Intraoperative muscle and fat metabolism in diabetic patients during coronary artery bypass grafting surgery: a parallel microdialysis and organ balance study


1Department of Cardiothoracic Anaesthesia, 2Department of Pharmacology and 3Department of Experimental and Clinical Medicine, Diabetes Research Centre, University Hospital, Linköping, Sweden
*Corresponding author: Department of Cardiothoracic Anaesthesia, Linköping Heart Centre, University Hospital, S-581 85 Linköping, Sweden. E-mail: zoltan.szabo@lio.se

Background. Surgical trauma causes stress and inflammatory reactions with elevated serum free fatty acids (FFA) and glucose levels characteristic of intraoperative insulin resistance. Our aim was to compare microdialysis findings with those using the classical organ balance technique and to test the clinical feasibility of microdialysis during cardiac surgery.

Methods. Nine diabetic and nine non-diabetic patients, undergoing routine coronary artery bypass grafting surgery, were studied using both microdialysis and the organ balance technique in the brachio-radial muscle of the forearm, and microdialysis in the pre-pectoral fat tissue. Glucose, lactate, and glycerol were measured in arterial and venous plasma and in the microdialysate before administration of heparin, at the release of the aortic cross-clamp, and before transfer to the intensive care unit.

Results. Glucose release from the diabetic muscle at the last sampling time was detected. This was confirmed by a negative glucose A–I (arterial–interstitial difference) in the muscle. No differences were observed regarding lipolysis in the fat tissue in terms of A–I of glycerol. Intergroup differences were detected at the first sampling time, where arterial plasma glucose and plasma insulin levels were higher and muscle interstitial glucose lower in the diabetic patients. Plasma insulin was higher in the diabetic patients even at the final measurement time.

Conclusions. In terms of lipolysis in the fat tissue and glucose transport in the muscle, the non-diabetic patients were metabolically ‘diabetics’ during surgery. Despite strict blood glucose control, disturbances in glucose homeostasis in the diabetic muscle persist. Microdialysis was easy to use during cardiac surgery.

Br J Anaesth 2009; 103: 166–72

Keywords: metabolism, glucose, lipid; muscle skeletal, metabolism; surgery, CABG, cardiovascular; technique, microdialysis

Accepted for publication: April 1, 2009

During cardiac surgery, the neurohumoral stress and the systemic inflammatory response cause trauma metabolism.1 This is characterized by increases in lipolysis and circulating free fatty acids (FFA) levels leading to insulin resistance.2 3 The major target tissues involved in insulin resistance are muscle and adipose tissue.4 The importance of insulin resistance during cardiac surgery is in the fact that the high blood glucose level in the critically ill is known to be associated with increased mortality after cardiac surgery and myocardial infarction.5–7 The efficiency of intraoperative insulin treatment and glucose control is still controversial.8

The increase in circulating FFA level blocks the glucose uptake in the skeletal muscle resulting in insulin resistance.4 Furthermore, the proportion of FFA oxidized in skeletal muscle increases. FFA oxidation consumes ~12% more oxygen than carbohydrates to produce the same amount of ATP.9 FFA also trigger apoptosis.10 The insulin resistance caused by the increase in FFA thus creates a dangerous inner environment should any organ ischaemia
occur during cardiac surgery, because of the increase in basal oxygen demand.

Since the skeletal musculature represents 40% of the body weight in humans, it is one of the main determinants of systemic oxygen demand. Monitoring metabolic changes in skeletal muscle and s.c. fat tissue assists in the optimal control of intraoperative stress metabolism. There are two ways to monitor these: one is the measurement of tissue concentrations of substrates and metabolites by microdialysis and the other is the classical organ balance technique based on the arterial–venous (A–V) difference and limb flow. Comparison of these two techniques is essential, if one has to understand and evaluate information obtained using the new technique.

Microdialysis in experimental anaesthesia was and still is used to study the pharmacokinetics of drugs and tissue metabolism. Microdialysis studies examining skeletal muscle metabolism during cardiac surgery are few and data from systematic comparison of microdialysis with the organ balance technique, as in diabetology, are not available. It was thus logical for us to begin data acquisition regarding skeletal muscle and fat tissue metabolism using parallel organ balance and microdialysis techniques studies similar to studies in diabetology. We used Gudbjornsdottr and colleagues’ study model to systematically describe the microdialysis findings in parallel to the classical organ balance technique and to test the feasibility of microdialysis as a clinical tool for the intraoperative monitoring of skeletal muscle metabolism during cardiac surgery.

Methods

This intraoperative clinical study was conducted at the Linköping Heart Centre between 2004 and 2006. The study was approved by the Local Ethical Committee, and all patients were included after obtaining the written informed consent.

Nine diabetic and nine non-diabetic patients with stable angina undergoing routine coronary artery bypass grafting (CABG) surgery were consecutively included in the study. The patients in the diabetic group were included if they had tablet- or insulin-treated type 2 diabetes mellitus. All non-diabetic control patients had a fasting venous plasma glucose below 7.0 mmol litre⁻¹.

Exclusion criteria were metabolic disorders other than type 2 diabetes mellitus, excessive bleeding, or other serious intraoperative clinical problem such as cardiac failure.

After an overnight fast, the patients took their ordinary beta-blocker, calcium blocker, or both, whereas angiotensin-converting enzyme inhibitors, insulin, and oral anti-diabetic medicines were withheld. All patients were operated on in the morning hours. The surgical theatre was air-conditioned with a constant temperature of 22.0 °C; the patient’s bladder temperature was 35.0–37.0 °C at all measurement times.

Premedication was acetaminophen 1 g orally and morphine sulphate 0.1 mg kg⁻¹ i.m. 1 h before surgery. Anaesthesia was induced with thiopental 3–5 mg kg⁻¹ (Pentothal Abbott) and fentanyl 0.3–0.5 mg (Janssen®). Muscle relaxation was achieved using rocuronium (Esmeron®) 0.6 mg kg⁻¹, and anaesthesia was maintained with isoflurane and intermittent i.v. fentanyl. The patients were ventilated with a gaseous mixture of oxygen in air with an Fio₂ of 0.5–0.6. Acetated Ringer’s solution 1000 ml was infused before commencing cardiopulmonary bypass (CPB) to counteract the hypotension after induction.

During the operation, the patients received a rapidly acting insulin infusion (Actrapid®) (1–2 u h⁻¹), if arterial blood glucose exceeded 7.0 mmol litre⁻¹. The infusion was started, if necessary, after the first blood sampling time. Insulin was required in seven of the nine diabetic patients and one of the nine non-diabetic patients.

Anticoagulation before CPB was with heparin 1 mg kg⁻¹ to achieve an activated clotting time of at least 480 s in whole blood coagulation as measured with the Hemochron® Junior. The heparin was reversed after CPB using protamine sulphate 1 mg to heparin 1 mg (ratio 1:1 mg). The extracorporeal circulation system was primed with crystalloid solution that did not contain carbohydrates other than acetate (Ringer Acetate Braun®) and mannitol. Glucose was avoided. During CPB, normothermia (bladder temperature >35.0 °C) and haemodilution to a haematocrit of 0.20–0.25 were maintained. The calculated arterial pump flow was 2.4 litre min⁻¹ m⁻². No patient required positive inotropic support during weaning from CPB. The adequacy of circulation immediately after the CPB was judged clinically based on haemodynamics, transoesophageal echocardiography, control of the mixed venous oxygen saturation (% SvO₂), and urinary output (ml h⁻¹).

Organ balance technique

A left radial artery catheter was routinely inserted under local anaesthesia before induction of general anaesthesia. A venous catheter (1.1 mm inner diameter) for venous blood sampling was introduced in the left antecubital vein. The blood flow in the left forearm was measured by standard venous occlusions double-looped mercury in silicone strain-gauge plethysmography (Pletismograph EC-6 with rapid cuff inflator Hokanson®, D.E. Hokanson, Bellevue, WA, USA) with the forearm at heart level and the strain-gauge at the maximal circumference. The device was zeroed and calibrated before each measurement [coefficient of variation (CV) 2%].

Microdialysis

Two microdialysis catheters (CMA60, CMA Microdialysis AB, Sweden with a membrane length of 30 mm and a cut off of ~20 kDa) were inserted immediately after induction.
of general anaesthesia, one in the left brachio-radial muscle and the other in the left pre-pectoralt s.c. fat tissue. The first microdialysis sample was obtained 60–90 min after the insertion of the microdialysis catheter. The catheters were perfused with lactate-free Ringer’s solution (Perfusion Fluid, CMA Microdialysis, Sweden) at a flow rate of 0.3 µl min$^{-1}$ using a CMA106 pump (CMA Microdialysis AB). No local or general complication caused by cannulation was observed intraoperatively and after operation.

Protocol

The three sampling times were: before the administration of heparin; on release of the aortic cross-clamp; and before departure from the operating theatre. The sampling times were chosen to capture relevant metabolic events in CABG surgery patients and to allow enough time to collect the microdialysis samples: (a) before heparin administration. This is a period with deep anaesthesia, relatively less surgical stimulation, and allows for microdialysis sampling 60–90 min after insertion. (b) On release of the aortic cross-clamp ~60 min after heparin. It is well known that the continuous flow delivered by the heart lung machine is unphysiological, the catecholamine levels are high, and there is haemodilution and a vasodilatation caused by the liberation of adenosine from the heart. (c) Just before the departure from the operating theatre ~60 min after the release of the aortic cross-clamp (the anaesthesia is light with all its endocrine consequences).

At every sampling time, measurements were made in the following sequence: first, the AV samples; thereafter, the forearm flow; finally, the microdialysis samples (first from the muscle then the fat tissue). The arterial and venous samples were drawn simultaneously by two persons. Gloves were used so as not to contaminate the samples. First obtained was the blood sample for plasma levels, then the arterial sample for blood gases. The vials were stored in ice-slush pending centrifugation. Blood gases were analysed at once as is usual clinical practice. The microdialysis samples were stored in ice-slush until the end of the operation.

The blood samples were centrifuged at 3000 rpm at 20°C using Hettich Universal K2S. Plasma and microdialysis samples were stored in microvials at –20°C pending analysis. The corresponding arterial, venous, and microdialysis samples were analysed simultaneously.

Glucose, lactate, and glycerol were measured. The analyses were done in CMA600 (the within-run CV was 1.8% for glucose, 4.0% for lactate, and 2.1% for glycerol).

The blood gas analyses were done using ABL700 Triolab Radiometer Copenhagen® (the CV was 1.5% for oxygen, 0.05% for pH, 1.4% for carbon dioxide, 0.7% for potassium, 4.12% for glucose, and 5.1% for lactate). Only the oxygen data were used in this study.

Plasma free insulin was analysed after polyethylene glycol precipitation as previously described. We used Mercodia Iso-Insulin ELISA (Mercodia, Uppsala, Sweden; the interassay CV for analyses of free insulin was 3%) a two-site enzyme immunoassay containing two monoclonal antibodies against insulin detecting human insulin and the insulin analogues lispro and aspart.

A–V and A–I differences were obtained by subtracting the venous or interstitial concentrations from the arterial ones. We calculated the plasma flow in the forearm as the product of blood flow and (1–haematocrit) at the given time: (flow ml min$^{-1}$ 100 g$^{-1}$)×(1–haematocrit).

We calculated the flux of substrates as a product of limb plasma flow and arterial–venous concentration difference. The oxygen consumption in the forearm was estimated by: (arterial–venous oxygen content difference)×(forearm blood flow). The oxygen content of the blood was given by [B-Hb×SO$_2$×(6.2×10$^{-4}$)]+(PO$_2$×0.01), where B-Hb is the blood haemoglobin in g litre$^{-1}$; SO$_2$ the oxygen saturation in %; PO$_2$ the partial tension of oxygen in the blood in kPa.

All analyses were done using a Hewlett Packard Compaq computer with XP operative system. We used Statistica version 7.1 (Statsoft Tulsa, OK, USA) to analyse the results. We present the results as mean (SEM). The groups (diabetic and non-diabetic patients) were compared using ANOVA for repeated measurements using Tukey’s honest significant difference test to analyse intergroup differences after the basal state and changes occurring with time.

The flux was tested against zero using the Mann–Whitney U-test adjusted for repeated measurements using the Bonferroni correction. Flux significantly lower than zero was considered release and uptake if significantly higher than zero. All differences were considered significant at $P<0.05$.

Results

The pre- and intraoperative data are described in Table 1. No hospital or 30 day mortality was observed.

### Table 1 Patient characteristics. Data are mean (range) or mean (SEM). BMI, body mass index; CPB, cardiopulmonary bypass; CC, aortic cross-clamp time; DM, diabetic group; Non-DM, non-diabetic controls; Hba1c, glycosylated haemoglobin. There were no significant differences

<table>
<thead>
<tr>
<th>Variables</th>
<th>Non-DM (n=9)</th>
<th>DM (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>63 (49–77)</td>
<td>65 (51–78)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77 (4)</td>
<td>84 (4)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.72 (0.03)</td>
<td>1.73 (0.02)</td>
</tr>
<tr>
<td>BMI (kg m$^{-2}$)</td>
<td>26 (1)</td>
<td>28 (1)</td>
</tr>
<tr>
<td>Female gender (%)</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>67</td>
<td>78</td>
</tr>
<tr>
<td>Number of distal anastomoses</td>
<td>4.4 (0.3)</td>
<td>4 (1)</td>
</tr>
<tr>
<td>CPB (min)</td>
<td>82 (10)</td>
<td>91 (8)</td>
</tr>
<tr>
<td>CC (min)</td>
<td>57 (7)</td>
<td>61 (6)</td>
</tr>
<tr>
<td>Long-term insulin treatment (%)</td>
<td>—</td>
<td>44</td>
</tr>
<tr>
<td>Hba1c (%)</td>
<td>—</td>
<td>6.52 (0.5)</td>
</tr>
<tr>
<td>30 day mortality</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Haemodynamics remained stable at all sampling times and throughout surgery in every patient. There were no intra- and postoperative clinical problems, and 30 day mortality was zero in the two groups studied. The present study caused no bleeding, infections, or other complications.

The results from muscle are listed in Table 2. Intergroup differences were detected at the first sampling time where arterial plasma glucose and plasma insulin levels were higher in the diabetic patients. At the last sampling time, the arterial plasma insulin level was higher in the diabetic group. Within-group differences are shown in Table 2.

When judging the plasma insulin levels, it must be kept in mind that seven of the nine diabetic patients and one of the nine non-diabetic patients received insulin 1–2 h⁻¹ after the first measurement. The substrate fluxes are shown in Table 3. Glucose uptake was only observed in the controls at the first two measuring times. A significant release of glucose from diabetic muscle was detected at the last measuring time as measured by the organ balance technique. This was supported by the negative arterial–interstitial concentration difference at that time as measured by microdialysis.

Glycerol release from the muscle was seen in the non-diabetic patients at first and third measuring times and in the diabetic patients at the last measuring time. Lactate release from the muscle was seen in diabetic patients during CPB at the second sampling time and at the end of the operation, and in the non-diabetic patients at the basal state and at the end of the operation. No changes in magnitude of the lactate flux and no
inter-group differences were seen between the groups at any time. Oxygen uptake was constant.

The results of tissue concentrations in the fat are presented in Table 4. The interstitial glycerol levels remained constant in the diabetic group. No significant within-group, intergroup, or both differences were recorded. Interstitial glucose levels were also stable, and no significant differences were recorded at any sampling time. Regarding the A–I differences for the three substrates, the only intergroup difference seen was glucose at the last sampling time in the non-diabetic group being higher than the diabetic. No within-group differences were seen at any time.

Discussion
To our knowledge, this is the first study in which parallel organ balance technique and microdialysis were used in coronary surgery. This study was designed to describe data using two parallel methods and to test the clinical application of microdialysis. The microdialysis technique we used was easy to handle and is potentially a useful clinical tool to monitor metabolism during cardiac surgery. In contrast, the organ balance technique was difficult to use and is not suitable for routine use in the operating theatre.

The main findings in this study are: (i) the general metabolic environment of both skeletal muscle and pre-pectoral s.c. fat in diabetic patients bore a close resemblance to non-diabetic patients during cardiac surgery; (ii) glucose release from muscle was seen at the end of the operation in the diabetic group. The diabetic and non-diabetic patients had similar glycerol levels in the fat tissue throughout the operation. We interpret this as a similar magnitude of lipolysis. Insulin infusion brought serum glucose levels to clinically acceptable levels, but the glucose metabolism in the diabetic muscle was still pathological with glucose release at the third sampling time. This is most probably due to accumulation and ‘spill over’ of glucose into the interstitial space since the rate-limiting step for uptake of glucose in the myocyte is the hexokinase conversion of glucose to glucose-6-phosphate and thereafter glycogen synthesis. This unusual release of glucose was also confirmed by a negative arterial–interstitial concentration difference in the muscle.

It is unclear why the increase in fat tissue glycerol, a marker of lipolysis, is not reflected in changes in plasma glycerol levels: we saw no correlation between the two parameters. It thus seems logical to measure tissue glycerol levels intraoperatively in cardiac surgery in order to monitor the lipolysis. Fat tissue glycerol levels as a surrogate for tissue FFA concentrations as measured by microdialysis cannot be translated to arterial plasma FFA levels. No differences were observed regarding lipolysis in the fat tissue in terms of A–I glycerol concentration difference.

The fact that the fat tissue glycerol levels were not statistically different means that the non-diabetic patients reacted similarly (in terms of lipolysis) to diabetic patients to the extreme stress situation of cardiac surgery. The increase in muscle oxygen uptake in non-diabetic patients concomitant with the cessation of glucose uptake can be interpreted as a normal metabolic reaction to stress-induced lipolysis. The pre-pectoral adipose tissue, which was easily accessible during cardiac surgery, is not known to which extent differs from the abdominal fat tissue as studies in this field are missing. Our results may thus only be partially comparable with those from other studies in the field of endocrinology.

A study by Mand’ak compared the normothermic and hypothermic CPB. In that, Mand’ak studied only the CPB period with microdialysis. Our results from the non-diabetic control group show somewhat higher values, especially in interstitial lactate and glycerol levels. These differences may be the result of changes in analysis techniques over the years, the low-dose insulin infusion we used, or biological differences in the two populations. We could also confirm an increase in muscle flow during CPB as did Mand’ak.

The mean basal plasma insulin level in the diabetic patients was two to three times higher than in the non-diabetics, despite both groups taking their beta-blockers before operation and being premedicated similarly. Our results may have been influenced by the fact that in the diabetic group seven of the nine and in the non-diabetic group one of the nine patients received rapidly acting insulin infusion Actrapid© 1–2 u h⁻¹ when clinically indicated in conformity with clinical routine. After the first measurement, we decided that the insulin infusion should be based on the blood glucose as measured by the blood gas machine (ABL700 Triolab Radiometer Copenhagen®) and not the plasma glucose values presented in this article.

Muscle blood flow was lower in the diabetic patients at the first sampling time when only anaesthesia influenced the limb plasma flow. A higher tissue flow due to isoflurane anaesthesia, haemodilution, the continuous flow delivered by the heart–lung machine and also the profound vasodilation caused by adenosine and other substances on release of the aorta cross-clamp may have influenced the results at the second measuring time. The papaverine injected (1–1.5 ml of papaverine solution 4 mg ml⁻¹) by the surgeon into the left internal thoracic artery before clamping it after harvesting (4–6 mg intra-arterial) may also have influenced the forearm flow at the last two sampling times.

Trauma metabolism causes an increase in stress hormones during cardiac surgery with concomitant lipolysis; this is also followed by changes in tissue perfusion. There are a number of limitations of this clinical study. We used glycerol as a marker of lipolysis, but we lacked parallel plasma FFA and catecholamine measurements. Also we used insulin infusion that could have effects of its own in some of our patients. We collected the muscle microdialysis sample after the flow measurement with venous occlusion
plethysmography. Therefore, even if the results partly mirror clinical reality, they should be interpreted cautiously. We have used Gudbjornsdottr’s study model and followed their method but with the s.c. microdialysis catheter placed in the pre-pectoral s.c. fat tissue; we do not know how pre-pectoral fat tissue reflects intra-abdominal or abdominal s.c. fat tissue. We used vein occlusion plethysmography to measure limb flow during CPB (vasodilatation due to the anaesthesia, haemodilution, and rapid shifts in the extracellular fluid space). Under these circumstances, vein occlusion plethysmography may give somewhat unstable results. The fluxes presented in this work must therefore be interpreted with caution. Moreover, highly perfused tissues with low uptake or release increases the possibility of type 2 error in this organ balance technique study. The use of conservative parametric statistics may have reduced the risk for type 1 error as 18 variables measured at three time points were compared between two groups in this small study.

In conclusion, the metabolic environment in both skeletal muscle and pre-pectoral s.c. fat in diabetic patients bore a close resemblance to non-diabetic patients during cardiac surgery. Glucose release from the muscle was seen at the end of the operation in diabetic patients. Diabetic and non-diabetic patients had similar glycerol levels in the fat tissue throughout the surgery indicating a similar magnitude of lipolysis. The insulin infusion used when clinically indicated brought the blood glucose levels to acceptable levels even in the diabetic patients, but the glucose metabolism in the diabetic muscle remained still pathological. Microdialysis is suited to the cardiac operating theatre and may become a suitable metabolic monitoring tool in the near future. The correlation between the organ balance technique and microdialysis presented here was not unequivocal. Larger, parallel microdialysis and organ balance technique studies are thus needed to obtain more knowledge on how the two different levels of biological organization behave from a metabolic point of view during extreme stress situations such as cardiac surgery. Moreover, such comparisons may help to establish the intraoperative optimal target blood glucose level and the insulin dosage during and after cardiac surgery with or without glucose infusion.

Acknowledgements
The authors are grateful to those 18 patients who accepted to take part in the study and also to Håkan Svensson and Patricia Linder as well as to Christina and Bettan from the Department of Clinical Physiology and to all staff at the thoracic operation theatre who kindly helped us to run this project.

Funding
The study was funded by (University Hospital Research Founding) US Forsknings Fond Project No. 03021022. The study received a coupled Grant from Orion Pharma and SFTAI (Swedish Association of Thoracic Anesthesia and Intensive Care).

References


Lindstrom T, Hedman CA, Arnnqvist Hj. Use of a novel double-antibody technique to describe the pharmacokinetics of rapid-acting insulin analogs. Diabetes Care 2002; 25: 1049–54