Peripheral tissue metabolism during off-pump versus on-pump coronary artery bypass graft surgery: the microdialysis study

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Received 14 September 2007; received in revised form 15 January 2008; accepted 24 January 2008; Available online 6 March 2008

Abstract

Objective: The aim of this study was to monitor and compare metabolic changes in the skeletal muscle during coronary artery bypass grafting surgery with and without cardiopulmonary bypass (CPB) by means of interstitial microdialysis. Glucose, lactate, pyruvate and glycerol were assessed as markers of basic metabolism and tissue perfusion. Methods: Twenty patients undergoing surgical myocardial revascularization were enrolled in this pilot study. Ten patients were operated on without CPB (group A, off-pump) and 10 patients using normothermic CPB (group B, on-pump). Interstitial microdialysis was performed by a CMA 60 (CMA/Microdialysis AB, Sweden) probe, inserted into the patient’s left deltoid muscle. Microdialysis measurements were performed at 30 min intervals. Glucose, lactate, pyruvate and glycerol were measured in samples using a CMA 600 Analysys (CMA/Microdialysis AB, Sweden). Results in both groups were statistically processed and the groups were compared. Results: Both groups were similar with regards to preoperative characteristics. Dynamic changes of interstitial concentrations of the measured analytes were found in off-pump (group A) and on-pump (group B) patients during the operation. There were no significant differences in dialysate concentrations of glucose and lactate between the groups. Significant differences were detected in pyruvate concentrations, lactate—pyruvate ratio and glycerol concentrations between off-pump versus on-pump patients. Pyruvate concentrations were higher in the off-pump group \((p < 0.05)\), the lactate—pyruvate ratios indicating the aerobic/anaerobic metabolism status were lower in the off-pump group \((p < 0.01)\) and the values of the concentrations of glycerol were lower in the off-pump group \((p < 0.01)\). Conclusion: Dynamic changes in the interstitial concentrations of the glucose, glycerol, pyruvate and lactate were found in both groups of patients (off-pump and on-pump). The presented preliminary results suggest that extracorporeal circulation during cardiac operations could compromise skeletal muscle energy metabolism.

Keywords: Cardiac surgery; Cardiopulmonary bypass; On-pump; Off-pump; Metabolism; Microdialysis

1. Introduction

Coronary artery bypass grafting (CABG) surgical revascularization of the myocardium ranks among the routine therapeutic methods of ischemic heart disease. Conventional coronary artery bypass (CCAB) grafting is performed with the use of cardiopulmonary bypass (CPB) and cardiac arrest. Despite advances in perfusion, cardiopulmonary bypass is still reported with profound physiological changes.

Off-pump coronary artery bypass grafting (OPCAB) has become an alternative technique to the conventional procedure for coronary revascularization. Off-pump surgery avoids some of the major side effects of cardiopulmonary bypass and reduces the disadvantages of the CPB. Several studies confirm the reduction of some postoperative complications associated with the cardiopulmonary bypass. The decrease in the systemic inflammatory response and lower activation of the inflammatory mediators following beating heart surgery [1], decrease in oxidative stress [2] or less myocardial injury with lower levels of myocardial markers [3].

Both types of CABG are associated with dynamic changes of blood circulation. In an operation for CPB, the blood flow in the peripheral and splanchnic circulation is redistributed. Hypoperfusion of the peripheral tissue caused by vasoconstriction, centralization of the circulation and possibly by hypothermia, can lead to severe postoperative complications. Off-pump surgery requires stabilization of the heart and good exposure of the target place of the anastomosis. In the period of performing distal anastomosis, reduction of the blood pressure caused by handling of the heart usually occurs. As a consequence, there could be a reduction of the...
blood supply in the peripheral tissue. Changes in tissue metabolic activity could influence the postoperative clinical outcomes.

It is difficult to assess the local changes of blood circulation in the peripheral areas, or to describe the metabolic activity changes. Direct measurement of the peripheral blood flow throughout the cardiac surgery is technically and ethically impracticable. The evaluation of the standard biochemical and hemodynamic parameters do not provide information on the regional metabolic changes.

For this purpose, the microdialysis method was used in our study for measurement of the concentrations of some substances in the interstitial fluid of the peripheral tissue. Glucose, lactate, pyruvate and glycerol were assessed as markers of basic metabolism and tissue perfusion.

The present study was designed to evaluate and compare the changes in the peripheral tissue metabolism during the off-pump CABG versus conventional on-pump CABG.

2. Patients, materials and methods

The study was carried out at the Department of Cardiac Surgery, University Hospital and the Faculty of Medicine in Hradec Králové, Charles University in Prague. The local ethics committee at the University Hospital and Faculty of Medicine in Hradec Králové approved the study. All subjects were given a detailed description of the study, and their written informed consent was obtained.

2.1. Patients

From February 2007 to June 2007 20 patients (16 male, 4 female) undergoing first-time CABG were included in the pilot study. All patients underwent an elective cardiac surgery procedure.

The exclusion criteria were concomitant surgery (valvar or aortic), an emergency procedure, patients with local or systemic infection or inflammation, severe left ventricular dysfunction (ejection fraction < 30%), renal failure (serum creatinine > 180 μmol l⁻¹ or active renal replacement therapy). The potential enrolee needed to meet the criteria for both off-pump and on-pump procedures. The surgeons had significant experience in both off-pump and on-pump CABG. The patients were randomly assigned to this study (n = 20), after the evaluation of all the candidates both clinically and angiographically, and randomly assigned to the off-pump or on-pump group.

Group A (off-pump, n = 10) underwent beating heart surgery whereas group B (on-pump, n = 10) underwent conventional myocardial revascularization with CPB and cardiopulmonary arrest of the heart. The patient’s preoperative characteristics (Table 1) and intraoperative and postoperative data (Table 2) were prospectively recorded.

2.2. Anesthetic and surgical technique

The anesthetic management, CPB and surgical procedures were standardized. Food and fluid intake was discontinued at midnight on the day preceding surgery. Anesthesia was induced with intravenous thiopental or midazolam and sufentanil, muscle relaxation with cisatracurium. Anesthesia was maintained by infusion of cisatracurium, sufentanil and propofol and dose changes were allowed to keep the patient adequately anesthetized and hemodynamically stable. Isoflurane was added in oxygen.

In all cases surgical approach was through median sternotomy. Then the left internal mammary artery and great saphenous vein were harvested.

2.2.1. Off-pump bypass surgery

Heparin was given at a dose of 1 mg kg⁻¹. The target activated clotting time (ACT) was above 250 s. Mechanical stability of the coronary arteriotomy area was achieved using a mechanical stabilizer Guidant (Guidant Acrobat, Vacuum Stabilizer System, Santa Clara, CA, USA) or Octopus (Octopus Evolution Tissue Stabilizer, Medtronic Inc., Minneapolis, USA) and soft silicon tourniquet put around the artery obtained.
hemostasis. The coronary was then opened and anastomosis was performed. Visualization was enhanced by using the surgical carbon dioxide blower (Guidant Axius Blower, Guidant, CA, USA). If signs of electrocardiographic or hemodynamic instability, or excessive bleeding appeared during the anastomosis, an intraluminal shunt (Guidant Axius Coronary Shunt, Guidant, CA, USA) was inserted. Reductions in arterial pressure caused by handling of the heart were compensated by sufficient volume filling and by placing the patient in a Trendelenburg position to maintain the mean arterial blood pressure above 50 mmHg. In some cases, vasopressors were administered for maintenance of adequate perfusion pressure. Following revascularization, the heparin effect was reversed with protamine.

2.2.2. On-pump bypass surgery

After median sternotomy and pericardiotomy, a cardio-pulmonary bypass was established by standard aortic cannulation and two-stage venous cannulation of the right atrium. Anticoagulation was induced before CBP with heparin (2.5 mg kg\(^{-1}\)) and the activated clotting time was monitored. Additional doses of heparin were given to maintain ACT time over 480 s. After cross-clamping of the aorta the cardiac arrest was instituted by an antegrade infusion of cold crystalloid potassium cardioplegia (St. Thomas solution, Ardeapharma, Ševčín, Czech Republic) or cold blood cardioplegia (blood to St. Thomas solution in ratio 4:1), repeated every 20 min, and topical cooling for myocardial protection were employed.

The extracorporeal circuit consisted of a hollow fibre membrane oxygenator with integral heat exchanger (Polystan Safe Maxi, Maquet Cardiopulmonary AG, Hirrlingen, Germany) and roller pump with non-pulsatile flow (Stöckert Safe Maxi, Maquet Cardiopulmonary AG, Hirrlingen, Germany). CPB involved normothermia and calculated blood flow 2.4 l m\(^{-2}\) min\(^{-1}\). Mean arterial pressure (MAP) during CPB was maintained at 50–75 mmHg, hematocrit was above 20%. The acid base status was kept using the alpha-stat perfusion strategy.

Once completing all distal anastomoses, the aortic cross-clamp was removed, and the proximal anastomosis was performed with partial clamping. Patients were weaned from CPB using inotropic support if necessary. Heparin was neutralized with protamine at 1:1 ratio. Additional doses of protamine were administered until the ACT had returned to the baseline or bellow.

### 2.3. Microdialysis technique

Microdialysis is a minimally invasive technique based on sampling of unbound molecules from the extracellular tissue space by means of a semipermeable membrane at the tip of a microdialysis probe. The basic principle is to mimic the function of a capillary blood vessel. In commercially available cannula, the inlet and outlet catheters are connected in a double-lumen fashion cannula with the semipermeable membrane at the tip. The probe is constantly perfused with the physiological solution at a constant low flow rate. The interstitial fluid is located between the cells and capillaries. The constitution of the extra cellular space is mostly influenced by local cell metabolism and local blood flow. Substances present in the interstitial fluid surrounding the microdialysis probe at concentration (\(c_{\text{tissue}}\)) diffuse through the membrane into the probe, resulting in a concentration (\(c_{\text{dialysate}}\)) in the perfusion medium. The composition of the microdialysate samples, obtained during the microdialysis procedure, reflects the actual metabolism state of the surrounding tissue. Samples of the microdialysates are collected and analyzed.

### 2.4. Experimental design (protocol)

Experimental subjects were in a supine position throughout the study period. Before surgical procedure, at the time.
of anesthesia introduction, one commercially available microdialysis probe (CMA 60; CMA/Microdialysis AB, Solna, Sweden) with a molecular weight cut-off of 20,000 Da and a polyamide membrane (length of 30 mm, diameter of 0.6 mm) was inserted under sterile conditions into the skeletal muscle tissue (left deltoid muscle) without prior local anesthesia.

The microdialysis probe was connected and constantly perfused with Ringer’s solution (Ringer’s solution, Braun Melsungen AG, Melsungen, Germany: Na⁺ 147 mmol l⁻¹, K⁺ 4 mmol l⁻¹, Ca²⁺ 2.25 mmol l⁻¹, Cl⁻ 156 mmol l⁻¹, 309 mosmol l⁻¹) at a constant flow rate of 0.1 ml h⁻¹ by means of a precision pump (Piéte A2 ISZ, Fresenius Vial, Le Grand Chemin, Brezins, France).

Prior to the start of the sampling period, probes were flushed with Ringer’s solution. The first sampling interval begins at the time of the skin incision after a sufficient washout period for the system with Ringer’s solution. Sampling of microdialysates was performed at 30-min intervals throughout the operation procedure (t₀.₅ = 30 min, t₁.₀ = 1 h, etc.). At the time of skin suture, when the microdialysis procedure finished, the probe was removed.

The lag time due to the dead volume between the microdialysis membrane and the point of dialysate collection was taken into account. To prevent evaporation, the microdialysis samples were collected in capped microvials (CMA Microvials). Microdialysis samples for biochemical analysis (glucose, lactate, pyruvate and glycerol) were frozen and stored until analysis.

2.5. Chemical analysis

CMA 600 Microdialysis Analyser (CMA/Microdialysis AB, Solna, Sweden) was utilized for the quantitative determination of glucose, lactate, pyruvate and glycerol. All procedures were conducted with adherence to the manufacturer’s instructions using the original (manufacturer-supplied) reagent kits and calibrators. The analytical methods were based on enzymatic colorimetric assays.

2.6. Statistical analysis

The data were processed by the NCSS 2004 and Statistica programs. Differences were considered statistically significant at the level of p < 0.05.

Demographic and perioperative data were reported as number, normally distributed data as mean ± standard deviation (SD); data that were not normally distributed are reported as median (minimum—maximum). Clinical variables were compared with two samples t-test or a Mann—Whitney U-test and a Kolmogorov—Smirnov test when indicated. The Fischer’s exact test and χ² test were used to analyze categorical data.

Values in figures are expressed as mean ± standard deviation. With respect to the small number of the patients, data were analyzed non-parametrically by Friedman’s test followed by a Mann—Whitney U-test (intergroup comparisons between variable at the same time point), and the Wilcoxon signed rank test (for differences within groups).

3. Results

3.1. Clinical characteristics and surgical data

Twenty patients were entered into this pilot study. The mean age (±SD) was 64.9 ± 6.28 years in the off-pump group and 67 ± 8.45 years in the on-pump group. The female/male ratio was 2/8 in both groups. Preoperative patient characteristics are presented in Table 1 and perioperative in Table 2. Complete revascularization was achieved in all patients. There were no significant differences between both groups in preoperative characteristics. Significant differences are in the intraoperative and postoperative data. Longer operation time, higher number of distal anastomoses and higher postoperative serum creatinine and urea concentrations were found in on-pump group. No death, acute myocardial infarction, acute renal failure, or stroke occurred.

3.2. Samples analysis — glucose, lactate, pyruvate and glycerol

3.2.1. Glucose

As shown in Fig. 1, the time course of the mean glucose concentrations in the interstitial space for the skeletal muscle during cardiac surgery off-pump and on-pump groups was very similar. Concentrations increased significantly in off-pump (200% at t₃.₀; p < 0.05) and on-pump (224% at t₂.₅; p < 0.01) group compared to baseline (i.e. t₀.₅ — time of the incision and grafts harvesting). However, there were no significant differences between the mean absolute concentrations off-pump versus on-pump.

3.2.2. Lactate and pyruvate

Lactate levels increased in both groups compared to baseline (p < 0.05) and reaching a peak increase of 185% in both groups (p < 0.05) at t₃.₀. Higher concentrations were observed in the on-pump group; however we found no statistical significant differences between these two study groups (Fig. 2).

The time course of the pyruvate levels was different in both groups as shown in Fig. 3. In the off-pump group the values increased from t₂.₀ significantly compared to the initial values reaching a peak of 296% (p < 0.01) at t₃.₀. In

![Fig. 1. Changes in glucose concentrations over time intervals. Values are expressed as mean ± SD (○, circles), off-pump group (n = 10); (□, squares), on-pump group (n = 10).](https://academic.oup.com/ejcts/article-abstract/33/5/899/447150)
the on-pump group pyruvate levels increased until t1.5 (cardiopulmonary bypass) with peak increase of 163% followed by a decrease to the baseline levels. The absolute concentrations were higher in the off-pump group than in the on-pump. We detected significant differences in pyruvate concentrations between groups ($p < 0.05$).

3.2.3. Lactate—pyruvate ratio

The levels of the lactate—pyruvate ratio (LPR) were higher at the end of the operation (t2.5 and t3.0) in the on-pump group compared to the basal value ($p < 0.05$) with the peak of 240% of the baseline ($p < 0.01$). The comparison between the LPR values between two groups showed higher concentrations in the on-pump group versus off-pump (from t2.0), and reached statistical significance ($p < 0.01$) (Fig. 4).

3.2.4. Glycerol

Lower concentrations of glycerol were measured in the off-pump group versus on-pump group ($p < 0.01$) (Fig. 5). Glycerol concentrations in the on-pump group increased during the operation, reaching a peak increase of 171% (t2.5) of the baseline (non-significant).

4. Discussion

The present study was the first to evaluate and compare the concentrations of glucose, lactate, pyruvate and glycerol in the extracellular fluid of the skeletal muscle during two types of CABG. Microdialysis has been used in this pilot study. This method represents an excellent tool for monitoring free substances directly and continuously in the interstitial fluid of the peripheral tissue, which surrounds the tissue cells, and thus reflects the metabolism status. In our study we have selected the analysis of the metabolism of the left deltoid muscle. This typical skeletal, peripheral, muscle is easily accessible (for handling by microvials) throughout the course of the cardiac surgery.

We selected glucose as a substance of basic energy metabolism when the concentrations are influenced among others by the oxygen supply. The glucose consumption is usually explained by the shift of the cellular metabolism from aerobic to anaerobic metabolism in ischemic tissue and with an acceleration of the glycolysis to sustain the energetically inefficient anaerobic glycolysis. The decreased levels of glucose in the period of the tissue ischemia were observed in studies of Bäckström and Franco-Cereceda [4] and Korth et al. [5]. Interestingly, we noted no differences between glucose concentrations in the off-pump versus on-pump group, but increased levels of glucose concentrations throughout the operation were observed in both groups. The explanation for these increased values could be the stress-induced secretion of adrenalin and β-mediated influence on the glucose homeostasis, or in some cases intravenous administration of glucose, despite non-increased levels of the serum concentrations. But the relationship between blood flow, glucose consumption and glucose concentration is a complex process. It also discussed the
possibility of enzymes inhibition.

In the present study, we have shown increased levels of the interstitial lactate in both groups, without any significant differences. The time course in the on-pump group is comparable to our previously reported results [6], and with the observation of the increased muscle lactate of Solligård et al. [7]. In the study of Bahlmann et al. [8] and also Heringlake et al. [9] plasma and interstitial levels of lactate were determined during CPB, and they noticed non-increased levels of plasma lactate compared to the increased myocardial values. These findings give evidence that microdialysis data reflect local tissue metabolism and not systemic changes in metabolite plasma concentrations.

Lactate is considered as an end product of the glycolysis in case of insufficient oxygen supply and is generally used as a marker of the tissue hypoxia and ischemia. However, we could not interpret our findings of increased lactate levels only as a result of the tissue hypoxia during the operation procedure. The production and the oxidation of lactate are closely associated with the pyruvate changes. The mechanism of the lactate and pyruvate conversion in the oxidative and non-oxidative phosphorylation involves enzymatic catalyzed processes. The lactate dehydrogenase and the pyruvate dehydrogenase have a predominant role in the regulation of turnover of lactate and pyruvate in their metabolic pathway. In some situations these could be enzymes influenced. Zemgulis et al. [10] described that pyruvate dehydrogenase complex (PDH) may be inhibited by the free radicals or influenced in different conditions, such as sepsis [11]. However, we detected no significant differences between off-pump versus on-pump group.

Interestingly, we measured higher levels of pyruvate at the end of the off-pump operation and non-increased, unchanged, concentrations during on-pump. Only pioneer studies with limited data on muscle or plasma pyruvate response to the CPB are available [6,12]. The experience with the determination of the muscle pyruvate is reported in the study of Rosendal et al. [13]. They reported about elevated concentrations of pyruvate in the trapezius muscle in the post-exercise period in healthy volunteers. They suggested that the source for pyruvate is either the glycolysis derived lactate or the uptake of the exogenous lactate, which is converted into pyruvate for further oxidation, and thus reflect the oxidative status of the area.

The lactate—pyruvate ratio, which describes redox state, is usually interpreted as an indicator of the hypoxia and marker of the tissue perfusion. We tried to calculate lactate—pyruvate ratio to be able to recognize tissue dysoxia. Although lactate—pyruvate ratio showed a profound rise in the on-pump group, this was more on account of stable tissue levels of pyruvate. Of note, the major difference seen in the ischemic index between the groups was not achieved by tissue accumulation of lactate in on-pump group, but elevated pyruvate in off-pump group.

In studies of brain damage, the increase of glycerol is usually interpreted as a marker of ischemic injury. Glycerol is the backbone of triacylglycerols and phospholipids, which are one of the main constituents of the cell membranes. During ischemia three pathways contributing to the glycerol release have been described. There is degradation of the cell membrane, caused by the failure of the calcium pump of the membrane, which initiates a breakdown of the membrane by the activating of the phospholipases. There is evidence that the glycerol could also be produced during hydrolysis of glycerol-3-phosphate derivated by glycolytic metabolism in the early phase if ischemia. Lipolysis of intracellular triacylglycerols during ischemia could be an alternative source of the glycerol [14,15].

Increased levels of the glycerol in the interstitial fluid monitored by microdialysis could reflect the disintegration of the cell membrane and ischemia. Bäckström and Franco-Cereda [4] and Pöling et al. [16] observed in the microdialysis studies increased levels of glycerol during myocardial ischemia and in the period of the reperfusion injury. We noted significantly higher concentrations of glycerol in the on-pump group, mainly in the time of the extracorporeal circulation. Local ischemic damage, supported by the findings of the higher LPR, but also inflammatory reaction or the release of the oxygen free radicals could explain these findings.

Microdialysis technique is a relatively safe and easy method for the measurement of unbound substances in the places that cannot be reached by other methods. The first publication on the application of microdialysis in humans dates back to 1987 and is a study on the characterization of the intestinal glucose concentration in healthy volunteers [17]. Although, the microdialysis is not routinely used in cardiac surgical practice, several studies on the myocardial metabolism evaluation have been published. Habicht et al. [18] performed this technique for the first time in human cardiac surgery, inserting the microdialysis catheter into the interventricular septum of the heart. Recently, Kennergren et al. [19], as well as Mantovani et al. [20] investigated the troponin-T and aspartate aminotransferase levels in the myocardial interstitium of the left ventricle during cardiac surgery. Many studies deal with the investigation of the myocardial tissue during cardiac surgery and the influence of the cardiopulmonary bypass and cardioplegia on the metabolism [8,21]. In addition, other authors in their experiments on animals published results of the myocardial energy metabolism related to the myocardial ischemia, cardioplegia and cardiac muscle metabolism [4,10,15,22]. Solligård et al. [7] reported the possibility of the endoluminal microdialysis to evaluate adequacy of splanchnic perfusion during coronary surgery with CPB. They observed increased bowel lactate concentrations during CPB as a consequence of impaired oxygen delivery.

Our experiences with microdialysis technique have been published in studies evaluating the metabolic changes, local blood flow and antibiotic tissue concentrations in the peripheral tissue during cardiac surgery and in the early postoperative period [6,23]. We noted higher perfusion of the skeletal muscle in those patients operated on in normothermia compared to the hypothermia. None of the patients from this pilot study had been included in our previous publications.

Off-pump CABG as a ‘less’ invasive technique and traditional on-pump CABG with its drawbacks of the extracorporeal system is nowadays still compared in many aspects. In this study we focused on the metabolic changes during two types of CABG in the peripheral tissue. We
suggested that episodes of less severe hypoperfusion and borderline tissue oxygenation of the peripheral tissue are relatively common. Niiniskosi and Kuttila [24] studied visceral and peripheral perfusion and oxygenation during cardiac surgery with CPB. They observed well-maintained visceral perfusion, while at the same time, those patients developed peripheral hypoperfusion and hypoxia.

The washout of lactate from previously hypoperfused tissues, after the restoration of the blood supply could explain elevated postoperative blood lactate levels [25]. This opinion could be supported by our findings of higher interstitial lactate levels in on-pump patients followed by the higher blood lactate levels at the time of intensive care unit admission; unfortunately the differences between groups were non-significant. We hypothesised that changes of the perfusion and consequently metabolic changes that occur in the peripheral tissue could be the reason for postoperative complications. In the next step it will be necessary to correlate microdiallytically measured concentrations of metabolic markers with postoperative variables.

The presented preliminary results suggest that extracorporeal circulation during cardiac operation could compromise skeletal muscle energy metabolism. The exact mechanism of this phenomenon remains to be determined. Further research with application of flow-tracers is needed to confirm and elucidate these findings. The procedure was well tolerated in our patients and no complications or side effects could be observed. The limitation of this pilot study is the relatively small number of patients in each group, but we noted statistical significant differences.

5. Conclusion

In conclusion, we have demonstrated that monitoring of glucose, lactate, pyruvate and glycerol in the skeletal muscle by the use of microdialysis is a reliable method to detect metabolic changes in the peripheral tissue during cardiac surgery. Supporting by our data, different changes in the peripheral energy metabolism occur during the off-pump compared to on-pump surgery, with higher skeletal glycerol and LPR values during the period of the cardiopulmonary bypass.

Acknowledgments

We acknowledge Dr Čermáková for her assistance with the statistical analysis of the data. We are also indebted to Mrs J. Nedvídková for her assistance with samples analysis.

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