Evidence for a significant myocardial contribution to total metabolic burden during hypothermic cardiopulmonary bypass: a study of continuously measured oxygen consumption and arterial lactate levels in pigs

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Objective: We assessed the causes of imbalance of oxygen transport by continuously measuring oxygen consumption (VO2) during hypothermic cardiopulmonary bypass (CPB) in pigs. Methods: Six pigs (17.2 ± 1.6 kg) underwent hypothermic (32 ± 2°C) CPB for 180 min with 120 min of aortic crossclamping (ACC). An AMIS 2000 mass spectrometer was adapted for the on-line measurement of VO2. Arterial lactate was measured at the beginning of CPB, the end of hypothermia, before and 10 min after ACC release, 20 min later, and at the end of CPB. Results: Arterial lactate increased from 1.8 ± 0.7 to 5.1 ± 1.8 mmol/L during CPB. Hypothermia reduced VO2 by 0.63 ± 0.29 ml/min/kg per °C, but lactate increased to 4.2 ± 1.5 mmol/L (p < 0.05). The most rapid rise of VO2 and lactate occurred during the first 10 min after ACC removal, accounting for 26% and 68%, respectively, of the total rise during rewarming. Conclusions: Inadequate tissue oxygenation persists throughout hypothermic CPB. The rise in systemic VO2 and lactate immediately after ACC release may reflect inadequate oxygen transport within the myocardium during ischemia and manifest on reperfusion. This simple technique may be used to provide important information regarding the dynamic balance of systemic and myocardial oxygen transport during ischemia–reperfusion. Perfus (2005) 20, 277–283.

Introduction

Cardiopulmonary bypass (CPB) remains a necessary element of surgery for most acquired and congenital heart diseases. The aim of CPB is to supply an adequate amount of oxygen (DO2) to match body oxygen demand (VO2).1,2 Despite the advances in CPB and protective techniques to ensure tissue oxygenation, inadequate DO2 may result from low pump flow, hemodilution and regional vasoconstriction. Indeed, high blood lactate levels are not uncommon, reflecting an imbalance between DO2 and VO2 and tissue hypoperfusion, and correlating with postoperative morbidity and mortality.3–7 However, the underlying mechanisms for the imbalance of oxygen transport remain incompletely understood. Many previous studies have examined intraoperative VO2 and its response to factors such as pump flow rate,2 hemodilution,8 therapeutic agents3 and temperature,9 but the changes of VO2 during the process of CPB remain poorly understood. This is largely because of the lack of a technique for its continuous measurement. In all previous studies, VO2 has been calculated intermittently by the reverse Fick principle.2,3,8 This intermittent measurement of VO2 does not take into account the highly dynamic nature of VO2 during CPB. We recently reported a technique to continuously measure VO2 during CPB using respiratory mass spectrometry, and obtained detailed information about the changes and absolute values of VO2 during hypothermic CPB in an animal model.10 We found that VO2 varied significantly between...
individuals at any given temperature, and was nonlinearly related to the change of temperature. More interestingly, we noticed an acute increase in VO₂ immediately after the release of the aortic crossclamp (ACC), which was attributed to myocardial oxygen consumption. Consequently, in this study, we aimed to further assess the balance between VO₂ and DO₂ in order to explore the causes of tissue hypoperfusion during the different phases of CPB, with focus on the potential myocardial contribution after the release of the ACC, in a similar porcine model, using continuous VO₂ measurement by respiratory mass spectrometry.

Materials and Methods

Anesthesia
After review and approval by the Institutional Animal Care and Use Committee of the Research Institute in the Hospital for Sick Children, six Yorkshire pigs weighing 14.4–19 kg (mean 17.2 kg) were studied. Premedication was given with intramuscular ketamine (33 mg/kg) and midazolam (0.3 mg/kg). Anesthesia was induced with inhaled isoflurane (5%). After the onset of anesthesia, the trachea was intubated, and muscle relaxation was obtained with peripheral intravenous infusion of pancuronium (0.1 mg/kg). Ventilation was initially controlled with a Servo 900C ventilator (Siemens Medical Systems, Solna, Sweden). Anesthesia was maintained with isoflurane 1.5–2%, and muscle relaxation by continuous infusion of pancuronium (0.8 µg/kg/min). The right carotid artery and jugular vein were cannulated for arterial pressure monitoring and infusions.

Cardiopulmonary bypass
A median sternotomy was performed. Venous drainage to the extracorporeal circuit was by a 32–24F two-stage venous cannula (Stockert Instrumente, Munich, Germany) placed in the right atrium via the right atrial appendage. The blood was circulated by a roller pump through a hollow-fiber membrane oxygenator (Dideco702, Dideco, Mirandola, Italy) and returned via a 14F arterial cannula (Jostra, Hirrlingen, Germany) into the root of the aorta. Before aortic cannulation, intravenous heparin (300–400 units/kg) was given to maintain an activated coagulation time greater than 480 s. The bypass machine was primed with 250 mL of whole blood obtained from a donor pig and 650 mL Plasma-Lyte148 solution (Baxter Co-operation, Toronto, Ont., Canada) together with 22 mEq/L NaHCO₃ and 5000 units of heparin. Venous blood temperature was continuously monitored by an in-line thermistor at the blood inlet of the membrane oxygenator. The lungs were not ventilated. Isoflurane was delivered into the gas inflow to the membrane oxygenator.

After establishing CPB, the aorta was cross-clamped and cardiac arrest was induced with cold cardioplegia (30 mL/kg) of 2:1 blood and crystalloid (BCD-Vanguard, COBE Cardiacvascular Inc, Mirandola, Italy). Active cooling was then performed using the integrated oxygenator heat exchanger until venous blood temperature reached 32°C. Animals underwent 120 min of ACC and cardioplegic arrest. Stable hypothermia was maintained until 20 min prior to the release of the ACC when active rewarming was commenced to restore the venous blood temperature to 36°C. Spontaneous ventricular fibrillation was reversed with the use of a defibrillator (43100A Defibrillator, Hewlett-Packard, McMinnville, OR, USA). CPB was continued for approximately 60 min. Throughout CPB, the nonpulsatile flow was maintained at approximately 90 mL/min/kg, increasing during rewarming to 97 ± 8 mL/min/kg, so that the mean arterial pressure ranged from 40 to 60 mmHg. No vasoconstrictors or vasodilators were used. The inflow oxygen fraction to the oxygenator was adjusted between 55 and 70% to maintain the arterial PaO₂ between 100 and 300 mmHg. Hematocrit was maintained between 20 and 25% with donor blood transfusion. Venous blood temperature was recorded at the same time.

Methods of measurements

Oxygen consumption. An AMIS 2000 quadrupole mass spectrometer (Innovision A/S, Odense, Denmark) was adapted for on-line measurement of systemic VO₂ during CPB. This is a highly sensitive and accurate method for continuous gas analysis, which allows simultaneous measurements of multiple gas fractions within a mixture. VO₂ was measured using the mixed expirate inert gas (argon) dilution method.11 Our adaptation for use during CPB has been described elsewhere.10

Oxygen delivery. Oxygen extraction ratio (E RO₂) and lactate. Arterial blood samples were taken after starting CPB (15 min), at the end of hypothermia (100 min), before removal of the ACC (120 min), 10 min after removal of the ACC (130 min), 150 min and close to the end of CPB (165 min) (Figure 1). Blood gases and lactate were measured by a gas analyser (I-STAT 1 portable clinical analyser, Abbott Laboratories Ltd, Mississauga, Ontario, Canada). DO₂ was then calculated as the pump flow rate
multiplied by arterial oxygen content. ERO₂ was the ratio of the measured VO₂ and calculated DO₂.

**Statistics**

Values are presented as mean ± SD. Comparisons were carried out by using the paired two-tailed Student's *t*-test. Correlation between two data sets was assessed using the correlation coefficient. The change of the data over the study period was analysed with one-way repeated measures of ANOVA. *p* values less than 0.05 were considered significant.

**Results**

Figure 2 shows the composite changes of venous blood temperature, VO₂, DO₂, ERO₂ and arterial lactate levels in the six pigs during hypothermia and rewarming periods of CPB. The rewarming period was further divided into four sections: 20 min before the release of the ACC, 10 min after the release of the ACC, the following 20 min and the last 15 min of stable euthermia.

During hypothermia, the venous blood temperature reduced by 1.3 ± 1.3°C (from 32.2 ± 1.3 to 30.9 ± 0.8°C, *p* < 0.01). VO₂ decreased by 0.83 ± 0.47 mL/min/kg (from 4.61 ± 0.13 to 3.78 ± 0.38 mL/min/kg, *p* < 0.01) so that, for every degree decrease in the temperature, VO₂ decreased by 0.63 ± 0.29 mL/min/kg. As DO₂ was maintained stable from 8.10 ± 1.33 to 8.28 ± 1.50 mL/min/kg (*p* > 0.05), ERO₂ decreased significantly from 0.58 ± 0.09 to 0.47 ± 0.08 (*p* < 0.05). There was a significant increase in arterial blood lactate levels from 1.8 ± 0.7 to 4.2 ± 1.5 mmol/L (*p* < 0.05) during the cooling period.

During the rewarming period, the venous blood temperature increased by 4.4 ± 0.7°C to 35.3 ± 0.5°C, and VO₂ increased by 2.12 ± 0.43 to 5.89 ± 0.54 mL/min/kg, resulting in an overall increase in VO₂ of 0.49 ± 0.13 mL/min/kg per °C. The changes in VO₂ relative to the change in the venous blood temperature varied during different periods of rewarming. During the 20-min period before the release of the ACC, VO₂ increased by 0.41 ± 0.24 mL/min/kg per °C. The period of the most rapid velocity of VO₂ rise relative to the rise in temperature was seen immediately after removal of the ACC, increasing at a rate of 1.59 ± 0.25 mL/min/kg in VO₂ per °C. During the subsequent 20-min period, VO₂ increased by 0.52 ± 0.35 mL/min/kg per °C. During the last period of CPB, both the venous blood temperature and VO₂ remained relatively stable, with the temperature being 35.3 ± 0.5°C and VO₂ 5.89 ± 0.54 mL/min/kg at the termination of CPB. The increase in VO₂ during this 10 min accounted for 26 ± 6% of the total increase during the entire rewarming period.

Arterial blood lactate levels increased during the rewarming period from 4.2 ± 1.5 to 5.1 ± 1.8 mmol/L at 150 min, which was followed by an insignificant drop to 4.9 ± 1.9 mmol/L at the end of CPB. The most marked increase occurred coincidentally, with the acute increase in VO₂ relative to temperature, during the 10-min period after the release of the ACC (from 4.4 ± 1.7 to 5.0 ± 1.6 mmol/L, *p* < 0.05), accounting for 68% of the total increase during the active rewarming period. When subtracting the amount of the increase in lactate during the 10-min period after the ACC removal from that during the whole rewarming period, the rise in lactate failed to show any significance when comparing the lactate levels between the beginning and end of the rewarming period (*p* = 0.94). Therefore, both increases in VO₂/°C and lactate were significantly greater during the immediate period after ACC removal, as compared to the 20-min period before ACC removal (*p* < 0.0001 for VO₂/°C and *p* < 0.05 for lactate) and the subsequent 20-min periods (*p* < 0.001 for VO₂/°C and *p* < 0.01 for lactate) (Figure 3). No correlation was found between the increases in VO₂ and arterial blood lactate levels during this period (*r* = 0.38, *p* > 0.05).

DO₂ remained unchanged until 150 min of CPB, when a slight increase was made to 8.66 ± 1.16 mL/min/kg (*p* > 0.05) by adjusting the pump flow rate. This resulted in a significant increase in ERO₂ to 0.61 ± 0.08 at 120 min (*p* < 0.0001), 0.67 ± 0.09 at 130 min (*p* < 0.001) and to 0.69 ± 0.08 at the end of CPB (*p* > 0.05). At the beginning of CPB, the lactate levels showed a close positive correlation to the ERO₂ (*r* = 0.92, *p* = 0.01), and negative to DO₂ (*r* = −0.79, *p* = 0.06), but not with
The increase in lactate during the cooling period tended to be positively correlated with the degree of decrease in VO₂ (r = 0.55, p < 0.05). At the end of hypothermia, lactate levels tended to be negatively correlated with VO₂ (r = 0.53, p < 0.05), but not with ERO₂ (r = 0.05) or DO₂ (r = -0.42). During the later period of rewarming after 150 min of CPB, lactate was negatively correlated with DO₂ and positively with ERO₂ (r = 0.67 and 0.64 at 150 and 165 min, respectively, p < 0.05), but not with VO₂. No such correlations were found during the early periods of rewarming, either before or after the release of the ACC.

All the pigs showed very poor cardiac contraction, with ventricular distension after the release of the ACC and four out of six failed to wean off CPB.

**Discussion**

This study, using continuous measurement of VO₂, helps to explain some of the known sequelae of hypothermic CPB with ACC. There is a continuous rise in arterial lactate levels, suggesting inadequate tissue perfusion, cellular dysfunction or a combination of the two. The fact that the relationship between arterial lactate levels and oxygen transport showed different profiles during different periods of cooling and rewarming suggests that different mechanisms contribute to this phenomenon. Interestingly, the acute increase in arterial lactate levels coincided with the acute increase in VO₂ relative to the venous blood temperature after the release of the ACC, and constituted the major component of their changes during the whole rewarming period. This phenomenon, which may be attributable to myocardial contribution, indicating the significant
impairment of myocardial oxygen transport during cardioplegic ischemia and reperfusion may have been missed with intermittent assessment, but was a consistent and obvious feature during continuous monitoring.

A major goal of CPB is to provide a balance between DO₂ and VO₂ to metabolically active tissues. Systemic DO₂ is a function of pump flow and arterial oxygen content. Pump flow is often lower than the ‘usual’ cardiac output, and hemodilution reduces oxygen content. Additionally, some other factors that affect the solubility of oxygen in the blood, such as temperature and pH, may, in turn, affect the delivery of oxygen to the tissues. Furthermore, CPB per se with its nonpulsatile flow and blood contact with the CPB circuit material, results in release of potent vasoconstrictors, such as endothelins, angiotensin II and catecholamines, with splanchnic vasoconstriction predominating.

In order to compensate for the marginally reserved capacity of DO₂, it is most usual to coincidentally reduce oxygen requirements, primarily by cooling to reduce cellular metabolism. Although hypothermia is used to decrease the metabolic rate and the vulnerability of tissue to ischemic damage, it also results in a series of undesirable physiologic responses that may limit DO₂ to the tissues. These responses include a leftward shift in the oxyhemoglobin dissociation curve, i.e., an increase in affinity of hemoglobin for oxygen, an increase in pH that further shifts the curve leftward, redistribution of blood flow away from splanchnic organs and a variable degree of vasoconstriction. Furthermore, rapid cooling by an integrated heat exchanger may result in nonuniformity of body temperature during the initial period of hypothermia, causing uneven tissue perfusion and VO₂. As a result, tissue oxygen extraction may be impaired, and tissue hypoperfusion may occur, even at seemingly sufficient systemic DO₂ values. This phenomenon is reflected in our data which showed that, although both VO₂ and ERO₂ decreased significantly during hypothermia, arterial lactate levels significantly increased from 1.8 ± 0.7 to 4.2 ± 1.5 mmol/L. Furthermore, as mentioned above, systemic VO₂ reflects oxygen need as well as tissue perfusion. In other words, the diminution in VO₂ during hypothermic CPB is not only simply the result of decreased metabolic rate for oxygen, but also partly due to impaired tissue oxygenation. This may explain our findings of the positive correlation between the decrease in VO₂ and the increase in lactate during the period of hypothermia, and the negative correlation between absolute VO₂ values and the arterial lactate levels at the end of hypothermia when the temperature was at its lowest, although not achieving statistical significance.

Lactic acidosis is largely attributed to anaerobic production by hypoxic tissues. However, reduced hepatic lactate metabolism should also be taken into account when interpreting the increase in lactate, especially during hypothermic CPB. Indeed, the reasons for elevated lactate levels frequently observed during CPB are complicated. Similar complexity also exists during rewarming. The continuous rise in lactate during rewarming may reflect ‘wash-out’ after restoration of flow to previously ischemic regional tissue beds and is magnified by imbalance between DO₂ and VO₂ induced by variable rewarming of tissues, resulting in an increased VO₂ in the presence of depressed DO₂. This appears to be a regional phenomenon. The splanchnic organs have been said to be the major source for lactate production during the rewarming phase of CPB. However, our data suggest that the myocardium may be an important source of arterial lactate release during rewarming.

The contribution of myocardial VO₂ and lactate production after the release of the ACC to the systemic changes during rewarming has not previously been studied in detail. Our study, based on the continuous measurement of VO₂, demonstrates that the major component of the increase in arterial lactate levels and VO₂ during rewarming was occurring during this brief period. The acute increase in VO₂, which we have previously described, was accompanied by an acute increase in lactate. In fact, the immediate post-ACC release period is the only period that lactate levels increased significantly and accounted for 68% of the total increase during the entire rewarming period. These findings suggest a significant imbalance of myocardial oxygen transport during cardioplegic ischemia and at the time of reperfusion. It is likely that, despite cold cardiopлегic arrest, anaerobic metabolism continues, exposing myocardium to oxygen debt, leading to lactate production and accumulation. Indeed, previous studies have reported a significant fall in myocardial ATP and a rise in directly measured myocardial lactate during cold cardioplegic arrest. These changes are strongly correlated with the duration of ischemia, particularly in young children, and postoperative release of myocardial troponin 1, a sensitive indicator of myocardial reperfusion injury. The prolonged cold cardiopлегic arrest of two hours in our study, therefore, may be expected to have induced a significant oxygen debt and lactate production, resulting in severe ischemia injury. In fact, all the pigs showed very poor cardiac function, with ventricular distension.
after the release of the ACC and four of six failed to wean from CPB. Repayment of oxygen debt and ‘washout’ of accumulated lactate at reperfusion may account, in part, for the significantly increased systemic VO₂ and arterial lactate levels after the ACC release in our study. The myocardial imbalance of oxygen transport may be further impaired at reperfusion. Reperfusion following cardiac arrest in CPB has been reported to be associated with an increase in myocardial oxygen consumption and a decrease in coronary perfusion due to impaired coronary endothelial function and subsequent myocardial injury.²²,²³ This is further amplified by ventricular fibrillation together with distension of the myocardium at reperfusion, which occurred in all of our pigs.²²,²⁴,²⁵ Due to the rigidity of our CPB protocol, we were not able to analyse the relationships between the changes in VO₂ and lactate, the potential determinants such as ACC duration and ventricular arrhythmia, and subsequent cardiac function. These changes are clearly less marked in the clinical setting, but a postoperative low cardiac output syndrome is not uncommon, despite optimal repair and ‘uneventful’ CPB procedure, and may account for 50% of postoperative morbidity and mortality.²⁰,²¹ Our simple method of continuous measurement of VO₂ should allow further studies, both experimental and clinical, in the future.

Limitations

The values of ERO₂ in our study were high. This may partly be an artifact because of the independent measurements VO₂ and DO₂. While VO₂ was directly measured, DO₂ was calculated from pump flow and arterial oxygen content. There may be some discrepancy between the two measurements. Indeed, in our previous study with the same protocol and technique, we found a significant underestimation of VO₂ calculated from the reversed Fick from the pump flow and arterial and venous oxygen contents as compared to the directly measured VO₂.¹⁰ Thus, ERO₂ derived in our study may be overestimated. Nonetheless, using the independent measurements of VO₂ and DO₂ avoids the problem of mathematical coupling when DO₂ is derived from VO₂. We believe, therefore, that the trend and interindividual variations of ERO₂ in our animals can be interpreted as reflecting real differences in tissue perfusion during CPB.

The CPB and ischemic protocol in our study was deliberately aggressive. While the changes in VO₂, ERO₂ and lactate may be more severe than they are in routine clinical practice, the protocol was designed to expose the major determinants of VO₂ and tissue perfusion, including temperature, DO₂, ERO₂ and myocardial contribution to these changes.

Lastly, we did not measure the coronary venous lactate levels. Those values may have provided direct information about myocardial oxygen transport during CPB. Nonetheless, it is reasonable to attribute the significant changes in systemic VO₂ and arterial lactate levels observed in our study to a myocardial contribution. Further studies to correlate these changes with coronary venous lactate levels will allow an assessment of the utility of our technique to assess myocardial oxygen transport during reperfusion.

Conclusions

Tissue hypoperfusion persists throughout hypothermic CPB due to an imbalance between VO₂ and DO₂ at both systemic and tissue levels. The major component of the changes in VO₂ and lactate during rewarming immediately after ACC release may be attributed to the myocardium, presumably reflecting events occurring during ACC and at reperfusion. Further clinical investigations using this simple technique to measure the changes in VO₂ and lactate during the immediate post-ACC release period may provide important information regarding the balance of global oxygen transport as well as myocardial responses during ischemia–reperfusion.

References
