may be affected by factors such as the gender of the patient (29).

Regardless, a diagnosis based on troponin measurements is a late one, being evident one or two days after surgery (30).

In attempts to obtain early diagnosis of myocardial ischemia, other markers have been considered, such as a myocardial fatty acid-binding protein. This marker peaks as early as one hour after the removal of aortic cross clamp (31-33), but it is also expressed by other tissues than the myocardium; its diagnostic value is also very low in the presence of renal failure (34), which is rather common after cardiac surgery.

Blood samples for the evaluation of metabolism:

Metabolic substrates are not specific for the myocardium. This is not a problem in experimental studies of the isolated working heart. Human studies require almost invariably simultaneous sampling of arterial blood and venous blood from the coronary sinus, in order to determine how much of the substances studied that are extracted or excreted from the heart.

1.4.3 Echocardiography

Echocardiography, especially if performed trans-esophageally, is a tool for monitoring graft function (35, 36), myocardial perfusion with echo-contrast (37), global and regional contractility (38) both during on-pump cardiac surgery and off-pump coronary surgery (39). Echocardiography is, however, biased by being operator dependent and, in addition, the acoustic window for trans-thoracic approach is often disturbed after cardiac surgery.

1.4.4 Myocardial biopsies

Sequential myocardial biopsies as a means of determining the metabolic status of specific areas of the myocardium (40) is an invasive technique offering a limited number of samplings and with a time delay for laboratory analysis of the sampled tissue.

1.4.5 Other methods

Nuclear magnetic resonance with 13C- and 31P-spectroscopy yields detailed information about myocardial metabolism of lactate and pyruvate (41, 42). Quantification of myocardial flow can be performed with 13N-ammonia or 15O-water positron emission tomography (43, 44). Positron emission tomography can be used to study myocardial oxidative metabolism employing 11C-acetate (45-47) and glucose metabolism employing 18F-fluorodeoxyglucose (48, 49). These methods generally require cumbersome equipment and can hardly be used during a standard cardiac surgical procedure.

1.5 Statistical methods

The comparison of different treatments is performed by statistical analysis of the results obtained. There are two possible types of error in the conclusion of a statistical test. The type I error is affirming that two groups are different when in reality they are similar. This type of error is also called α; the commonly reported P value represents the probability of making this type of error when one claims that two groups are different.

The type II error, also called β, is affirming that two groups are similar when in reality they are different.

The power of a statistical model is defined as $1-\beta$ and denotes the ability of that model to find a difference between two or more groups. The power of a statistical model depends on the number of cases, the standard deviation for normally distributed continuous variables and the magnitude of the difference of the effect between the groups.

Table I shows how many cases are needed in each arm of a randomized study in order to demonstrate a risk reduction with 0.05 α error ($P< 0.05$) and 0.1 β error (corresponding to 90 % power).

Table I

Risk reduction	Frequency of the adverse event				
	1 %	2%	3 %	4 %	10 %
10 %	197750	97924	64649	48011	18064
50 %	6253	3100	2049	1524	578

When seldom-observed adverse events such as operative death or other major complications are used as end-points, very large numbers of subjects are usually needed. Subsequently, studies employing microdialysis, by being more sensitive and specific, may require smaller numbers of subjects than studies with clinical end-points.

1.6 Calibration of microdialysis

The concentration of a substance in the microdialysate depends on the recovery rate of the probe. Major determinants of the recovery rate include the surface area of the membrane, the membrane pore diameter, the flow rate, the chemical properties of the membrane, the temperature and the tissue pressure. The recovery rate in vitro is not necessarily representative of the recovery rate in a tissue, so various methods for calibrations are needed.

1.6.1 Zero flow

The concentration of the chosen substance is measured in the dialysate at different flow rates. The interstitial concentration at zero flow is calculated by regression analysis, assuming that total equilibration is reached between the interstitium and the dialysate when the flow into the probe is zero (8, 50).

1.6.2 Zero transfer

The chosen substance is added to the perfusion solution at different concentrations. When the perfusion solution has the same concentration as the interstitium, no difference will be registered in the solution entering the probe and that leaving the probe (9).

1.6.3 Near equilibrium

The perfusion flow is kept very low, allowing the microdialysate to reach almost the

equilibrium with the interstitium (51). This method has a low time resolution.

1.6.4 Internal reference

A known amount of the chosen substance, marked with a radioactive isotope, is added to the perfusate, taking care to avoid a concentration equal to the expected concentration of the interstitium. The amount of isotopic marked substance, which is lost to the tissue during the perfusion, equals the unlabeled amount, which leaves the interstitium to the dialysate. This method was previously used in adipose tissue (52), in skeletal muscle (53) and in skin (54).

2. AIMS AND DESIGN OF THE STUDIES

I. Aim

To ascertain whether a microdialysis probe causes insertion damage in the myocardium and whether this damage could be differentiated from the ischemia induced by the surgical procedure itself.

I. Design

Low-risk coronary patients were studied. Two microdialysis probes were implanted in each patient in the anterior and lateral areas of the left ventricle as early as possible after sternotomy. Thereafter troponin-T was determined serially both in peripheral blood and in microdialysate, along with clinical monitoring.

II. Aim

To ascertain whether microdialysis could study the energetic metabolism of the heart by detecting changes in and levels of interstitial glucose and lactate during and after cardiac surgery.

II. Design

Low risk patients were studied. Two microdialysis probes were implanted in the same area of the heart of each patient. Lactate and glucose levels were determined in arterial blood, in venous blood from the coronary sinus and in microdialysates before, during and after the procedures. The microdialysis probes were calibrated with the internal reference method.

III. Aim

To develop a new microdialysis probe with improved characteristics including better mechanical stability, accuracy and time resolution. The aim was also to gather reference data for future clinical use and metabolic control.

III. Design

Temporary myocardial ischemia was caused by snaring the left anterior coronary artery in an open-chest experimental pig model. Two microdialysis probes were implanted in each animal,

one in the ischemic area and one in a control area. Glucose, lactate, pyruvate and glycerol were measured before, during and after ischemia.

IV. Aim

To test whether microdialysis could detect ischemia and metabolic differences in small groups of low risk patients undergoing alternative strategies for coronary artery bypass grafting.

IV. Design

Low-risk coronary patients were randomized to undergo coronary bypass graft on the beating heart without heart-lung machine or on the arrested heart with cardioplegia and heart-lung machine support. One microdialysis probe was implanted parallel to the left anterior descending coronary artery of each patient and glucose, lactate, pyruvate, glycerol and urea were measured before, during and after the procedure.

3. MATERIALS AND METHODS

3.1 Ethics

The study protocols were reviewed and approved by the local ethics committees. The use of isotopes in study II was approved by the Radiation Safety Committee of the Sahlgrenska University Hospital. All patients gave their informed consent. The studies were conducted according to the Helsinki declaration (www.wma.net).

3.2 Subjects and sampling

Studies I, II and IV included forty-six patients, each participating in one study only (Table II). Study III included eighteen crossbred male pigs (Swedish Landrace, Eskilstuna, Sweden) with a mean weight of 22,6 ± 2,1 kg.

Table II. Some clinical characteristics of the subjects

Variable	Study I	Study II	Study III	Study IV
Subjects	humans	humans	pigs	humans
No. of subjects	7	13	18	26
Gender F/M	0/7	3/10	0/18	9/17
Age (years)	55 ± 11	61±13	-	66 ± 7
IHD/N-IHD	7/0	4/9	healthy	26/0
LVEF (%)	55 ± 9	60 ± 10	normal	57 ± 10
Use of CPB	all	all	none	14/26
ACC (minutes)	52 ± 12	65 ± 20	-	29 ± 13
LAD occlusion (minutes)	-	-	20	12 ± 4

ACC = aortic cross clamp time; CPB = cardio-pulmonary bypass; F = female; IHD = ischemic heart disease; LAD = left anterior descending coronary artery; LVEF = left ventricular ejection fraction; M = male; N-IHD = non-ischemic heart disease.

Sampling intervals in studies I and IV were 15 minutes from implantation until approximately two hours after reperfusion, thereafter vials were changed every hour until the end of the observation. In study II the sampling interval was 10 minutes during the operation and one hour after the operation. In study III the vials were changed every ten minutes until one hour after reperfusion and every 30 minutes later on. The total amount of analyzed vials was approximately 2500.

3.3 Microdialysis probes

A probe developed and produced in cooperation with the Department of Histology, University of Gothenburg, was used in studies I and II.

Two different modifications of the commercially available CMA-70 microdialysis probe (CMA Microdialysis AB, Solna, Sweden) were tested in study III. They were named CMA-70-A and CMA-70-B respectively.

Study IV was performed with CMA-70-B probes as described in study III.

3.3.1 University of Gothenburg probe

The probe was constructed from double channel, non-toxic, medical grade polyethylene tubing (outer diameter 1,5 mm). A 9 mm long segment of the outflow lumen was cut at a distance of 20 mm from the probe tip and a tubular dialysis segment was glued in its place. The dialysis segment was a 12 mm piece of hydrophilic polymer tubing (CPC/PE, outer diameter 0,6 mm, Gambro dialys, Lund, Sweden). The molecular weight cutoff of this membrane was approximately 70 kDa. An epicardial pacing wire was secured to the tapered tip of the probe. The distal end of the pacing wire was connected to a curved surgical needle. The inlet tubing for the perfusion medium had a length of approximately 2000 mm, allowing the CMA-100 pump to be placed outside the sterile field. A vial adapter for sample collection was connected to a 470 mm long piece of outlet tubing giving a 60-minutes delay in collecting the microdialysate.

In study II, in adjunct to standard probes, a shorter version was implanted for intra-operative sampling, equipped with a 30 mm long outlet tube, in order to minimize the time delay for collecting the dialysate.

3.3.2 CMA-70 probe

The standard commercially available CMA-70 probe has an outer diameter of 0,6 mm. The 10 mm long dialysis membrane is placed at the tip of the probe and the two lumina are coaxial. The molecular cutoff is 20 kDa. In study III the probe was modified by gluing a stainless steel temporary pacing wire to the tip (CMA-70-A) in order to implant it with the same technique as with the University of Gothenburg probe. The second modification tested in study III was without the pacing wire but with a cuff of rolled medical grade adhesive tape or a small piece of autologous pericardium attached to the shaft of the probe at a distance of 15 mm from the membrane (CMA-70-B). The latter probe model was also employed in study IV, however, only the version with an autologous pericardial cuff.

3.4 Perfusion of the probes

In studies I and II the probes were perfused with sterile isotonic NaCl solution at a flow rate of 2.5 microliters/minute by a CMA-100 microdialysis pump (CMA Microdialysis AB, Solna, Sweden).

In study III and IV the probes were perfused at a flow rate of 1 microliter/minute with a sterile isotonic solution (Na+ 147 mmol/L; K+ 4 mmol/L; Ca++ 2.3 mmol/L; Cl- 156 mmol/L; pH 6; osmolality 290 mosm/kg) by a CMA-107 pump.

3.5 Implantation of the probes

The implantation technique had some common steps that were kept constant in the four studies.

3.5.1 Common implantation steps

One or more probes were primed before the surgical procedure started.

A longitudinal median sternotomy was performed and the pericardium opened. Pericardial stay sutures were used to gently tilt the heart out of the pericardium in order to expose the implantation site. The probes were inserted through the chest wall from the left subcostal region. The vial connectors were secured to the skin of the left lateral chest wall: their position allowed easy vial changes during and after the surgical procedure. At the end of the studies, the probes were pulled out percutaneously in the same fashion as temporary pacing wires.

3.5.2 Pacing wire technique

This technique was used in studies I and II with the University of Gothenburg probes and in study III with the CMA-70-A probe. The distal needle of the pacing wire attached to the tip of the probe was inserted in the ventricular wall with the same technique as used for temporary pacing leads (Fig. 1). The pacing wire was then pulled out until the full length of the dialysis membrane was embedded in the myocardium. The pacing wire was cut approximately 1 cm from its exit point and bent in order to prevent reverse displacement of the probe. In addition, a 6-0 monofilament suture was used to stabilize the neck of the probe, proximally of the membrane.

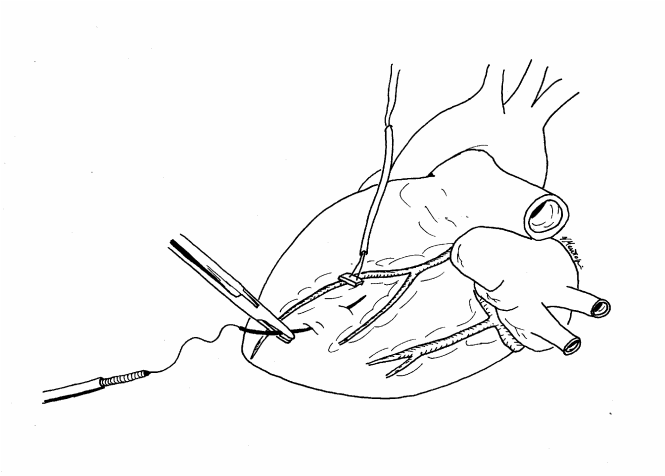

Fig 1. Pacing wire technique for implantation of the microdialysis probe

3.5.3 Injection needle technique

This technique was used in study III for the CMA-70-B probe and in study IV.

A 1.2 x 50 mm injection needle was prepared by bending it to an appropriate curve and by removing the rear connector. The probe was inserted into the rear lumen of the needle, slightly forcing the cuff against the rim of the opening, in order to ensure stability during implantation (Fig. 2, Step 1). The loaded needle was inserted into the myocardium parallel to the epicardium for a length of approximately 30 mm, and was then removed at the exit point (Fig. 2, Step 2). The cuff on the shaft of the microdialysis probe prevented the probe from following the needle when the latter was removed. Subsequently, the membrane of the probe remained in a stable intramyocardial position. The probe was finally secured to the epicardium with a 7-0 monofilament suture around the shaft, proximal to the cuff.

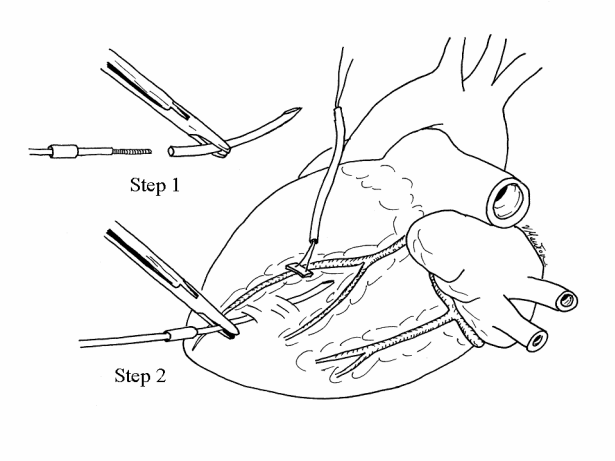

Fig. 2. Injection needle technique for implantation of the microdialysis probe.

3.6 Internal reference calibration

In situ calibration for study II was performed according to the internal reference technique (52), by adding 5 μCi/ml [^{14}C]lactate and 5 μCi/ml [^{3}H]glucose (Amersham, Buckinghamshire, UK) to the perfusate. The administration of isotopes was done before and during cardioplegia and in 3-hour pulses at 25 and 35 hours postoperatively, in order to take into account the fluid shifts and temperature changes expected during the observation. Continuous perfusion with isotopic markers was avoided to prevent accumulation of the label in the ambience of the catheter. The loss of radioactivity over the microdialysis membrane was used to calculate relative recovery.

3.7 Troponin-T analysis

Troponin-T levels in study I were determined with a one-step sandwich ELISA assay using streptavidin technology (Boehringer, Mannheim, Germany). Determinations were always duplicated. Microdialysates were diluted with saline, 1:5 and 1:20. The intra-assay coefficient of variation was 3.13.

3.8 CMA-600 analyzer

The CMA-analyzer (CMA/Microdialysis AB, Solna, Sweden) was used in studies III and IV. The device is mounted on a trolley and can be placed in the operating room or bedside in the intensive care unit. The vials containing microdialysate can be placed in the device directly after sampling from the patient or can be refrigerated for delayed batch analyses. Determinations of glucose, pyruvate, lactate, glycerol and urea are obtained within 60 to 120 seconds and are displayed on a computer monitor with a proprietary software, which can also store patient data from other monitoring sources. Analyses are performed with a colorimetric method and enzyme reagents.

3.9 Statistical analyses

Continuous unpaired data were compared with Student t-test or Mann-Withney U test as appropriate. Categorical variables were analyzed with chi-square or Fisher's exact test as appropriate. Time series were tested for differences within and between groups with repeated-measures ANOVA and post-hoc tests.

4. RESULTS

4.1 Study I

Intramyocardial troponin-T monitoring with microdialysis in coronary artery bypass surgery.

The implantation of the probes did not induce any side effects according to ECG monitoring and measurements of enzyme release in blood.

Serum troponin-T was undetectable before the operation; subsequently it increased linearly and reached a mean level of 0.42 microgram/l three hours after reperfusion. This concentration was maintained for approximately 20 hours.

Troponin-T in microdialysate in the majority of the probes showed a pattern including three peaks. An initial peak corresponding to the insertion trauma was followed by a period of 70–80 min, including the cardiac arrest time, with low troponin-T concentrations, i.e. 10–15% of the first peak concentrations. A second peak was registered three hours after reperfusion, with a mean value of 22.9 microgram/l, 50 times higher than the corresponding serum level. While serum troponin-T remained fairly constant, microdialysate troponin-T decreased rapidly allowing the detection of further peaks in three patients or the prolongation of the third-hour peak in two patients. The course of microdialysate troponin-T corresponded to some hemodynamic and electrocardiographic changes that were not mirrored in serum troponin-T.

4.2 Study II

Myocardial interstitial glucose and lactate before, during, and after cardioplegic heart arrest.

No microdialysis-related complication occurred.

The interstitial level of glucose decreased significantly after the infusion of cardioplegic solution and remained low throughout the period of cardioplegic arrest. It returned to pre-cardioplegic levels one hour after reperfusion and it decreased again 25 to 35 hours after surgery to the same level as attained during cardioplegia.

The interstitial level of lactate showed a non-significant decrease immediately after the administration of cardioplegic solution, followed by a significant increase throughout the

period of cardioplegic arrest. A significant decrease of interstitial lactate was registered again 25 to 35 hours after surgery.
The arterial-interstitial difference for both glucose and lactate increased significantly 25 hours after surgery.

4.3 Study III

Microdialysis for myocardial metabolic surveillance: developing a clinical technique.

The CMA-70-A probe proved to be easy to implant, confirming the validity of the pacing wire method. It performed well in terms of metabolic analyses: the induced ischemia was clearly detected with highly significant drops of interstitial glucose and pyruvate and peaks of lactate, glycerol and lactate/pyruvate ratio. Time resolution was good. However, the durability of the probes was suboptimal: four out of 18 probes showed technical failure at some time during the one-hour reperfusion time.
The needle implantation technique developed for CMA-70-B was as easy to perform as the pacing wire method of CMA-70-A. CMA-70-B also detected the induced ischemia with significant changes of all measured substances and had equal time resolution to CMA-70-A. The CMA-70-B outperformed CMA-70-A in terms of durability and reliability, since only one probe out of 18 ceased working during a reperfusion time that was prolonged to six hours. The time course of analyzed substances was very similar with both types of probes. Glucose did not show any implantation response: its level was constant from the time of probe implantation throughout the whole equilibration period. Lactate, glycerol and lactate/pyruvate ratio showed an implantation response, levels decreased after implantation and a baseline was reached during the equilibration period. Pyruvate showed a constant increase from the implantation time: a plateau was not observed with CMA-70-A due to the shorter observation time. In the CMA-70-B study the pyruvate level stabilized after approximately three hours.

4.4 Study IV

Myocardial metabolism assessed by microdialysis: A prospective randomized comparison in off-pump and on-pump coronary artery bypass surgery.

The microdialysis technique now performed clinically as in study III proved easy to use, produced reliable results and showed no evidence of complications. The patient randomization resulted in two comparable groups regarding the preoperative variables. One patient randomized to off-pump surgery crossed over to on-pump surgery because of a deep intramyocardial course of the left anterior coronary artery requiring on-pump techniques and was excluded from statistical analyses. No difference between the two groups could be demonstrated regarding major clinical endpoints: all patients were alive and free from major adverse events after 19 months of follow-up. On-pump patients postoperatively had a significantly higher release of troponin-T in peripheral blood. No significant difference between the groups was noted in glucose or lactate sampled from peripheral blood.

All substances analyzed in microdialysate had similar levels in the two groups at the beginning of the observation, before the start of the cardiac procedure. Significant differences within and between groups were registered thereafter. Microdialysate levels of glucose, pyruvate and urea were stable during the operation in the off-pump group, while in the on-pump group these substances showed significant decreases during cardioplegic cardiac arrest and significant increases after the removal of aortic cross clamp. No difference between groups was registered for glycerol or lactate/pyruvate ratio. Lactate was higher in the off-pump group. In the late phase of the postoperative course, the on-pump group showed higher microdialysate levels of glucose, lactate and urea, while pyruvate was lower, compared to the corresponding values of the on-pump patients.

One on-pump patient, the crossover case, experienced acute occlusion of the mammary artery graft shortly after the anastomosis was finished, requiring a second cardioplegic arrest to suture a vein graft as substitution for the arterial graft. The patient recovered uneventfully but microdialysate levels of all analyzed substrates differed from those of the other on-pump patients.

One off-pump patient underwent a coronary angiography 18 hours after the operation due to major electrocardiographic changes suggestive of ongoing acute ischemia, despite normal hemodynamics. The angiography documented good graft function. The microdialysate levels of substrates were similar to those of the other off-pump patients.

5. DISCUSSION

The main findings of study I-IV are that:

- the implantation of a microdialysis probe into the myocardium coincided with a local and short-lasting release of interstitial troponin-T. Later, the probe could register further releases of troponin-T with patterns that were different from those of peripheral blood analyses and that were probably representative of cardiac events,

- microdialysis could register the absolute levels of glucose and of lactate in the interstitial fluid of the myocardium not only before and after surgery, but also during cardioplegic cardiac arrest and that interstitial glucose was not totally depleted during the cardioplegic arrest,

- commercially available probes could be adapted for use in cardiac surgery allowing the metabolic status of the myocardium in general and the occurrence of ischemia in particular to be detected with high sensitivity and short time delay,

- microdialysis could detect significant differences in the myocardial response to different surgical techniques in two randomized groups of patients, where the group sizes would not allow the detection of any difference in clinical outcome. Furthermore, clinically relevant events were correctly and promptly registered by microdialysis.

* * *

Cardiac surgery is presently scrutinized, partly due to the need of cost containment. Medically, patients referred for surgery are getting older and have more comorbidities than in the past, possibly increasing the surgical risk. Not least, malpractice issues may be a concern. The general shift towards evidence-based medicine applies also to cardiac surgery, i.e. when comparing two treatments. Two conditions must be met in order to draw valid conclusions; first, the two groups of subjects must be comparable, second, the study must have enough statistical power. The condition of comparability is best fulfilled by prospective randomized studies while the large numbers necessary to achieve statistical power are sometimes only

possible to achieve in retrospective reviews of large cohorts. A typical case is coronary surgery with or without cardio-pulmonary bypass (CPB).

5.1 OPCAB versus CABG with cardiopulmonary bypass

Untoward effects are described in the brain and in the systemic inflammatory system from the use of CPB, partly explained by unavoidable aortic manipulation and blood contact with foreign surfaces (55, 56). Since the early nineties, an increasing number of off-pump coronary artery bypass (OPCAB) cases have been performed in the hope of avoiding the morbidity caused by CPB. The systemic inflammatory response to cardiac surgery seems to be lower if CPB is avoided (57, 58), since blood is not exposed to a foreign surface. The proportion of OPCAB is varying widely in different centers and countries. In some centers, no patients are currently scheduled for on-pump CABG at all (59, 60). In the United States it is estimated that 25% of all CABG:s performed yearly are OPCAB:s (61). For Canada the number is reported as 16% (62), while in India OPCAB is estimated to account for more than 50% of coronary cases (63). Retrospective studies of very large databases have shown significant advantages of off-pump technique regarding risk-adjusted mortality, renal failure, brain damage and lung function (64, 65). Graft patency after OPCAB was similar to that of conventional on-pump technique (66, 67). Prospective randomized comparisons of small groups of patients in addition showed the superiority of off-pump technique regarding blood-products requirement, hospital stay and myocardial enzyme release (68-70). Furthermore OPCAB is attributed to decrease the risk of post-operative atrial fibrillation (71), renal damage (72, 73) and is associated with better glucose homeostasis (74). Avoidance of CPB is also associated with advantages in neurological and cognitive functions (75, 76).

However, many other reports question the superiority of OPCAB over conventional CABG. Part of the inflammatory response is caused by the surgical trauma independently of CPB (77). Tissue phospholipids are liberated during the course of surgery, which are known to activate the extrinsic loop of the coagulation system. On the other hand, the damage caused by CPB to the coagulation system may improve graft patency (78, 79). Some retrospective studies on large patient cohorts could not show any difference in operative mortality (80), neither could some small randomized studies show a reduction in peri-operative morbidity (81). Neurocognitive advantages of OPCAB were not proven by some studies (82, 83). Long term graft patency has been reported to be lower after OPCAB (84), but criticism was raised

about the fact that investigators made randomized comparisons without having sufficient experience in OPCAB (85-87).

A meta-analysis of 18 randomized trials (88) showed that OPCAB reduced the relative risk at one year by 34% of combined end-points: mortality, stroke and myocardial infarction. This result was not statistically significant because of low numbers, in spite of the fact that this meta-analysis included 1584 patients.

None of the small prospective randomized studies could demonstrate any advantage in mortality because of low numbers. Large studies are in most cases retrospective, which makes the results less valuable, even when using appropriate methods for risk adjustment.

In conclusion, there is no consensus about potential OPCAB advantages over traditional on-pump CABG. The American Heart Association has stated that the superiority of one technique over the other cannot be declared so far and that a large-scale prospective randomized study is required (89). This request is legitimate but may be a problem from a theoretical point of view. If whatever technique had to be judged based on its effect on major end-points such as operative mortality, very few innovations would be accepted in cardiac surgery. Table I shows the number of cases needed in randomized studies to detect differences in results with low α and β errors. The numbers reported in Table I should be considered in view of the number of coronary bypass cases performed per year in Sweden, which is in the range of 5000. The Department of Veterans Affairs sponsored the prospective randomized study “Outcomes Following Myocardial Revascularization: On and Off Cardiopulmonary Bypass” (ClinicalTrials.gov Identifier: NCT00032630). This study started in April 2002 and was aimed at randomizing 2200 patients within four years. The status of the study in January 2005, as reported by Jones (90), was that more than 8800 patients had been screened and only 1327 randomized.

All the above-mentioned data show the difficulty in evaluating new techniques by using traditional end-points. One solution could be to identify as end-points other variables allowing powerful analyses with a low number of subjects. Monitoring the cellular metabolism might be such a solution. There is a need for tools that allow such monitoring in an easy and affordable way in clinical settings, also offering real-time results.

5.2 Substances

The aim of study I was to test whether the implantation of the probe itself would cause myocardial damage that could interfere with the interpretation of measurements of the cardiac response to surgical trauma. Troponin-T was chosen since it is considered to be a specific and reliable marker of myocardial damage and is routinely assessed in clinical settings. The high molecular weight cut-off of the microdialysis membrane allowed sampling of this relatively large protein. The insertion peak was evident in all probes but two; it was always distinct from subsequent releases. The concentration of troponin-T in microdialysate was 50 times higher than in peripheral blood, but since the probes were not calibrated it is difficult to draw any conclusion about the absolute interstitial levels of troponin-T. The levels of troponin-T in serum were consistent with studies not employing microdialysis (91-95). The time kinetics of troponin-T in the interstitium differed distinctly from those in serum and could be correlated to subtle events, such as temporary electrocardiographic changes, which are a common finding after cardiac surgery, but whose interpretation is unclear (24). On the other hand, the accumulation of troponin-T in serum prevented the detection of further releases that might have been caused by sub-clinical events. Subsequently, troponin-T measurements in the interstitium provided more information than peripheral blood sampling.

The background of study II was that a better understanding of the regulation of delivery and uptake of nutrients in the heart is required to optimize the myocardial metabolic balance during cardioplegic arrest. Previous studies have shown that serious derangement of myocardial metabolism occurs after cardiac surgery (96-101). In most studies myocardial metabolism has been analyzed utilizing blood samples from peripheral vessels and/or from the coronary sinus. During cardioplegic arrest the heart is not perfused and blood cannot be sampled from the coronary sinus. To our knowledge, this was the first report of interstitial levels of glucose and lactate during cardioplegia. Glucose and lactate were chosen because they are important myocardial nutrients, they could be measured in blood and they could be marked with radioactive isotopes. The internal reference calibration method was used to determine the real levels of glucose and lactate in the interstitium and the calibrations were repeated at different time points to ensure optimal reliability of the results. Coronary sinus blood sampling was also performed in order to compare the results of microdialysis with an accepted method for the assessment of myocardial metabolism. Arterial/interstitial differences of glucose and lactate levels were not identical to arterial/coronary sinus differences,

demonstrating that microdialysis offers more information than blood sampling. Unique, new information was gained by this study, which hopefully will help in improving myocardial protection during cardioplegic arrest.

5.3 Microdialysis probes

The first two studies of this thesis were performed with a probe developed in our laboratory specifically for myocardial use and previously used by us in experimental and human studies (21, 22, 102). That probe had some unique features. It was very easy to use, since the implantation technique was the same as for temporary pacing leads, which are a routine part of most adult cardiac surgical procedures. In addition, the dialysis membrane was protected behind a reinforced tip. The length of the outflow tubing allowed the vial connector to be placed outside the patient's chest for postoperative use. An important drawback of this probe was the dead-space, the delay from the membrane to the vial being one hour at the flow rate used. The analysis of traditional ischemic markers used, such as troponins, had to be performed in a laboratory, causing further delay between a cardiac event and the results of analyses. After study II the probe developed at the University of Gothenburg could no longer be used since no resources were available to certify the production process according to the new European rules.

A microdialysis probe dedicated for cardiac surgery was not available on the market when planning study IV. Subsequently, study III was aimed at adapting a commercially available probe, produced according to CE requirements, to the specific conditions of cardiac surgery.

The flow rate in the probe was decreased from 2.5 to 1 microliter/minute when we shifted from the University of Gothenburg to the CMA probe. The flow rate is important both for the relative recovery and the time resolution of microdialysis. A slower flow rate determines a higher recovery of substances from the interstitium; a higher flow rate increases the output per time unit, thereby allowing vials to be collected more often and decreasing the time delay from the membrane to the vial. The CMA-70 is much thinner than the original probe and the time delay of the microdialysate from the membrane to the vial is not exceeding ten minutes at a flow rate of 1 microliter per minute whereas the University of Gothenburg probe had a time delay of one hour at a flow rate of 2.5 microliters per minute. The shift to CMA-70 thus represented a major advantage in obtaining real time monitoring

The CMA microvials used can be analyzed in the CMA-600 analyzer. This machine is mounted on a trolley and can be placed in the operating room or in the intensive care

department. The CMA-600 can analyze metabolic substrates within a few minutes but cannot analyze large molecules such as troponins. Given the possibility of monitoring the energetic metabolism of the heart bedside and in real time, we decided that it was no longer necessary to look for myocardium-specific markers of ischemia. Since the molecules targeted were very small (glucose, lactate, pyruvate and glycerol), the molecular cut-off (20 kDa) of the probes chosen was sufficient. Membranes with larger pores are commercially available but we wanted to exclude large molecules not studied from sampling.

In study III, we first tried to reproduce our original implantation technique by gluing a temporary pacing wire onto the tip of a CMA-70 probe, naming it CMA-70-A. This probe proved very easy to implant in a desired position. The analytical capability of CMA-70-A was good, since ischemia could be detected very early from onset and with significant changes of all measured substances. However, the mechanical properties of CMA-70-A were suboptimal. The likely reason was that the dialysis membrane constitutes the tip itself of the CMA probe. Subsequently, the pacing wire exerted traction directly on the membrane rather than on the reinforced tip as in probes of the Gothenburg University model.
Therefore, a new probe design was developed in order to avoid traction on the tip of the CMA-70 and to avoid shear stress on the membrane. This probe was named CMA-70-B. The implantation method used for CMA-70-B differed radically from the one most commonly described in the literature for CMA probes and recently used in humans by another group (23). That method requires the implantation of a plastic catheter in the heart. Subsequently, the needle of the catheter is removed and the microdialysis probe is introduced into the tip of the catheter, which is then retracted. That technique requires a series of actions rather than a single one, thus being both more complicated and hypothetically more prone to damage the membrane. The CMA-70-B proved very easy to use, the analytical capabilities were as good as CMA-70-A, while the mechanical stability was much better.

5.4 Implantation response

In addition to validating the use of a commercially available probe for the heart, study III provided interesting information about the time course of glucose, lactate, glycerol and pyruvate after the probe implantation. An implantation response was evident for lactate and glycerol, with high levels recorded immediately after the implantation. These peaks were interpreted as implantation damage, similar to that observed in humans for troponin-T in

study I. Both lactate and glycerol then reached a baseline, similar to the levels observed after ischemia. Glucose did not show any implantation response and levels were stable after implantation.

5.5 Pyruvate

The pyruvate course was peculiar with a constant increase after the probe implantation and stabilization only after three hours. Our study model did not allow a further explanation of the pyruvate course. Under normal conditions cardiac cells base their energy production on fatty acid oxidation, therefore the glucose catabolytes produced by glycolysis are normally found at a very low level in cytoplasm. The absence or reduction of oxygen, necessary for fatty acid oxidation, changes the metabolism from aerobic to anaerobic, increasing the level of glucose catabolytes in cytoplasm. In particular, the NADH availability and lactate dehydrogenase activity increase lactate concentration transforming the pyruvate produced by glycolysis.
The increase of pyruvate in cytoplasm may be the result of a variety of metabolic events acting in concert. One proposition is the decrease of pyruvate dehydrogenase activity (23), which can oxidize and decarboxylate pyruvate to acetyl CoA in mitochondrion, another possibility is a decrease of the pyruvate transporter, which carries out the transport of pyruvate into mitochondria (103). A further possible explanation for pyruvate increase in cytoplasm is malic enzyme activity, which produces pyruvate and $NADPH+H^+$ from malate. This important reaction is coupled with fatty acid synthesis. The fatty acid synthesis could be an expression of a repair process in the cells after microdialysis probe insertion, that can produce a minimal local injury, as shown by glycerol increase. Cytoplasm pyruvate can also derive from transamination of alanine. Conversely, several amino acids can be converted into pyruvate. Thus, transamination is a major link between amino acid and carbohydrate metabolism. Figure 3 depicts metabolic pathways to pyruvate.

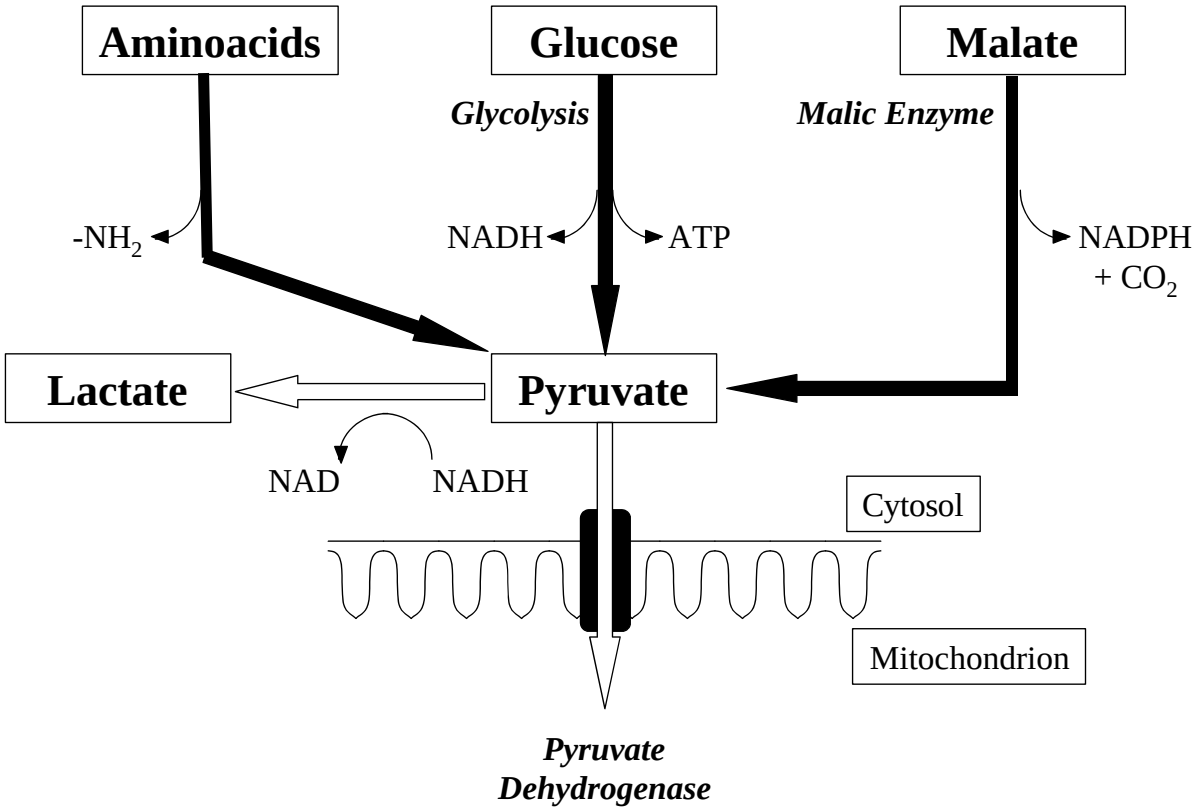

Fig. 3. Main metabolic pathways of pyruvate.

5.6 Future clinical application

If cardiac microdialysis is adopted for clinical myocardial monitoring, surgeons might implant probes at the end of surgery to monitor the ischemic status of the myocardium downstream to a specific coronary anastomosis. Microdialysis could be used after valve surgery as well since it is common to observe electrocardiographic alterations related to the area of the right coronary artery after declamping the aorta. Microdialysis might help distinguish between temporary ischemia caused by air bubbles and permanent ischemia caused by coronary embolization. In such situations, an equilibration time is not possible and it would be necessary to recognize in advance the pattern of the implantation response for various substances.

In study IV we compared on-pump and off-pump techniques for myocardial revascularization due to the clinical relevance of these two techniques. Patients were allocated to two different groups by prospective randomization. This conferred a high grade of validity to the

comparisons. There was no difference in clinical outcome, which was not a drawback but rather the premise of the study, since we wanted to detect whether microdialysis could demonstrate differences in techniques that apparently offer similar results. The higher release in blood of troponin-T in on-pump cases was expected and is in agreement with studies not employing microdialysis (104, 105). The two groups had similar interstitial levels of all analyzed substances at the beginning of the observation time, which demonstrates the validity of subsequent comparisons. Significant differences between the two groups were registered for glucose, pyruvate, urea and lactate, while glycerol and lactate/pyruvate ratio were probably biased by too short an equilibration time. Further interstitial differences were detected in the late postoperative course. Two clinically relevant events occurred in this series: an acute occlusion of a graft in one case and pathological electrocardiographic alterations with normal graft flow in another. In both cases microdialysis correctly registered the events, demonstrating severe ischemia in the first one and ruling out ischemia in the second.

6. CONCLUSIONS

- The initial increase of interstitial marker levels seen after implantation of a microdialysis probe is probably caused by the implantation itself, subsequent changes are caused by ischemia or other factors affecting the metabolic status of the myocardium (I).

- New information regarding interstitial levels of glucose and lactate before, during and after open-heart surgery has been obtained (II).

- A modified, commercial microdialysis probe could easily, efficiently and safely be used in an animal model to measure glucose, lactate, pyruvate and glycerol (III).

- This probe was clinically validated and used to show significant metabolic differences, not clinically evident, between two groups of patients treated with two surgical methods for revascularization (IV).

In summary, this study shows that microdialysis can be used in cardiac surgery to study and monitor ischemia and metabolism without delay and with high precision. The behavior of several markers during and after cardiac surgery has been described for the first time.

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