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BY
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AALBORG UNIVERSITY
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CEREBRAL AND MUSCLE BLOOD METABOLISM AT LOW BLOOD FLOWS DURING CARDIOPULMONARY BYPASS

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PhD dissertation

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PREFACE AND ACKNOWLEDGEMENTS

The present PhD thesis is based on studies carried out from 2010 to 2016 during my part-time PhD programme. The PhD studies were carried out during my employment at the Department of Thoracic Anaesthesiology & Intensive Care Medicine, Aalborg University Hospital, Aalborg, Denmark, where the humane study was conducted (study I). The animal studies were carried out at the Institutes of Clinical Medicine, Skejby University Hospital, Aarhus, Denmark, (study II) and at the Department of Nuclear Medicine & PET Centre, Aarhus University Hospital, Aarhus, Denmark, (studies III and IV). The studies have been presented at both national and international meetings.

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Sisse Anette Thomassen

January 2017

ENGLISH SUMMARY

In clinical practice, systemic measurements such as pulse, blood pressure, cardiac output, oxygen-derived variables, and blood lactate levels are used to assess tissue perfusion and thereby tissue oxygenation. The systemic measurements are global assessments and often require invasive techniques that limit an early detection of shock. During shock and low blood flow states, complex regulatory mechanisms ensure a stable supply and delivery of oxygen to the brain at the expense of other organs such as muscle tissue. Also, during cardiopulmonary bypass (CPB) the non-pulsatile blood flow produced by the CPB is distributed according to an organ hierarchy, preserving blood flow to the brain as the top priority at the expense of muscle tissue that has the lowest priority. Cerebral and muscle metabolism can be assessed by regional measurements that extend from advanced methods leading to calculation of tissue perfusion using dynamic positron emission tomography (PET-CT) and cerebral and muscle tissue metabolomics measured by microdialysis to non-invasive and continuous near-infrared spectroscopy (NIRS) that reflects the tissue oxygenation balance in superficially located organs, e.g. brain and muscle tissue, thereby providing information on tissue oxygenation in the highest and the lowest prioritised organs during hypoperfusion.

The overall aim of this PhD thesis was to investigate the tissue metabolism of the brain and muscle using cerebral NIRS (human study) and muscle NIRS (experimental studies) and evaluate the measurement with more advanced regional monitoring methods, i.e. microdialysis and PET-CT during low blood flows using CPB, thereby gaining insight into metabolism and oxygenation balance at the extreme ends of the organ hierarchy during hypoperfusion.

In study I, we hypothesised that cerebral oxygenation saturation (rSO_2) and hence perfusion measured by NIRS was preserved in 22 cardiac surgery patients during CPB. The study was a cross-over study with random allocation to a CPB blood flow for 20 min based on either the calculated flow (2.4 L/min/m^2) or the individual measured CO prior to CPB, and then a switch to the opposite blood flow for another 20 min. rSO_2 was preserved during both CPB blood flows, with the individual estimated blood flow ranging from 1.9 to 3.1 L/min/m^2 in cardiac surgery patients.

In study II, we hypothesized that muscle tissue oxygenation saturation (StO_2) measured with NIRS 1) was a fast reacting indicator of global hypoperfusion during CPB and 2) that it follows the changes in systemic acid/base balance and in metabolism in remote organs in an experimental porcine model. Twelve pigs were coupled to CPB initiated with a blood flow of 60 mL/kg/min for 1 hour, and hereafter randomised to a CPB low blood flow of 47.5 or 35 mL/kg/min for 1 hour and returned to a CPB blood flow of 60 mL/kg/min for 1 hour. StO_2 was compared with microdialysis obtained from muscle tissue, intestinal tissue, and brain tissue during all CPB blood flows. During inadequate systemic circulation, StO_2 reacted

rapidly and indicated insufficient global tissue perfusion even when systemic circulation was restored.

In study III, we hypothesized that changes in muscle tissue perfusion measured with PET-CT would be reflected by parallel changes in StO_2 measured by NIRS. In eight pigs, normothermic CPB was established with a blood flow of 60 mL/kg/min for 1 h. Thereafter pigs were randomised to a blood flow of either 47.5 or 35 mL/kg/min for 1 h followed by a blood flow of 60 ml/kg/min for an additional hour. PET-CT scans were performed at spontaneous circulation and each blood flow. StO_2 was compared with values of the dynamic PET-CT scan of the corresponding regional muscle at all blood flows. StO_2 remained high until muscle blood flow had decreased to about 50%, after which StO_2 almost linearly followed the fall in muscle blood flow.

In study IV, we hypothesised that CPB blood flow is more important than mean arterial blood pressure (MAP) for cerebral perfusion during normothermic CPB. This study is part of study III. During the study period, no intervention was undertaken with regard to the changing MAP. At the end of each blood flow stage, MAP and cerebral blood perfusion (CBF) measured with PET-CT using ^{15}O-labeled water were determined. We demonstrated that CPB blood flow is a main determinant of CBF and that a high MAP does not protect against cerebral hypoperfusion.

In summary, this thesis indicates that the blood flow during normothermic CPB may be safe at a wider range (1.9–3.1 L/min/m^2) in uncomplicated cardiac surgery patients evaluated by cerebral NIRS, but in an experimental porcine model with very low blood flows during CPB, we found CBF to be flow dependent and that an increased MAP did not protect against cerebral hypoperfusion. NIRS measurements above the lowest prioritised organ, the muscle tissue, demonstrated that StO_2 to react rapidly due to insufficient global tissue, but StO_2 remained high until muscle blood flow had decreased to about 50%. In conclusion, muscle NIRS could serve as an early warning signal of insufficient muscle metabolism, but further studies are needed.

DANSK RESUME

I klinisk praksis anvendes systemiske parametre, såsom puls, blodtryk, minutvolumen, afledte oxygen variabler (blandet eller central venøs saturation), samt blod laktat til at vurdere sufficient vævsgennemblødning og dermed iltforbruget af periferier vævene. De systemiske parametre er globale målemetoder og kræver ofte invasive teknikker og derfor er disse målemetoder af begrænset værdi, når shock tilstande skal erkendes tidligt i forløbet. I forbindelse med shock samt tilstande med nedsat vævsgennemblødning styres fordelingen af systemiske blodvolumen til organerne af komplekse reguleringsmekanismer der sikrer sufficient blodgennemstrømning og dermed ilttilførsel til hjernen på bekostning af andre organer såsom muskelvæv. Ligeså når der anvendes hjertelungemaskine fordeles den non-pulsatile blodgennemstrømning produceret af hjertelungemaskinen efter et organhierarki, hvor blodgennemstrømning til hjerne prioriteres først, og muskelvæv til sidst. Hjernens og musklernes metabolisme kan måles med regionale målemetoder, strækkende sig fra omfattende 1) avancerede målinger med dynamisk positron emission tomografi (PET-CT), der via komplekse beregninger angiver den regionale vævsgennemblødning 2) vævsbiomarkører der måles med mikrodialyse til 3) non-invasive og kontinuerlig infrarød spektroskopi (NIRS) monitorering, der afspejler det organspecifikke iltforbrug i overfladiske beliggende væv, såsom hjerne og muskelvæv. Dermed opnår man indsigt i det organspecifikke iltforbrug i vævene i forhold til disses prioritering (prioriteret højeste og lavest) i organ hierarkiet.

Det overordnede formål med PhD afhandlingen var, at undersøge den organspecifikke metabolisme i hjerne og muskler ved brug af cerebral NIRS (humant studie) og muskel NIRS (eksperimentalt studie), samt at sammenholde NIRS målingerne med mere avancerede regionale monitoringsmetoder såsom mikrodialyse og PET-CT ved nedsat vævsgennemblødning ved brug af hjertelungemaskinen, og dermed få indsigt i organspecifikke metabolisme og iltforbrug i det højeste og laveste prioriterede væv i organ hierarkiet.

I studie I, var hypotesen, at den cerebrale iltmætning (rSO_2) og dermed perfusion målt ved NIRS var bevaret i 22 hjertekirurgiske patienter under CPB. I et cross over studie blev de hjertekirurgiske patienter randomiseret til én af to grupper. Den ene gruppe startede med det beregnede blod flow (2.4 L/min/m^2) i 20 minutter efterfulgt af yderligere 20 minutter med det individuelt thermodilutionsmålte blod flow målt forud for hjertelungemaskinen. Den anden gruppe startede med det individuelt thermodilutionsmålte blod flow i 20 minutter efterfulgt af det beregnede blod flow (2.4 L/min/m^2) i 20 minutter. Hjernens iltmætning målt med NIRS var bevaret under begge blod flows med det individuelle blod flow fra 1.9 til 3.1 L/min/m^2 i de hjertekirurgiske patienter.

I studie II var hypotesen, at muskelvævs iltmætning (StO_2) målt med NIRS 1) var en hurtigt reagerende, indirekte markør for global hypoperfusion under CPB og 2) at StO_2 reflekterede ændringerne i systemisk syre/base balance og metabolisme i de perifere organer undersøgt i en grisemodel. Tolv grise blev tilkoblet hjertelungemaskine med et blod flow på 60 mL/kg/min i en time og herefter randomiseret til et lavt blod flow 47,5 eller 35 mL/kg/min i en 1 time og til sidst 1 time på det oprindelige blod flow 60 mL/kg/min. StO_2 blev sammenlignet med mikrodialyse i muskelvæv, tarmvæg og i hjernevæv under alle CPB blod flows. Ved utilstrækkeligt blod flow reagerede StO_2 hurtigt og indikerede utilstrækkelig systemisk vævsgennemblødning, selv når systemisk cirkulation blev reetableret efter perioden med systemisk hypoperfusion.

I studie III var hypotesen, at ændringer i muskelvævsgennemblødningen målt med PET-CT ville blive afspejlet i parallelle ændringer i StO_2 målt ved NIRS. Otte grise fik etableret normothermisk CPB med blod flow 60 mL/kg/min i 1 time. Efterfølgende blev de randomiseret til et blod flow på enten 47,5 eller 35 mL/kg/min i 1 time, og til sidst efterfulgt af 1 time med blod flow på 60 mL/kg/min. PET-CT blev udført under spontan cirkulation og efter hver blod flow periode. StO_2 blev sammenlignet med den PET-CT beregnede muskel blodgennemstrømning. StO_2 forblev høj indtil muskel blodgennemstrømning var faldet til ca. 50%, hvorefter StO_2 næsten faldt lineær med muskel blodgennemstrømning.

I studie IV var hypotesen, at blod flowet leveret af hjertelungemaskinen var vigtigere end middel arterieblodtrykket (MAP) for den cerebrale perfusion ved lavt blod flow. Analyserne var lavet på data indsamlet under studie III. Under studiet blev MAP ikke farmakologisk behandlet. Ved afslutningen af hver blod flow periode blev MAP og den cerebrale blod perfusion (CBF) målt med PET-CT, og den præcise blodgennemstrømning af hjernen beregnet og sammenlignet med MAP. Studiet viste, at et CPB leveret blod flow var en vigtig determinant for hjernes blodgennemstrømning og at et højt MAP ikke sikrede mod cerebral hypoperfusion.

Opsummerende viste studierne, at ilttilbuddet til hjernen var bevaret ved CPB blod flow 1.9-3.1 L/min/m^2 vurderet med NIRS i ukomplicerede hjertekirurgiske patienter, men i den eksperimentelle grisemodel med utilstrækkelig systemisk cirkulation var det CPB leverede blod flow vigtigere for opretholdelsen af hjernens vævgennemblødning, da et øget MAP ikke sikrede mod nedsat hjerne vævgennemblødning. Ved brug af NIRS på det lavest prioriterede organ, - muskelvæv viste resultaterne, at StO_2 reagerede hurtigt og indikerede utilstrækkelig systemisk vævsgennemblødning, når CPB blodflow blev reduceret signifikant. StO_2 forblev høj indtil muskel blodgennemstrømningen var faldet til cirka 50%. I visse kliniske tilstande kan StO_2 måske være en tidlig markør for utilstrækkelig ilttilbud til muskel og dermed metabolisme, men yderligere studier er nødvendige.

PUBLICATIONS

This PhD dissertation is based on the following papers referred to in the text by their roman number.

I. Thomassen SA, Larsson A, Andreasen JJ, Bundgaard W, Boegsted M, Rasmussen BS. **Should blood flow during cardiopulmonary bypass be individualized more than to body surface area?** Perfusion. 2011 Jan;26(1):45-50. doi: 10.1177/0267659110382062.

II. Thomassen SA, Kjærgaard B, Sorensen P, Andreasen JJ, Larsson A, Rasmussen BS. **Regional muscle tissue saturation is an indicator of global inadequate circulation during cardiopulmonary bypass. A porcine study using muscle, intestinal and brain tissue metabolomics.** Perfusion doi:10.1177/0267659116674271, 2016

III. Thomassen SA. Kjaergaard B, Alstrup AKO, Munk OL, Frøkiær J, Larsson A, Rasmussen BS. **Muscle tissue saturation compared to muscle tissue perfusion during low blood flows: an experimental study**. Submitted to Journal of Cardiothoracic and Vascular Anesthesia.

IV. Thomassen SA, Kjærgaard B, Alstrup AKO, Munk OL, Frøkiær J, Larsson A, Rasmussen BS. **Pump blood flow and not arterial blood pressure determines cerebral perfusion during normothermic cardiopulmonary bypass. An experimental porcine study.** Submitted to Journal of Cardiothoracic and Vascular Anesthesia.

ABBREVIATIONS

CPB	Cardiopulmonary bypass
BSA	Body surface area
LBM	Lean body mass
BMI	Body mass index
SvO_2	Mixed venous saturation
CO	Cardiac output
CBF	Cerebral blood flow
CI	Cardiac index
DO_2	Oxygen delivery
VO_2	Oxygen consumption
SaO_2	Arterial oxygen saturation
ICU	Intensive Care Unit
LPR	Lactate/pyruvate ratio
NIRS	Near-infrared spectroscopy
rSO_2	Regional cerebral tissue oxygen saturation
StO_2	Regional muscle tissue oxygen saturation
CABG	Coronary artery bypass grafting
CEA	Carotid endarterectomy
PET-CT	Positron emission tomography
$PbtO_2$	Oxygen tension in brain tissue

PaO_2	Partial pressure of arterial oxygen
$PaCO_2$	Partial pressure of arterial carbon dioxide
ICP	Intracerebral pressure
CPP	Cerebral perfusion pressure
MAP	Mean arterial blood pressure
ROIs	Regions-of-interest
VOI	Volume-of-interest
LME	Linear mixed effects models

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Figure 12. The relationship between normalised muscle blood perfusion obtained by PET and StO_2 obtained by NIRS. The line depicts the best fit.

Figure 13. Cerebral tissue perfusion in % of baseline (spontaneous circulation) during the different CPB blood flows. Pigs 1–3 (low blood flow 35 mL/kg/min); pig 4 prolonged low flow (47.5 mL/kg/min); pig 5 profuse bleeding from the nose (47.5 mL/kg/min); and pigs 6–8 (low blood flow 47.5 mL/kg/min).

CHAPTER 1. BACKGROUND

1.1. CARDIOPULMONARY BYPASS

In 1953, John Gibbon performed the first open-heart procedure (1), but first years later were a series of open-heart procedures performed using a modification of the Gibbon heart-lung machine (2). Today cardiopulmonary bypass (CPB) is used worldwide for cardiac surgery (3), and in the Western Region of Denmark CPB is used in 88% of all heart surgery procedures (4), with a 30-day mortality rate of 1.5% [95% CI 1.0–2.2%] after cardiac surgery (4). Although the mortality rate is low, complications still occur, e.g. severe bleeding, systemic inflammation response, stroke or neurocognitive dysfunction, and different degrees of multiorgan dysfunction and failure (3). The underlying mechanisms are highly complex and depend on several factors, e.g. patients' comorbidity, embolic episodes, and periods of tissue hypoperfusion during CPB (5). During low flow conditions, the blood flow is distributed according to an organ hierarchy where the brain is of highest priority, then followed by the kidneys, the intestines, and finally the muscle tissues (6). The brain is protected by a strong autoregulation that keeps cerebral blood supply carefully titrated to the oxygen demand during both normal and low flow conditions (7). In contrast, muscle tissue blood flow is governed by central and humoral mechanisms leading to vasoconstriction and centralisation of the muscle blood flow to vital organs such as the brain (8). Muscle tissue acts as a reservoir of volume (8) that can be recruited, and therefore monitoring muscle tissue may serve as an early warning signal during low flow conditions.

1.2. CALCULATED BLOOD FLOW DURING CARDIOPULMONARY BYPASS

CPB acts as a mechanical and physiological substitute for the heart and the lungs and ensures oxygen delivery to all tissues (2, 9–10). A circular system with a roller pump or centrifugal pump delivers a non-pulsatile blood flow and an oxygenator provides a gas exchange of oxygen and carbon dioxide. The level of non-pulsatile blood flow during normothermic CPB is primarily calculated in litres per minute and based on the product of body surface area (BSA) and a constant of 2.4 (11–12). The most commonly used BSA formula was described by Dubois and Dubois in 1916, but is based on a very small sample size (12). The rationale for using BSA is that, on average, each square metre of the body mass has the same metabolic rate (11–12). The metabolic rate, and thereby the oxygen requirements, is primarily determined by lean tissue. In obese patients, a larger portion of the body mass is fat, and several studies have demonstrated that the Dubois formula is inaccurate in these patients (13–19). Recent studies (20–21) demonstrate that lean body mass (LBM) rather than BSA or body mass index (BMI) correlates with mixed venous saturation (SvO_2)

during CPB (20), and in obese cardiac surgery patients fewer complications and a smaller number of blood transfusion are seen when ideal weight according to height is used to calculate BSA, indicating a more optimal blood flow during CPB (21).

1.3. PATIENT POPULATION IN CARDIAC SURGERY

Cardiac surgery is challenged by an increase in the elderly population in the Western world (22–23). Future cardiac surgery patients are therefore increasingly characterised by higher co-morbidities, in particular cerebrovascular disease, chronic obstructive pulmonary disease, left ventricular dysfunction, renal dysfunction, peripheral arterial disease, and diabetes mellitus (24–27). Indeed, increased comorbidities tend to be associated with a high rate of complications in cardiac surgery patients (24). Old age, previous stroke, hypercholesterolaemia, hypertension, and diabetes are all predictors of postoperative neurologic complications (25, 28–29).

1.4. CEREBRAL AND MUSCLE PERFUSION

To obtain an optimal CPB, blood flow is important because an insufficient blood flow leads to tissue hypoperfusion and thereby induces organ damage; on the other hand, hyperperfusion (too high blood flow) increases the risk of air embolism, the need of blood transfusion, and the development of a systemic inflammatory response (30-31). The following theories exist on how organ blood flow and hence tissue perfusion is governed: 1) The organ perfusion depends on arterial blood pressure and vascular resistance of the organ; 2) The distribution of blood flow to each organ is determined by the cardiac output (CO) because each organ receives a percentage of CO based on the organ's metabolic needs; 3) The CPB produced blood flow is distributed due to an organ hierarchy where the brain is of first priority, then kidneys and intestines, and finally the muscle tissue, which has the lowest priority (6, 32).
Cerebral blood flow (CBF) is regulated by powerful mechanisms in order to match cerebral metabolic demand and supply (32). It is presumed that a fluctuating blood pressure will not lead to changes in CBF if blood pressure is kept within the autoregulatory range of 50 to 150 mmHg (33). However, in a study in which a lower body negative pressure technique was used to imitate an acute decrease in central blood volume, an association between CO and CBF was demonstrated in young healthy subjects. Each percentage of change in CO corresponds to a 0.35% change in CBF, e.g. a 30% reduction in CO leads to a 10% reduction in CBF (34). A recent study in 31 healthy elderly subjects (35) failed to demonstrate an association between CO and CBF using magnetic resonance imaging techniques, but the study was done during rest and without any acute blood volume decrease. However, the study showed that when fractional CBF (total CBF/cardiac CO) is calculated, the fractional CBF is inversely correlated with cardiac index (CI (CO/BSA)), e.g. when

CI (and CO) decreases the fractional CBF will increase and the brain will receive a larger amount of CO. The peripheral regional blood flow of muscle tissue is also tightly regulated as it serves as a reservoir of volume during low CO. Indeed, muscle tissue has the ability to maintain its function even if oxygen delivery (DO_2) is dramatically reduced (8). The blood flow to muscle tissue is governed by a vascular basal tone. An inhibitory stimulus to basal vascular tone induces a vascular vasodilatation, while an excitatory stimulus induces a vascular vasoconstriction (8). During low CO the most important regulatory mechanism is the central nervous system that activates baroreceptors and chemoreceptors, causing vasoconstriction and thereby a shunting of the blood flow from the periphery to the brain.

1.5. OXYGEN DELIVERY DURING CARDIOPULMONARY BYPASS

Besides the regulatory mechanism that governs organ specific blood flow, haemoglobin concentration and oxygen content are other factors influencing the DO_2 to the tissues and thereby organ functions. If DO_2 falls due to a decrease in blood flow, the extraction of oxygen from the blood will increase, but when a critical low DO_2 is reached, a drop in tissue oxygen consumption (VO_2) is seen, because the extraction of oxygen is limited. This phenomenon is characterised by a shift in the VO_2-DO_2 relationship from aerobic to anaerobic metabolism (36). In an experimental pig study using fluorescent microspheres (6), an organ-specific hierarchy of DO_2 during CPB was demonstrated. The DO_2 of the brain and kidneys was maintained at blood flows in the range 1.7 to 2.3 L/min/m^2, in contrast to significant decreased DO_2 in muscle tissue. These findings indicate that blood flow to the brain may be preserved at the expense of blood flow to the muscle tissue. It might also indicate that each organ has its own organ-specific critical DO_2, so when the systemic critical DO_2 value is reach, some organs may be well below their critical DO_2, whereas others organs are above their critical DO_2, as critical systemic DO_2 presents the sum of all organ DO_2 values.

Individualised therapy has gained acceptance in many clinical specialties. It has been shown that postoperative complications after major surgery can be reduced by the use of individualised circulatory therapy (37). Cardiac surgery patients are characterised by increasing co-morbidities, age, and overweight, therefore there is a need for further refinement of CPB strategies by adapting CPB blood flows to individual patient needs. Nowadays, as mentioned above, the CPB blood flow is based on a calculation of BSA, but new monitoring technologies may help in the evaluation of systemic and regional perfusion and oxygenation and thereby the metabolism of regional tissues.

1.6. SYSTEMIC MONITORING

The blood flow and perfusion pressure are interdependent during CPB. They are determined by arterial impedance, the degree of haemodilution, temperature, and arterial cross-sectional area. Changes in haemodilution and temperature are the most important determinants of DO_2 and VO_2 in tissues. VO_2 can be assessed by continuous measurement of SvO_2. SvO_2 depends on several factors:

$$SvO_2 = SaO_2 - (VO_2 / (CO \times (haemoglobin) \times k)$$

Where SaO_2; arterial oxygen saturation and k a constant. The normal value for SvO_2 is > 65%. However during CPB, an SvO_2 within the normal range does not guarantee sufficient oxygenation of all tissues as organ perfusion may be unevenly distributed (38). Indeed, during normothermic CPB, tissue hypoperfusion seems to be more accurately assessed by measuring serum lactate compared to measuring SvO_2 (39).

Hyperlactatemia is defined as blood lactate levels between 2–5 mmol/L without metabolic acidosis. Lactic acidosis occurs when lactate levels rise above 5 mmol/L together with metabolic acidosis (40). Indeed, when DO_2 is limited, the majority of intracellular pyruvate is converted into lactate and hyperlactatemia develops (41). This is named type A lactate acidosis and typically occurs after initiating CPB, or within the first 24 hours of the postoperative period (41–42). It is relatively common, with an incidence of 10 to 20% in cardiac surgery patients using CPB (43-44) and is associated with an increased risk of a complicated postoperative course with an 8- to 10-fold increase in mortality (44–46). Multiple factors contribute to the development of early hyperlactatemia (40), and the lactate clearance can be affected because of hepatic dysfunction (41). In a recent study, hyperlactatemia with a lactate > 3 mmol/L during CPB was associated with the need of a intra-aortic balloon pump, a longer duration of mechanical ventilation, and a longer length of stay in an intensive care unit (ICU) (47). In general, the lactate to pyruvate ratio (LPR) is approximately 10:1, but lactate production can also occur with normal LPR, called type B lactate acidosis (42). Late-onset hyperlactatemia occurs in approximant 14-20% of adult patients following cardiac surgery but is not associated with an increased risk of adverse events (40, 42). The pathophysiology behind late-onset hyperlactatemia is unclear (42).

Both systemic parameters display obvious advantages, but also disadvantages. SvO_2 is a fast reacting parameter, but it may overestimate oxygen delivery during low flow conditions due to centralisation of blood flow (38). Lactate is a more sensitive marker of tissue hypoperfusion but is late reacting and is not ideal for monitoring insufficient blood flow due to its slow elimination (41). Both systemic parameters are global parameters, and therefore they do not provide information about the underlying regional tissue perfusion during low blood flow conditions.

1.7. REGIONAL MONITORING

1.7.1. Near-infrared spectroscopy

Near-infrared spectroscopy (NIRS) is a non-invasive technique based on the transmission and absorption of near-infrared light as it passes through the underlying tissues. The infrared light penetrates tissue in several centimetres depth and is not attenuated by skin, bone, or other organs (48–49). The pioneer Jobsis discovered that metalloproteins (haemoglobin, myoglobin, and cytochrome c oxidase) acts as chromophores that absorb near-infrared light differently based on their concentration and state of oxygenation (oxy- or deoxygenated haemoglobin). Oxy-haemoglobin and deoxy-haemoglobin constitute the largest part of the chromophores (50), whereas the cytochrome c oxidase (a mitochondrial enzyme) only accounts for 2 to 5% of the chromophores. However cytochrome c oxidase is responsible for more than 90% of cellular oxygen consumption because of its ATP production (48–49). The deoxy- and oxy-haemoglobin and cytochrome c oxidase absorb infrared light at different wavelengths: deoxy-haemoglobin 650–1000 nm, oxy-haemoglobin 700–1150, and cytochrome oxidase aa3 at 820–840 nm (49). The NIRS devices use multiple wavelengths and calculate the tissue's optical density at each wavelength based upon the reflected light. The calculation is based on a modification of the Beer-Lambert Law (50–51). The NIRS devices use wavelengths between 700 and 850 nm (INVOS 730 and 810 nm) because the wavelengths of total haemoglobin and oxy-haemoglobin are clearly separated, thereby calculating regional tissue saturation (52). The NIRS light transmission is an elliptical distribution. The mean depth is proportional to the separation of the optodes by a factor of approximately 1/3 (53). If the optode distance is increased, the depth of penetration increases, and the signals from the extra-cerebral tissue decrease, improving the NIRS signal. However, increasing optode distance increases the risk of direct thermal tissue damage (53). The maximal optode distance is 5 cm, resulting in penetration depth of approximately 2 cm (53). To improve the NIRS signal, two receiving optodes can be used. The closer receiver optode (e.g. 3 cm separation) detects the NIRS signal from superficial tissue, whereas the more distant receiver optode (e.g. 4 cm separation) detects the NIRS signal from deeper tissue. A built-in subtraction algorithm enables calculation of the deeper tissue saturation (53). The NIRS signal reflects the oxygen balance in small vessels below 1 mm in diameter, because larger vessels contain large concentration of iron containing heme groups that absorbs all infrared light (52).

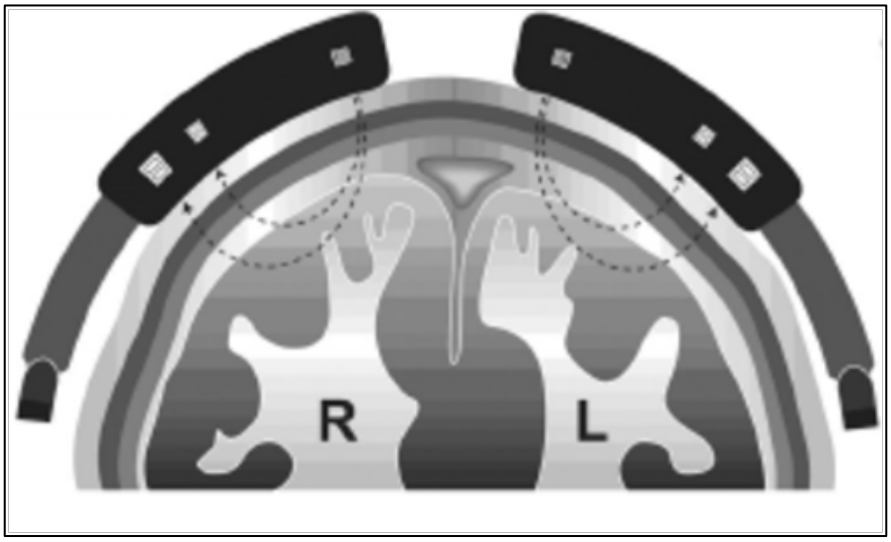

Figure 1. The image demonstrating a schematically cross-section of the brain with NIRS sensor placed over the frontal lobe bilaterally. The NIRS optode transmits infrared light in elliptical distribution through the extracranial tissue and brain tissue. The reflected NIRS signal is collected by the two receiver optodes. The closer receiver detects the NIRS signal from the extra-cranial tissue, whereas the distant receiver detects the NIRS signal from deeper tissue, e.g. brain tissue.

Despite the built-in subtraction algorithm, extra-cranial tissue influences the NIRS signal as demonstrated in a study where three different NIRS devices were tested (54). In healthy persons, a pneumatic head cuff was inflated. Indeed, after 5 min (post-inflation) a decrease in NIRS signal was seen from baseline for INVOS mean decrease 16.6%, SD ± 9.6% ($P = 0.0001$), FORE-SIGHT mean decrease 11.8%, SD ± 5.3% ($P < 0.0001$), and the EQUANOX mean decrease 6.8%, SD ± 6.0% ($P = 0.0025$). The authors concluded that extra-cranial tissue appears to significantly influence the regional cerebral tissue oxygen saturation (rSO_2) measured with NIRS. Other studies have indicated that the use of vasoconstrictive agents influence extra-cerebral tissue and thereby rSO_2 (55–58). Another study has shown that muscle tissue oxygenation saturation (StO_2) is influence by body surface cooling in healthy persons (59). The main mechanism was vasoconstriction, but the study did not allow any conclusion regarding whether skin or muscle tissue is the major contributor to the changes in StO_2. A recent study (60) investigated the effect of normothermic (36.6 C) versus hypothermic CPB (32 C) on the rSO_2 signal in cardiac surgery patients. rSO_2 values were significantly higher during normothermic CPB, but SvO_2 values were lower compared to hypothermic CPB. No explanation could be given. The NIRS signal is also influence by melanin pigmentation found in hair, which weakens the infrared light transmission. This is in contrast to melanin in the skin, which is located in the epidermal layer (50–100 μm) (61). Another concern is that haemoglobin and myoglobin share similar optical properties. Myoglobin may

therefore influence StO_2 values. Myoglobin has a higher affinity for oxygen than haemoglobin; therefore myoglobin will have high oxygen saturation during tissue hypoperfusion and result in StO_2 overestimated (61). NIRS devices also show intra-individual and inter-individual variability in baseline rSO_2 (52). In cerebral and muscle tissue, the average distribution of arterial/venous ratio is 30% arterial and 70% venous (52), but there is a considerable biological variation in arterial/venous ratios between patients. All NIRS monitors have predefined arterial/venous ratios (INVOS 25%/75%) therefore a trend monitoring is more appropriate. Indeed, the different NIRS devices have different solutions to these technological challenges that make it difficult to compare studies. Last but not least, the single-use NIRS sensors are relatively expensive, so cost-benefit analysis may also be considered before routine clinical application (62).

1.7.1.1. Near-infrared spectroscopy of the brain in cardiac surgery

During cardiac surgery rSO_2 sensors are placed on the patient's forehead covering the so-called watershed area (the area receiving blood from the anterior and medial cerebral arteries), thus reflecting the oxygen balance in the grey cortex (63). Murkin et al. (64) conducted a prospective randomised study in which patients were randomised to an intervention group (n = 100), where intraoperative measured rSO_2 was kept above 75% of preoperative baseline values (INVOS) using an intervention protocol, while the non-intervention group underwent blinded rSO_2 monitoring (control, n = 100) without intervention. The stroke incidence was insignificant between the groups, but the patients in the no intervention group died or developed organ morbidity significantly more often than those in the intervention group. The no intervention group had a lower baseline and mean rSO_2 values, and an increased number of episodes of intraoperative cerebral desaturation. Also longer ICU stay and hospital length of stay were demonstrated in the non-intervention group. In another prospective randomised study including 265 coronary artery bypass grafting (CABG) patients (65), the intervention group received active treatment if rSO_2 decrease more than 20% from baseline compared to the non-intervention group. Mean rSO_2 values were 58% and 61% in the intervention and control group, respectively. In the intervention group, rSO_2 was not restored in all patients. The incidence of postoperative cognitive decline was assessed by a neurocognitive and neuropsychological examination but no difference was found. In a post hoc analysis, a prolonged cerebral desaturation time was defined as rSO_2 score greater than 3.000%/second, meaning an rSO_2 value below 50% for 3000 seconds or more. A prolonged cerebral desaturation >3000% seconds was associated with postoperative cognitive decline and extended hospital length of stay. However, because treatment was initiated to reverse desaturation after the rSO_2 threshold had been reached, it may be that potential benefits are obtained from the rapid intervention and reversal of cerebral desaturation. This theory is supported by an animal study (66), and in a prospective observational study of cardiac surgery patient using CPB (67) in which a lowest value of rSO_2 below 35% or the areas under the curve of rSO_2 below 40% for

more than 10 minutes significantly increased the incidence of neurological events determined by neurologic tests. Heringlake M et al. (68) designed a prospective observational study of 1178 patients undergoing cardiac surgery. A low preoperative rSO_2 was significantly correlated with postoperative biomarkers of both cardiac and renal injury, and with preoperative cardiac dysfunction (ejection fraction <30%). rSO_2 was lower in patients who had died by day 30 compared with those who survived, and the preoperative rSO_2 <50% was an independent risk factor for 30-day and 1-year mortality. The authors suggest that preoperative rSO_2 could be a surrogate marker of other organ perfusion, and rSO_2 may be of use as in risk stratification of patients undergoing cardiac surgery.

1.7.1.2. Near-infrared spectroscopy of muscle tissue

StO_2 has been well studied in trauma patients. Crookes et al. (69) conducted a prospective observational study that includes normal human volunteers (n = 707) and patients admitted to a Level I trauma centre (n = 150). Using the In-Spectra tissue spectrometer, they establish the normal ranges of thenar StO_2 (mean 87%, SD ± 6%). In addition, the thenar StO_2 was able to discriminate the no shock patients from patients with severe shock. Likewise, thenar StO_2 values were able to predict those who survived and those who did not survive at an early stage. Indeed, they correlated a 10% decrease in thenar StO_2 to an increase in mortality using an In-Spectra device (70). If the trauma patient was able to maintain StO_2 above 75%, there was a high probability of not developing organ dysfunction or death after severe trauma (71–72). In cardiac surgery, StO_2 has been investigated in the perioperative setting using CPB. In the perioperative period conflicting results exists, as StO_2 does not correlate with global systemic parameters (73–74). However, using CPB, StO_2 declined by 13%, with a delayed increase in lactate and base deficit. Interestingly, the minimum muscle StO_2 value preceded the maximum lactate level by an average time of 94 min, suggesting that StO_2 measured with In-Spectra identifies hypoperfusion much earlier than conventional metabolic markers during CPB (75).

1.7.2. Validation of near-infrared spectroscopy

Regional cerebral tissue oxygen saturation (rSO_2):

Clinical validation and cerebral ischaemic thresholds for rSO_2 are derived from retrospective studies involving patients for carotid endarterectomy (CEA) in which rSO_2 is compared using clinical neurologic examinations (76). During anaesthesia for CEA, 10 of 99 patients developed neurologic symptoms, and they had a significantly greater reduction in rSO_2 from baseline (rSO_2 > 20%). In a larger study using general anaesthesia for CEA, a much smaller reduction of 11.7% from

baseline was identified as predictor of postoperative neurologic symptoms (77). Indeed, both studies demonstrate that a reduction in baseline less than 20% seldom results in neurologic complications, but on the other hand, a decrease below 20% may not necessarily cause neurologic complications (76-77). Today, there is no definition of what is a critical decrease in rSO_2 from baseline that correlated with or leads to postoperative neurologic symptoms after cardiac surgery. However, the most common ischaemic thresholds for rSO_2 during cardiac surgery are obtained from the above-mentioned studies even though anaesthetics and temperature differs in cardiac surgery. A previous study (68) demonstrated rSO_2 values in room air mean 62%; cardiac patients inspired 100% oxygen mean rSO_2 66%; and increasing rSO_2 values compared to preoperative rSO_2 when anaesthetised with total intravenous anaesthesia or sevoflurane (68, 78). During cardiac surgery, the rSO_2 ischaemic threshold is a 20% reduction from baseline or an absolute rSO_2 value below 50% (62). The "normal" cerebral range lies between 60% and 75%, with a variation of approximately 10% for absolute baseline values (79).

Regional muscle tissue oxygen saturation (StO_2):

The StO_2 measured with NIRS has been validated in in vitro studies (80), in animal models (81–82), and in studies in humans. In a human study, muscle oxygenation and venous saturation of the forearm was closely related during exercise, and muscle oxygenation level decreased or increased with an intravascular administration of norepinephrine and nitroprusside, respectively (83). Indeed several studies demonstrate that StO_2 correlates with venous blood in the underlying tissue (84–87), but only if the venous blood sampled represents the tissue region studied by NIRS. StO_2 may also reflect muscle blood flow. Using NIR1000 and recording forearm StO_2 values during different inspired oxygen fraction, the forearm muscle blood flow was calculated from the obtained StO_2 values using complex calculations. The NIRS calculated forearm blood flow was compared with muscle blood flow measured with venous occlusion plethysmography ($R^2 = 0.95$) (87). Furthermore, in a complex study muscle VO_2 measured with NIRS (88) showed a good correlation with forearm muscle blood flow measured by strain-gauge plethysmography ($r = 0.93$).

Multiple peripheral sites for measuring StO_2 are possible, but only the thenar eminence and the forearm have been validated (89). Traditionally, the thenar eminence is used due to the minimal fat layer, minor inter-individual variation, and the lesser risk of systemic oedema. The disadvantage of using the thenar eminence is the difficult fixation of the sensor (89). The forearm is a predominant site of vasoconstriction during low CO, so the vascular response may be earlier and more marked (90–91). Indeed, a previous study demonstrated that the forearm StO_2 is more sensitive than the thenar StO_2 in detecting hypovolemia (92). Alternative measurement sites for StO_2 are pectoral muscle, deltoid muscle, vastus lateralis muscle, and the paravertebral region (93–94). The disadvantage of these sites is that they represent more central measurement sites and therefore they may be less sensitive in detecting vasoconstriction.

1.8. ADVANCED REGIONAL MONITORING

1.8.1. Microdialysis

Microdialysis is designed to mimic a blood capillary and is used for continuous measurement of biomarker concentrations in the extracellular fluid of tissues (95). The measured biomarkers of microdialysis are glucose, pyruvate, lactate, and glycerol (96). As blood flow decreases the regional metabolism changes. Initial glucose delivery to the tissue decreases with decreasing blood flow, and as the cells use the available glucose, the glucose concentration in the interstitial tissue will decrease. During anaerobic metabolism, pyruvate is converted into lactate, shifting the equilibrium towards lactate. The LPR thereby reflects whether metabolism is determined by an aerobic or anaerobic mechanism. During severe anaerobic conditions, the decreasing ATP production and pH decrease, leading to enzyme failure. Finally, degradation of cell membranes leads to a release of phospholipids that are converted into glycerol and free fatty acids, which accumulate in the extracellular tissue fluid. Therefore, increased levels of glycerol measured by microdialysis fluid may indicate cell membrane disintegration due to ischaemia (97-99). Measuring biomarkers concentration may indirectly indicate whether the regional metabolic blood flow is sufficient.

Several limitations must be remembered when microdialysis is used. It has been shown that implantation of a microdialysis catheter can disturb microcirculation, rate of metabolism, or physiological barriers, such as the blood–brain barrier (99). It is important that sufficient time is allowed after insertion of the microdialysis catheter for recovery and establishment of a new steady state. Another important consideration is to distinguish between the absolute recovery and the relative recovery. The absolute recovery is the amount of substance obtained in the dialysate. The relative recovery is the ratio between the concentration of dialysate and the concentration in the tissue. The tissue and dialysate concentrations may be different because the equilibration over the membrane is not complete. The absolute and the relative recovery are inversely related to each other and to the flow rate, as seen in Figure 2.

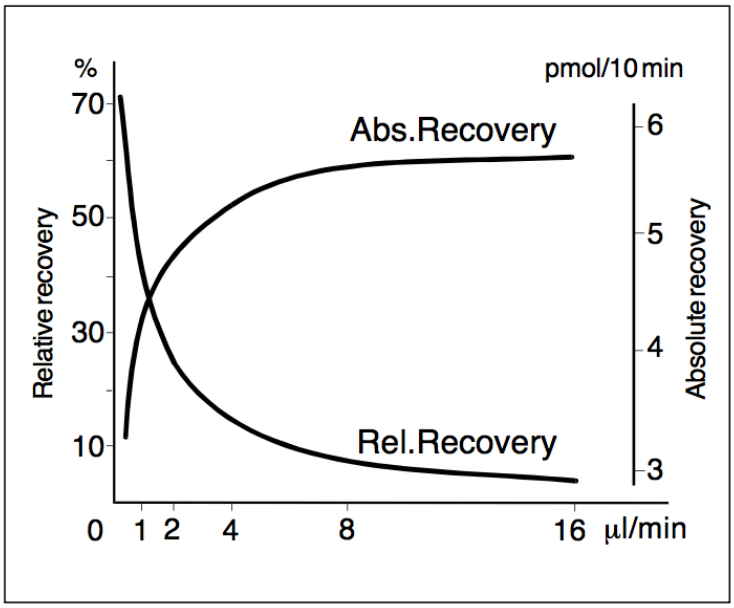

Figure 2. The absolute and relative recovery time as a function of flow rate.

The highest absolute recovery is obtained by high flow rate, but will in addition result in dilution of the dialysate and reduced concentration of the biomarker. The relative recovery is largely dependent on the flow rate: the lower the flow rate, the higher the recovery. However, in practice the flow rate cannot be decreased too much since the sample volume obtained for analysis will be too small. The collected biomarkers contain a mean concentration during a sample interval, and therefore it is the relative changes in concentrations that are relevant (95). Furthermore, the microdialysis results reflect regional metabolism, but with a time delay in proportion to the perfusion rate, length of connecting tubes, and the time applied for analysis of the dialysate, which must be taken into account when interpretation the microdialysis results.

1.8.2. DYNAMIC POSITRON EMISSION TOMOGRAPHY

Dynamic positron emission tomography (PET-CT) scan uses a short-lived radioactive tracer that is inhaled or injected into the blood circulation (100). After an equilibrium period, the injected radioactive tracer becomes concentrated in tissues of interest. During the following scan, a record is made of the tissue concentration as the tracer decays. Thereafter a resulting map is constructed showing the tissues in which the radioactive tracer has become concentrated (Figure 2a). Further image

analysis and kinetic modelling result in calculation of regional tissue perfusion of brain and muscle tissue (Figure 2bc). Limitations to the widespread use of PET-CT arise from the high costs of cyclotrons needed to produce the short-lived radionuclides for PET-CT scanning and the need for a special setup for handling radioisotope preparation. In addition, during PET-CT scanning, the radiation exposure is around 23–26 mSv (101). In comparison, the radiation dosage for chest x-ray is 0.02 mSv and for CT scan of the chest 6.5–8 mSv (102).

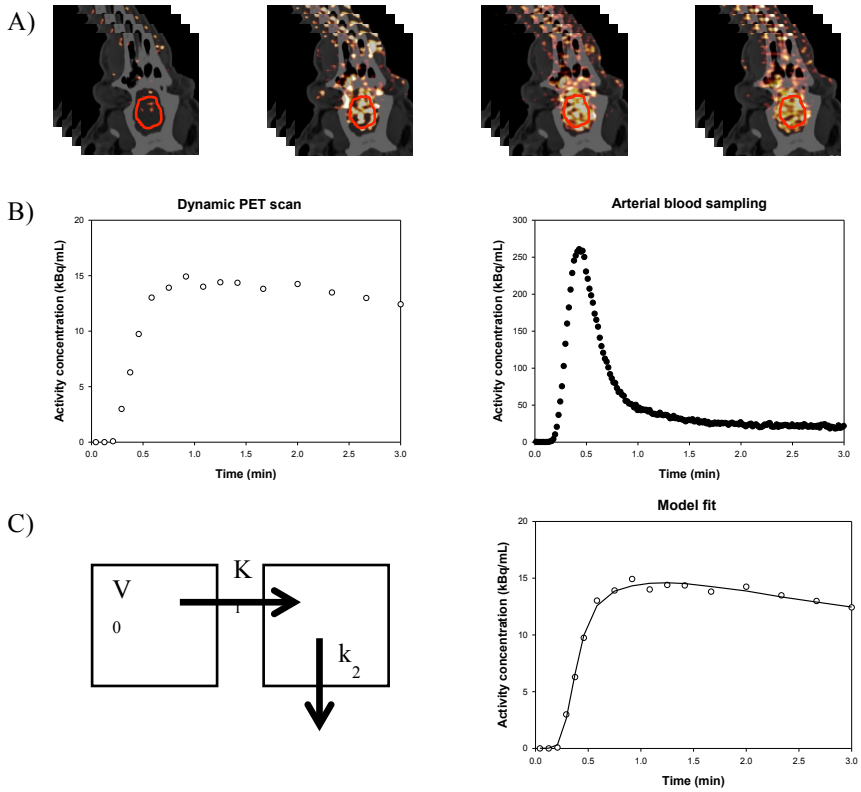

Figure 3. Flow process diagram of handling PET images in study IV. A) Selected slices from the dynamic PET scan following $H_2{}^{15}O$ injection. The volume-of-interest (VOI) is shown in red and is made up of regions drawn in adjacent slices. The four sets of slices correspond to time points t = 0.3 min, 0.6 min, 0.9 min, and 2.3 min. B) Time-activity curves in the brain VOI (dynamic PET) and in arterial blood (automatic blood sampling). C) The one-tissue compartment model with three parameters K_1, k_2 and Vo, where K_1 is an estimate of CBF. The best fit of the model (black solid line) to the brain time-activity curve (open circles) using the arterial time activity curve.

1.8.3. BRAIN OXYGENATION

Oxygen tension in brain tissue ($PbtO_2$) is measured with a Licox microcatheter that uses a polarography technique (a Clark electrode) to measure the oxygen tension. $PbtO_2$ reflects both the perfusion and diffusion characteristics of oxygen in brain tissue (103). $PbtO_2$ is affected by several factors: partial pressure of arterial oxygen (PaO_2), partial pressure of arterial carbon dioxide ($PaCO_2$), CBF, intracerebral pressure (ICP), cerebral perfusion pressure (CPP) and diffusion time, (103) and, haematoma formation and tissue oedema. A normal $PbtO_2$ value obtained in animal studies is in the range of 25 to 30 mmHg (104), and $PbtO_2 < 10$ mmHg has been associated with ischaemic CBF thresholds, and a decrease in jugular venous saturation below the critical threshold of 50% correlated with a brain $PbtO_2$ of 8.5 mmHg (105).

CHAPTER 2. OVERALL AIM AND HYPOTHESIS

The overall aim of the PhD thesis was to investigate tissue metabolism of the brain and muscle using cerebral NIRS (human study) and muscle NIRS (experimental study) and evaluate the measurement with more advanced regional monitoring methods, i.e. microdialysis and PET-CT during low blood flows using CPB, thereby gaining insight into the extreme ends of the organ hierarchy during hypoperfusion.

2.1. HYPOTHESES

Study I

We hypothesised that cerebral perfusion is preserved during CPB at blood flows equalling the cardiac index measured prior to CPB evaluated by cerebral NIRS during CPB.

Study II

We hypothesised that StO_2 measured with NIRS 1) is a fast-reacting indirect indicator of global hypoperfusion during CPB and 2) follows the changes in systemic acid/base balance and in metabolism in remote organs.

Study III

We hypothesised that changes in muscle tissue perfusion measured with PET-CT would be reflected by parallel changes in StO_2 measured by NIRS.

Study IV

We hypothesised that CPB produced blood flow is the main determinant of cerebral perfusion compared to mean arterial blood pressure for cerebral perfusion during normothermic and normocapnic low flow CPB.

CHAPTER 3. MATERIALS AND METHODS

3.1. STUDY DESIGN

Study I design

Twenty-two adult patients scheduled for elective cardiac surgery were included. All cardiac surgery patients had a left ventricular ejection fraction > 50%, no history of cerebral insult, head trauma, or verified carotid artery stenosis. A randomised crossover study was performed, where blood flow during normothermic CPB for 20 minutes was based on either the calculated flow (2.4 L/min/m^2) or the individual thermodiluted measured CI prior to CPB, and then a switch to the alternative blood flow for another 20 minutes. After the last study period, the CPB blood flow was adjusted to the standardised 2.4 L/min/m^2. During the two different blood flows, systemic parameters and bilateral rSO_2 were measured.

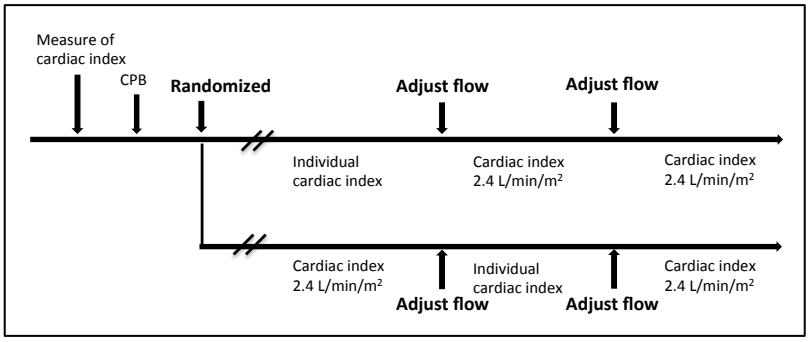

Figure 4. The flow chart in study I.

Study II design

Twelve pigs (80 kg) were anesthetised and normothermic CPB was established at 60 mL/kg/min, thereafter blood flow was reduced to either 47.5 mL/kg/min (Group I) or 35 mL/kg/min (Group II) for another hour and finally returned to a blood flow of 60 mL/kg/min for 1 hour. StO_2 and microdialysis were performed in the musculus gracilis of the non-cannulated leg, and microdialysis was performed in intestinal tissue and brain tissue in addition to Licox measurements of the brain at each blood flow.

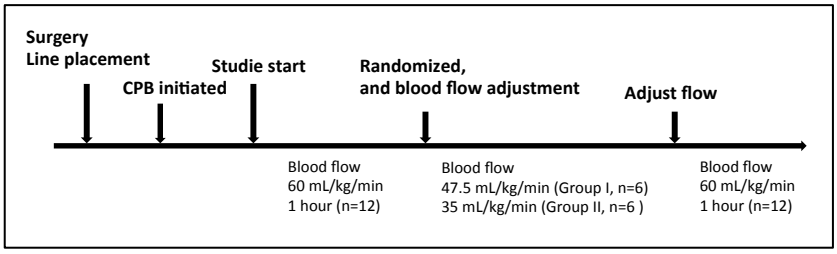

Figure 5. Flow chart in study II.

Studies III and IV design

Eight pigs (69–71 kg) were anesthetised and baseline PET-CT was performed. The animals were connected to normothermic CPB with a blood flow of 60 mL/kg/min for 1 hour, thereafter blood flow reduction to either 47.5 mL/kg/min (Group I) or 35 mL/kg/min (Group II) for another hour and finally returned to a blood flow of 60 mL/kg/min for 1 hour. PET with ^{15}O-labelled water was performed to evaluate the blood perfusion in muscle tissue (study III) and cerebrum (study IV) during each blood flow.

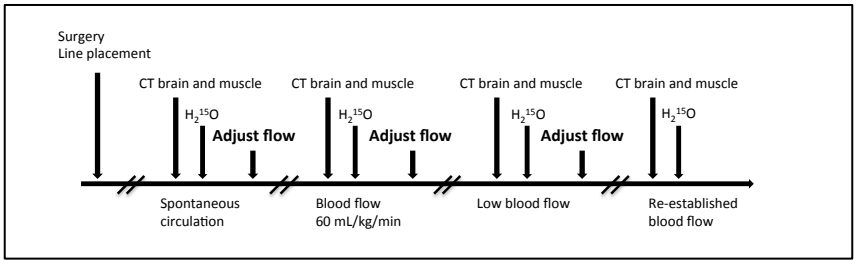

Figure 6. Flow chart in studies III and IV.

3.2. MONITORING METHOD

In the four studies, systemic and different regional measurements were used to evaluate tissue perfusion, oxygenation, and metabolism during normal (study I) and low CPB blood flows (studies II–IV), with emphasis on cerebral and muscle tissue.

3.2.1. MONITORING METHODS STUDY I

Systemic oxygen consumption was estimated as arterial and venous blood samples and withdrawn every 5 minutes during the 20 minutes of each study period and analysed. Mean arterial blood pressure (MAP), SvO_2, and CI were recorded at the same time. The regional rSO_2 was measured with NIRS (INVOS 4100) continuously using sensors placed bilaterally on the patient's forehead prior to the induction of general anaesthesia. Cerebral oximeter readings were recorded and stored.

3.2.2. MONITORING METHODS STUDY II

The muscle tissue oxygen balance was estimated by continuously StO_2 measurements with NIRS (INVOS 5100). The sensor was placed on the skin above the musculus gracilis of the non-cannulated back leg. In addition, a microdialysis catheter (CMA 60, CMA Microdialysis AB, Solna, Sweden) was inserted into the musculus gracilis through a skin incision next to the NIRS sensor. Tissue perfusion was performed with a physiologic solution (CMA perfusion fluid) at a pump flow rate of 1.0 μL/min (CMA 102, CMA Microdialysis AB, Solna, Sweden). The muscle tissue CMA60 catheter has an inner diameter of 15 mm, a 20 mm shaft, and a 105 mm outlet tube. The calculated dead space is 0.18 μl per cm outlet tube. No adjustment for time delay was done, as the dead space time of 2.25 minutes was considered low compared to the total sampling time (20 minutes).

Systemic parameters (arterial and venous blood samples), regional brain microdialysis and $PbtO_2$, and regional microdialysis of intestinal colon were used to evaluate the level of ischaemia during the two low blood flows. A microdialysis catheter was inserted into the brain (CMA 70 brain, CMA Microdialysis AB, Solna, Sweden) and the intestinal tissue of the colon thorough a drill hole and mini-laparotomy, respectively. One hour of dialysis was performed to obtain a steady state in all tissues, and the dialysates were discarded. During the study period, dialysates were collected in vials every 20 minutes. Vials were maintained at 4ºC during the study period and finally stored at -80ºC until analysed for glucose, lactate, pyruvate, and glycerol (CMA 600 Microdialysis Analyser, CMA Microdialysis AB, Solna, Sweden). No adjustment for time delay was done during brain and intestinal sampling. The CMA microdialysis catheter for the brain was specified with a cutoff

at 20,000 Daltons and a membrane length 10 mm, and the CMA microdialysis catheters for the intestinal mucosa and muscle tissue were specified with a cutoff at 20,000 Daltons and a membrane length 30 mm with a flow rate of 1.0 μl/min. In our study, the relative recovery was not determined.

Cerebral NIRS could not be measured due to the thickness of the porcine skull of > 4 cm thick. Instead, a Licox microcatheter was inserted through a drill hole, and $PbtO_2$ and temperature were recorded every 20 minutes (Licox CMP Monitoring System Integra Neurosciences, Plainsboro, NJ, USA).

3.2.3. MONITORING METHODS IN STUDY III AND STUDY IV

In studies III and IV, PET was performed by a Siemens Biograph 64 Truepoint PET/CT camera (Siemens AG, Erlangen, Germany) following a PET/CT scan protocol which consisted of: 1) a topogram, 2) a CT scan, and 3) a 5-min PET list-mode scan after 500 MBq $H_2{}^{15}O$ intravenous injection. After each PET scan, a period of at least 6 half-lives (12 min) was allowed for the previous tracer to decay. During the PET acquisition, arterial blood was sampled repeatedly, and radioactivity was measured every 0.5 s by use of an automatic blood sampler connected to the right carotid artery branch (Allogg AB, Mariefred, Sweden). Dynamic PET data and arterial blood data were corrected for radioactive decay from the start of the tracer administration. Using fused PET/CT images, regions-of-interest (ROIs) were drawn in muscle tissue (study III) and brain (study IV). For each dynamic scan ($H_2{}^{15}O$), ROIs were defined in adjacent transaxial slices of an image of the mean radioactivity concentration. The NIRS sensor was identified at the muscle surface, and ROIs were draw precisely beneath the sensor at levels 1–2 cm, 2–4 cm, and 4–6 cm (study III). Likewise ROIs were drawn of the brain with no differentiation between the grey and the white substance (study IV). The ROIs were summed to a volume-of-interest (VOI) from which the time course of muscle tissue (study III) and brain (study IV) concentration of $H_2{}^{15}O$ was generated. Tissue blood perfusion was estimated by fitting the one-tissue compartment model to the muscle time-activity curve (dynamic PET) using the arterial input function (blood samples). In this way, robust fits were obtained using three parameters (blood perfusion, blood volume, and time delay) and fitting only the early 2 minutes of PET data. The non-linear regressions were made using in-house developed software iFit (www.liver.dk/ifit.html).

The systemic oxygen consumption was estimated by arterial and venous blood samples withdrawn every 15 minutes. In study III, StO_2 was measured with NIRS (INVOS 5100) continuously using sensors placed on the skin above the musculus gracilis of the non-cannulated back leg. In study IV, rSO_2 could not be measured due to the thickness of the porcine skull, i.e. > 4 cm thick.

3.3. STATISTICS

In study I, a linear mixed effects model (LME) that included fixed and random effect analysis was used to examine the change of biomarkers over time in the two treatment groups (calculated cardiac index group and individual thermodiluted measured cardiac index group). All data were tested for normal distribution. Non-normal distributed data were log-transformed to achieve normality before further statistical analysis. Parametric statistics were used for normally distributed data. Results shown as mean +/- SD. P-values less than 0.05 were considered statistically significant. The model was implemented and analysed with statistical software package R, Version 2.9.1[8] (using the contributed package nime for mixed effects modelling).

In studies II-IV, a non-parametric statistical method was used. Values were giving as median (25–75 percentiles), and each pig served as its own control.

In study II, the Wilcoxon-Mann Whitney test was used to compare the baseline values between the groups. Friedman test was performed for each parameter at 60 minutes during each period in the two groups to estimate a significant level from baseline during the different blood flows.

In study III, the relationship between StO_2 and muscle blood flow was described by nonlinear regression using the Global Curve Fitting (Sigma plot 12.5, Systat Software Inc., San Jose, CA, USA) and the R^2 was reported.

In study IV, individual cerebral tissue perfusion and MAP, haemoglobin, and $PaCO_2$ values were compared during the course of the experiment using Spearman's correlation analysis.

Statistical analyses were performed using Stata® (version 14.0), and the level of significance was set at $p < 0.05$.

CHAPTER 4. SUMMARY OF RESULTS

4.1. STUDY I

Mean individual blood flow (measured prior to the CPB) versus the calculated blood flow was 2.1 L/min/m^2 and 2.4 L/min/m^2 (Figure 7), respectively. In both study periods, the SvO_2 was >60 %, base excess 3–4 mmol/L and lactate less than 2.0 mmol/L. There were no differences between the groups regarding CI, MAP, rSO_2left, rSO_2right, or SvO_2.

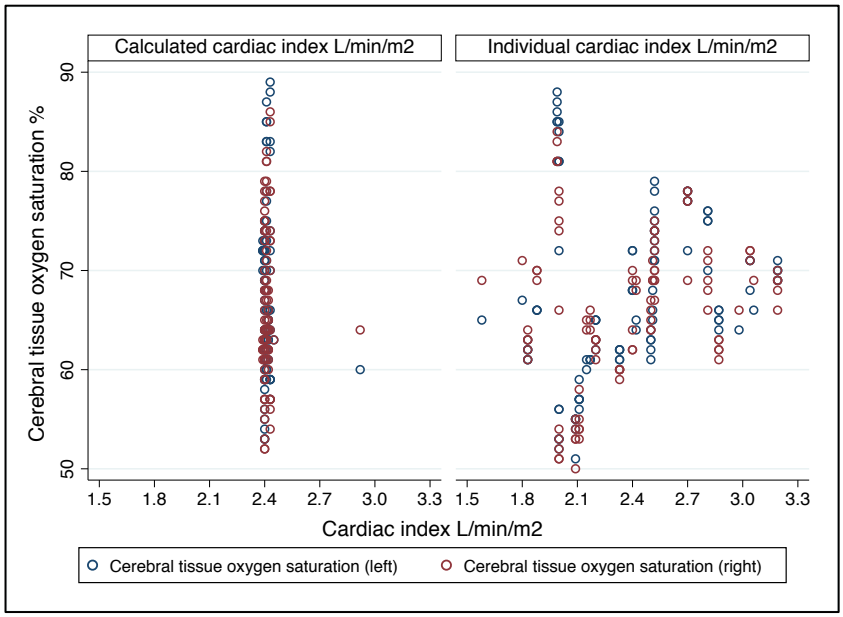

Figure 7. The two figures show rSO_2 values measured with NIRS at the y-axis during calculated cardiac index of 2.4 L/min/m^2 (left figure) and individual cardiac index (right figure).

4.2. STUDY II

The systemic parameters demonstrated systemic ischaemia with a significant decrease in SvO_2 (Group I: p =0.02, Group II: p =0.01) and an increase in serum lactate in Group II (p =0.01) during low blood flow. The intergroup differences were also significant (SvO_2: p =0.02, lactate: p =0.01). However, in Group II, SvO_2

increased and was almost normalised during reperfusion in contrast to lactate (Figure 8).

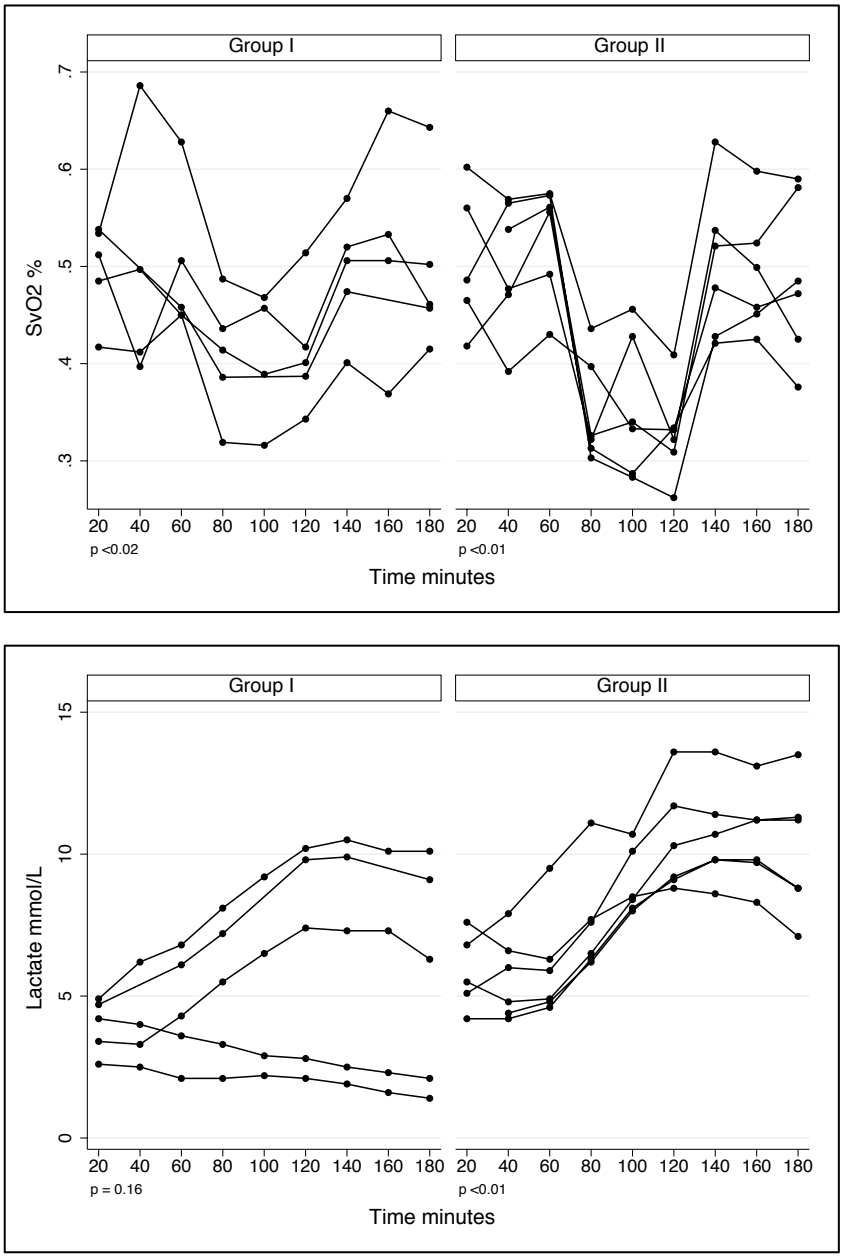

Figure 8. Time course of SvO_2 and lactate measured every 20 minutes at each blood flow. Blood flow of 60 mL/kg/min^2 from 0–60 minutes, low blood flow from 80–

120 minutes, and re-established blood flow of 60 mL/kg/min^2 from 140–180 minutes.

The StO_2 showed only minor changes in Group I (p =0.06), but in Group II a significant decrease (p =0.01) during the low flow period was seen (Figure 9). During reperfusion, StO_2 increased; however, the increase was less pronounced in Group II. The decrease in StO_2 occurred rapidly within the first 5 minutes, as seen in Figure 10. A significant correlation was seen between SvO_2 and StO_2 (Spearman; r: 0.29, p <0.005) and between lactate and StO_2 (Spearman; r: -0.35, p <0.001).

Changes in the metabolites in muscle tissue corresponded with muscle StO_2 values; In Group II significant changes were seen for muscle pyruvate and muscle glycerol, as no changes occurred in Group I. No correlation could be demonstrated between blood lactate and LPR of muscle. Likewise, changes in intestinal and brain tissue metabolites paralled muscle StO_2. However, $PbtO_2$ did not change significantly.

One pig (Group I) was excluded due to severe blood loss during preparation for CPB, and the StO_2 values were missing in one pig (Group I) due to technical problems wit h the equipment.

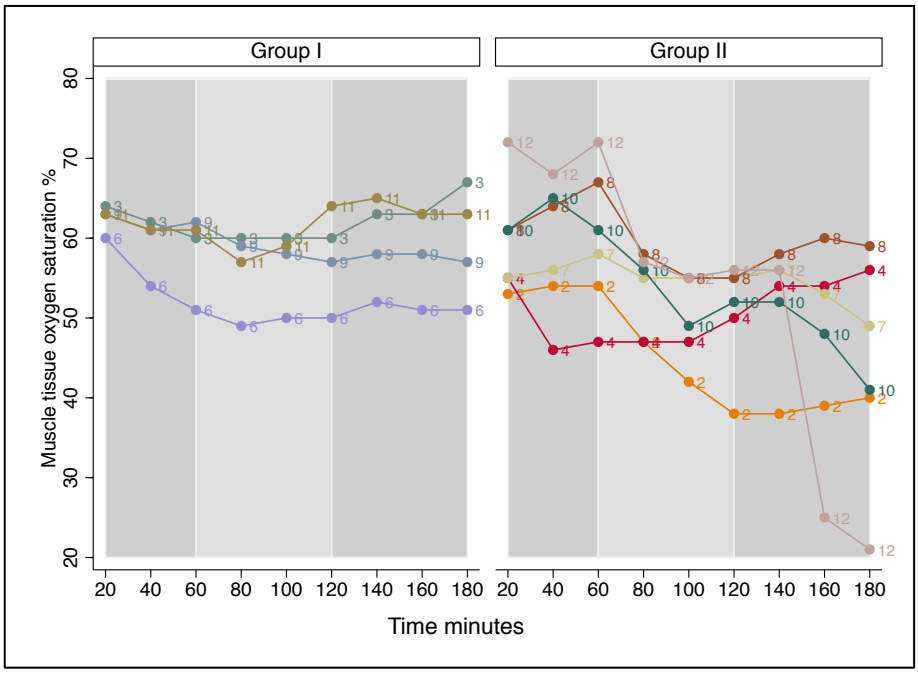

Figure 9. The two figures show the individual StO_2 Group I (47.5 mL/kg/min (left figure)) and Group II (35 mL/kg/min (right figure)) during the time course of the study. The dark grey zone resembles the blood flow of 60 mL/kg/min and re-

established blood flow of 60 mL/kg/min, and the light grey zone resembles low flow.

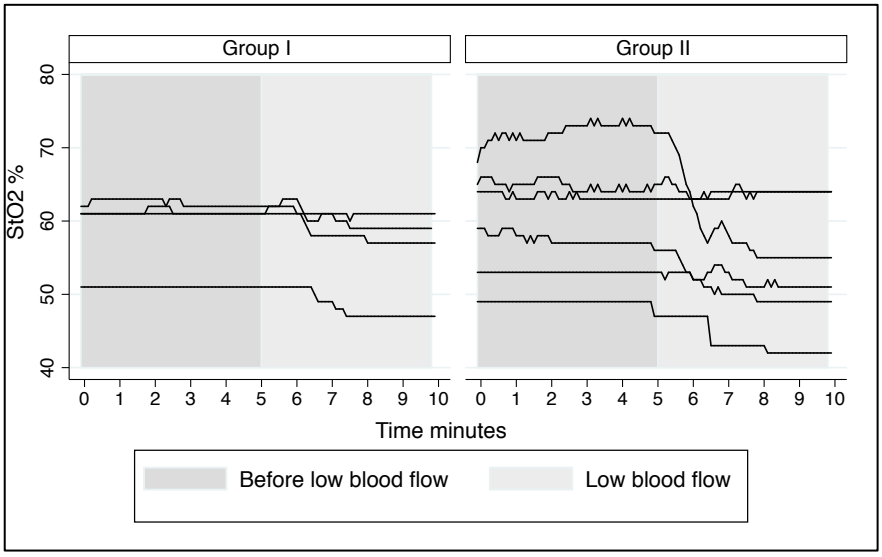

Figure 10. The two figures show the individual StO_2 values in percentage at the y-axis 5 minutes before blood flow reduction to low blood flow and 5 minutes after initiating low blood. Left figure Group I (47.5 mL/kg/min) and right figure Group II (35 mL/kg/min).

4.3. STUDY III

Systemic ischaemia was demonstrated during the two low flow periods. Muscle tissue perfusion was measured at three different depths, 1–2 cm; 2–4 cm, and 4–6 cm, in the hind limb skeletal muscle. Accordingly the StO_2 was measured over the same region. Muscle tissue perfusion was high before initiating CPB but decreased during CPB according to the CPB blood flow (Figure 11). Indeed, StO_2 remained high until the muscle tissue perfusion had decreased to about 50%, after which StO_2 almost linearly followed the fall in muscle tissue perfusion. There was an excellent non-linear relationship between the normalised values for muscle tissue perfusion and StO_2, with an R^2 of 0.85 (p <0.00001) (Figure 12).

In studies III and IV, two pigs had complications; 1) One animal due to severe bleeding from a nasal temperature probe (#4); 2) one animal due to technical problems with dynamic PET (#5).

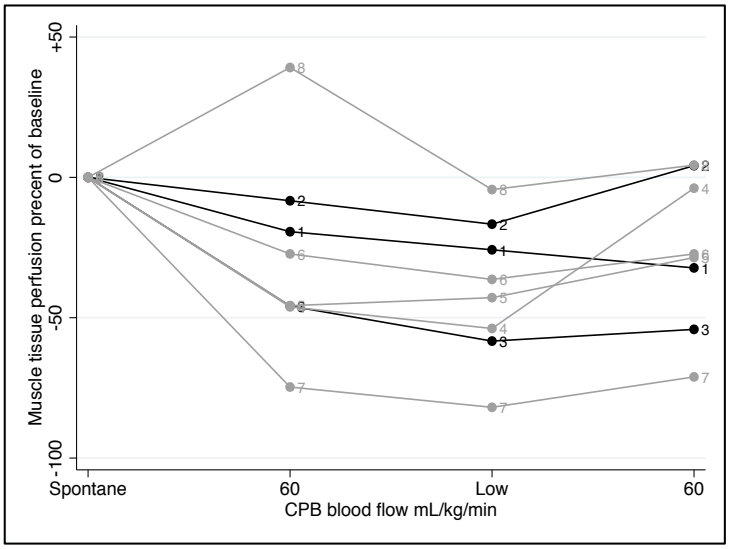

Figure 11. Muscle tissue perfusion in % of baseline (spontaneous circulation) during the different CPB blood flows. Pigs 1–3 (low blood flow 35 mL/kg/min); pig 4 prolonged low flow (47.5 mL/kg/min); pig 5 profuse bleeding from the nose (47.5 mL/kg/min); and pigs 6–8 (low blood flow 47.5 mL/kg/min).

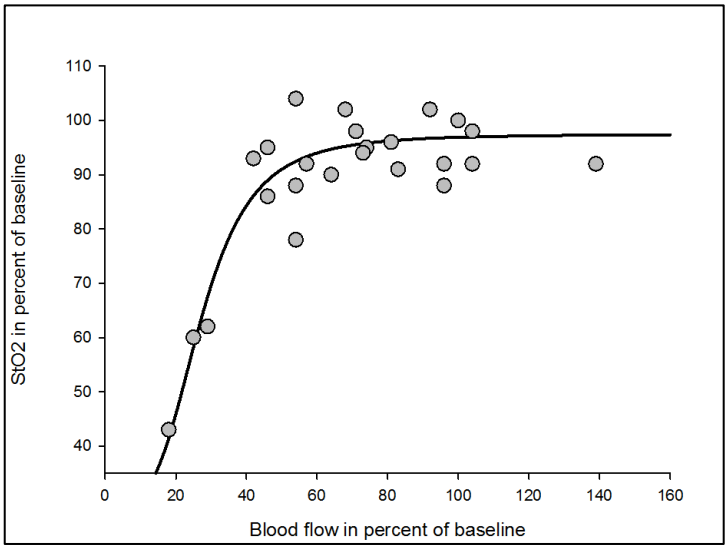

Figure 12. The relationship between normalised muscle blood perfusion obtained by PET and StO_2 obtained by NIRS. The line depicts the best fit.

4.4. STUDY IV

Initiating blood flow of 60 ml/kg/min from spontaneous circulation increased CBF (Figure 13). Furthermore, CBF and CPB pump flow were positively related (R^2 = 0.37), while CBF and MAP showed an inverse relationship (R^2 = 0.30). No relation between CBF and haemoglobin (R^2 = 0.01) or CBF and $PaCO_2$ (R^2 = 0.03) was found. rSO_2 was not measured due to the thickness of the pig's skull.

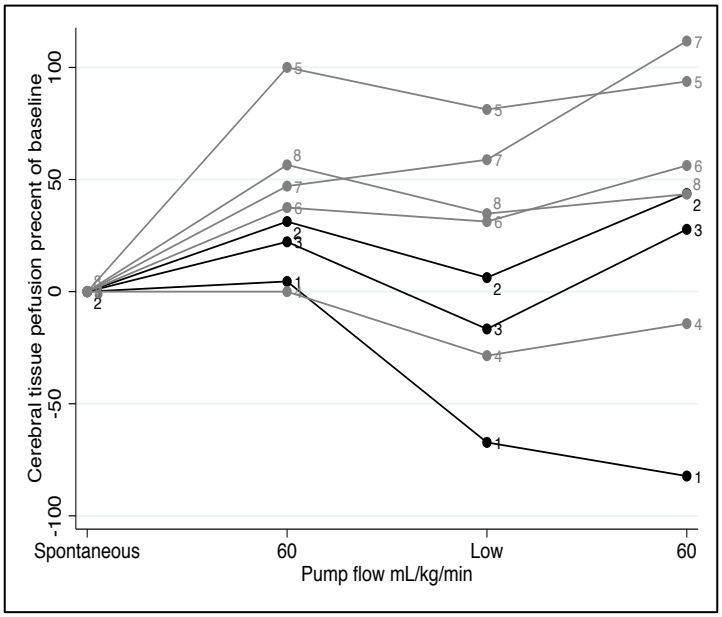

Figure 13. CBF in % of baseline (spontaneous circulation) during the different CPB blood flows. Pigs 1–3 (low blood flow 35 mL/kg/min); pig 4 prolonged low flow (47.5 mL/kg/min); pig 5 profuse bleeding from the nose (47.5 mL/kg/min); and pigs 6–8 (low blood flow 47.5 mL/kg/min).

CHAPTER 5. DISCUSSION

In clinical practice, systemic measurements of haemodynamics are used as a surrogate marker to assess regional perfusion and hence tissue oxygenation and metabolism. The main disadvantage of systemic haemodynamic measurements is that they are global assessments, and require invasive techniques (38, 41) therefore time-consuming for early detection of shock states (106). Measurement of regional tissue perfusion, oxygenation balance, or metabolism has several advantages. In early stages of shock, blood flow is recruited from peripheral tissues (muscle tissue) to vital organs (brain tissue) (6, 8), therefore monitoring perfusion in peripheral tissues could be an early marker of systemic hypoperfusion (90–91). Furthermore, peripheral tissues are easy to monitor using non-invasive techniques thereby facilitating earlier initiation of monitoring (106).

The overall aim of the PhD thesis was to investigate tissue metabolism of brain and muscle using cerebral NIRS (human study) and muscle NIRS (experimental study) evaluated by more advanced techniques, thereby studying both extremes (brain and muscle tissue) of of the well-defined organ hierarchy during low CPB blood flows. The main findings were that (1) during short period of CPB, a cardiac index of 1.9–3.1 L/min/m^2 is sufficient to sustain cerebral oxygenation measured with cerebral NIRS in cardiac surgery patients; (2) during CPB mediated inadequate systemic circulation StO_2 decreased and indicated insufficient global tissue perfusion even when systemic circulation was restored after a period of systemic hypoperfusion in an experimental animal model; (3) during CPB mediated inadequate systemic circulation StO_2 remained high until muscle blood flow had decreased to about 50%, after which StO_2 almost linearly followed the fall in muscle blood flow in an experimental animal model; (4) and during normocapnic and normothermic CPB, pump flow is a main determinant of cerebral perfusion compared to MAP during low blood flow in an experimental animal model.

5.1. STUDY I

Cardiac surgery patients are characterised by high co-morbidity (24-27), age (22-23), and weight (20-21). Consequently it is increasingly important to tailor optimal treatment for the individual patient (37). As early as 1959, Gollan et al. suggested SvO_2 as an individual guidance for blood flow requirements during CPB (107). Indeed, a recent study used SvO_2 to tailor individual therapy and showed that keeping SvO_2 above 75% led to a better preserved DO_2 than BSA based blood flow (108). In study I, the BSA-based blood flow was compared to an individualised CI (patient's own measured CI prior to CPB) showing that the blood flow during normothermic CPB is safe at a wider range than the recommended (3) blood flow of 2.2–2.5 L/min/m^2. In fact, the present study showed that during elective heart

surgery patients with a normal LVEF, an individual CPB flow of 1.9 L/min/m^2 to 3.1 L/min/m^2 resulted in cerebral and global oxygenation similar to that of CPB flow based on BSA. Only a few studies have challenged BSA calculated blood flow (20, 21,108). However, in hypothermic cardiac surgery patients (109), an increase in blood flow from 2.5 L/min/m^2 to 3.0 or a decrease from 2.5 L/min/m^2 to 2.0 L/min/m^2 led to an increase or a decrease in rSO_2, respectively. Restoring MAP with phenylephrine made rSO_2 decrease even further at a blood flow 2.0 L/min/m^2 (109). Comparing the later study (109) with our study is difficult due to different study designs. In our study, we compared calculated blood flow with individual blood flow, so some cardiac surgery patients were above and others below the calculated blood flow during the individual blood flow period. Furthermore, in our study I the rSO_2 values were compared over 20-minute periods, whereas in the later study (109), rSO_2 was measured 5 minutes after blood flow change. Other factors that might have caused the conflicting results are the use of hypothermic CPB, as hypothermia is known to cause vasoplegia and to influence CBF (110). In addition, hypothermia also induces vasoconstriction of the skin that might influence the rSO_2 values (110). Furthermore, when phenylephrine was used to correct MAP, a decreased in rSO_2 value was seen (109). One explanation might be that phenylephrine caused extra-cerebral vasoconstriction as demonstrated in previous studies (55-58). In our first study, we challenged the organ hierarchy by using individualised CI and demonstrated that rSO_2 was reserved in all cardiac surgery patients. However, we did not monitor StO_2, and therefore we do not know whether the preserved rSO_2 was caused by recruitment of blood from the peripheral muscle tissue.

5.2. STUDY II

During low cardiac output states, a vascular response is initiated though a central mechanism that acts via baroreceptors and chemoreceptors to maintain blood volume, causing vasoconstriction in peripheral tissues and thereby markedly reducing muscular perfusion in haemodynamic shock (8). This vascular response implies that muscle is one of first tissues where perfusion is down-regulated during insufficient systemic circulation, and the last one where perfusion is re-established after adequate systemic circulation has been restored (8). Indeed we found that StO_2 was a fast-reacting parameter that decreased during low blood flow and reflected insufficient muscle tissue oxygenation in Group II (35 mL/kg/min) in accordance with the ischaemic metabolic changes detected by microdialysis in muscular tissue. Our finding is supported by a previous animal haemorrhage model (111) in which StO_2 of the right hind limb was compared to systemic parameter during resuscitation. The main conclusions were that StO_2 measured in the hind limb was reliable as an index of severity of shock and resuscitation as systemic parameters. Although the studies have different setups and causes of low blood flow, our CPB model also simulates severe shock at the lowest level of blood flow (35 ml/kg/min that equals a reduction >50 % of blood flow). However, we also observed a rapid

decrease in StO_2 in Group I (47 mL/kg/min), but the decrease was insignificant and may be explained by the small number of animals in group I. In our study, there was a correlation between systemic metabolism (SvO_2 and lactate) and StO_2. On the contrary, we could not show a correlation between the regional measured metabolomics (LPR muscle, gut, or brain) and StO_2 (a surrogate marker of global hypoperfusion) or the regional measured metabolomics (LPR muscle, gut, or brain) and lactate. However, several studies have shown that the correlation between systemic parameters and peripheral microcirculation is difficult to prove (112-114). Oxygen transport to the cells is determined by 1) convective flow, i.e. the transport of red blood cells to capillary compartment and 2) a passive diffusion, the oxygen diffusion from red blood cells to the mitochondria for ATP production. During resuscitation the convective flow is optimised, but it may not lead to an optimisation of the passive diffusion of oxygen (114-115). Indeed in our study, we were only able to demonstrate a correlation between systemic metabolism and the decreasing StO_2 (convective level), but not with the extracellular metabolic changes measured with microdialysis (diffusion level), which reflects intracellular concentrations of biomarkers that easily diffuse across cell membranes, such as lactate and pyruvate. Indeed, in our study, one of the challenges is in terms of defining tissue ischaemia using microdialysis, as systemic lactate levels are increased in our low flow animal model. Thresholds are known for LPR of brain (116) and muscle tissue (117), but no validated LPR threshold of colon ischaemia has been described. However, systemic lactate levels may influence luminal lactate concentration when serum lactate exceeds 5 mmol/l (118). Therefore, systemic lactate values in the present study may have affected measured tissue lactate. In study II, we challenged the organ hierarchy by construction of a low blood flow porcine model, where we demonstrated that StO_2 was a fast-reacting parameter, but we were unable to demonstrate a correlation between StO_2 and the muscle tissue metabolomics.

5.3. STUDY III

Muscle tissue has a low resting metabolic rate and has a high tolerance to ischaemia (6). The NIRS monitor primarily reflects the oxygen balance of regional tissue (52). As blood flow is reduced, muscle tissue DO_2 falls; to counteract the decrease in DO_2, an increased extraction is seen, but if the compensatory mechanisms are exceeded, the muscle tissue VO_2 will fall and the DO_2-VO_2 relationship may become linear. Due to the low metabolic rate of muscle tissue, the VO_2-DO_2 relationship may initially be linear when muscle blood flow is severely reduced if haemoglobin concentration and oxygen content are stable. In study III, we found that during inadequate systemic circulation, StO_2 remained high until muscle blood flow had decreased to about 50%, after which StO_2 almost linearly followed the fall in muscle blood flow. Our result may explain the conflicting results regarding the ability of NIRS to detect blood loss and hypovolemia in the perioperative setting. In a previous study (119), a 500-ml blood loss during blood donation did not lead to changes in StO_2 variables in awake volunteers. This is in contrast to a study using

lower body negative pressure technique to mimic central hypovolemia, where StO_2 did decrease in awake volunteers (92). From the perspective of our study, it seems that a rather dramatic systemic blood flow decrease is needed before StO_2 decreases. Indeed, a compensatory mechanism like that shown in a recent study (120) where a haematocrit less than 0.22 resulted in an unchanged StO_2 due to an increased regional blood flow may also be a part of the explanation. Another important issue when StO_2 is used to detect hypovolemia is the application site and the sensors measuring depth. The underlying muscle groups have a variable sensitivity with regard to detecting central hypovolemia (92), and the sensors measuring depth are likewise important as they determine the influence from non-muscular tissue on the StO_2 value (54, 90-91). In animal research, the measuring sites and depth of muscle tissue are poorly validated, although it has been proposed that the muscle of the hind limb in pigs reflects the severity of shock and resuscitation in a trauma porcine model (70). We estimated the muscle tissue perfusion in three different depths 1–2 cm, 2–4 cm, and 4–6 cm in the musculus gracilis of the non-cannulated back leg and found that muscle tissue perfusion showed the same trends at the three depths. This is in accordance with a previous human study (92). In study III, we demonstrated that StO_2 first decreased when muscle tissue perfusion was decreased by 50%. Consequently StO_2 may not be the optimal marker of peripheral hypoperfusion.

5.4. STUDY IV

In major cardiac surgery neurologic complications remain high (121-122). Indeed, after CABG 1.6–5% of patients experience a stroke (121) and cognitive decline is even more common, although it decreases to 10–20% of the patients after 1 year (123). It is well described that the brain's oxygen supply is maintained by a stable CBF governed by cerebral pressure-flow autoregulation as long as MAP is within the range of 50 to 150 mmHg (33, 123). Likewise it has been demonstrated that CBF is independent of MAP evaluated by a static methodology, the ^{133}Xenon clearance technique during CPB (7, 123). However, a more recent study using transcranial Doppler demonstrated that CBF is CPP pressure dependent during CPB. In his study, the CPP varied between 30 and 70 mmHg, while the CBF velocity was measured with transcranial Doppler. A positive slope with increasing CPP was demonstrated that likely reflects similar slope changes in CBF (124). Our study II points in the same direction because the cerebral metabolism was decreased when CPB blood flow was reduced to >50%, indicating that CPB blood flow might be as important as MAP.

Indeed in the experimental study IV, we found that CPB blood flow was a main determinant of CBF measured by PET-CT during low blood flow. Furthermore, high MAP did not protect against cerebral hypoperfusion. This corresponds with the findings of Soma et al., concluding that MAP does not govern CBF during hypothermic CPB and a blood flow above 40 mL/kg/min is enough to achieve an adequate cerebral perfusion (125). However, Soma et al. (125) used hypothermia,

which reduces the metabolic rate, causing decreased VO_2 demands, and thereby allowing a lower CPB blood flow to adequately meet cerebral perfusion demands (126). Indeed, conflicting results exists with regard to what constitutes sufficient CBF to meet cerebral metabolism when normothermic CPB blood flow is used in experimental models. During normothermia CPB, CBF is preserved using blood flows of 80–100 mL/kg/min (6, 127–129). However, reducing normothermic CPB blood flow to 70–50 mL/kg/min for 2 hours led to increasing cerebral metabolic derangement (130). Using nearly the same CPB blood flow (47.5 mL/kg/min) for 1-hour, cerebral metabolism was preserved in our study II. Similarly, O'Dwyer et al. using radioactive microspheres also showed that CBF is preserved at a normothermic CPB blood flow of 50 mL/kg/min (127). Furthermore Boston et al. showed preserved cerebral blood flow over wider range of CPB blood flows of 1.7–2.3 mL/min/m^2, corresponding to 33–45 mL/kg/min (6).

The use of different 1) animal models, 2) CPB blood flows, and the different 3) cerebral monitoring devices makes comparison very difficult. Indeed, in our study IV we found that going from pulsatile to non-pulsatile blood flow of 60 mL/kg/min led to an increased CBF. This is in contrast to a lamb study where initiating a CPB blood flow of 150 mL/kg did not increase CBF (131). Animals have lower brain size in relation to body weight (132) compared to humans and therefore the normal relative flow to the brain is higher in humans than in all other species. Thus, a CPB blood flow of 60 mL/kg/min in our pigs might produce a luxury perfusion of the brain. However we did not collect rSO_2 values due to the thickness of the pig's skull, so luxury brain oxygenation cannot be confirmed.

CHAPTER 6. ADVANTAGES AND LIMITATIONS

6.1. STUDY DESIGN

In study I, the crossover design was strong, as patients were their own control. However, the study included only cardiac surgery patients with a normal ejection fraction, and individual CI may not be sufficient in cardiac surgery patients with a low ejection fraction because their ability to compensate this is reduced.

In studies II–IV, we chose to randomise CPB to two lower blood flows, and return to the initial blood flow of 60 mL/kg/min, thereby permitting the pig to be its own control. One concern is that the initial blood flow of 60 mL/kg/min could be viewed to be in the lower end for pigs weighing 80 kg. However, the literature is very sparse concerning optimal or normal CPB blood flow rates for pigs, but most studies consider a CPB blood flow of 70–100 mL/kg/min to be optimal in porcine models (133), and therefore we chose a blood flow of 60 mL/kg/min to prevent hyperperfusion in our porcine model. Processing the results of the study II, the initial blood flow of 60 mL/kg/min resulted in SvO_2 values of 50–60%, but it should be pointed out that 60 mL/kg/min is in the lower end of a normal optimal blood flow. In our studies III and IV using the same blood flow of 60 mL/kg/min, we found median values of SvO_2 to be 57% and median lactate values to be 1.1 mmol/L.

Indeed, the study design itself can be discussed. Our aim was to construct a porcine model to test whether StO_2 was able to identify low blood flow during CPB. Another study design than the one we used could have been considered. A stepwise decrease in CPB blood flow would lead to a spill over from the previous blood flow steps. An alternatively design CPB blood flow could be to decrease to a lower blood flow, then return to the starting CPB blood flow before a new lower CPB blood flow was chosen. However, this study design is time consuming, and if perfusion time is prolonged, the CPB itself will affect both the systemic and regional parameters.

6.2. CHOICE OF ANIMAL MODEL

Many different animal models have been used for haemodynamic evaluation over the past years. Rodents have yielded some of the information, but many differences exist between the cardiovascular systems of rodents and humans such as heart rate, oxygen consumption, and adrenergic receptor ratios (134). Pigs have gained favour over dogs, in part because of marked differences in the coronary anatomy of dogs

and humans (134). The porcine heart closely resembles the human heart in terms of its coronary circulation and haemodynamics. The physiological functions are comparable in size with humans, allowing the testing of devices that can also be used in humans. For these reasons, the Danish Landrace pig was chosen as experimental model. However, in order to be able to compare animal studies, it is important that the animals are weight- and age-matched (134).

CHAPTER 7. MAIN CONCLUSIONS

The four studies forming this thesis contributed with novel findings regarding the interpretation of metabolism in cerebral and muscle tissue during individually defined blood flow (study I – human study) and low flow states (studies II–IV – animal studies). Study I indicates that the blood flow during normothermic CPB may be safe at a wider range than 2.2–2.5 L/min/m^2. Thus, although the individual blood flow did not improve cerebral or systemic perfusion during CPB, the present study showed that during elective heart surgery an individual CPB flow of 1.9 L/min/m^2 to 3.1 L/min/m^2 had a similar cerebral and global oxygenation as did a CPB flow calculated on the basis of the individual BSA. Furthermore, in study IV we found that CPB blood flow is important for maintaining CBF and that increased MAP does not protect against cerebral hypoperfusion in an experimental porcine model using PET-CT for evaluation of adequate CBF.

During inadequate systemic circulation, StO_2 reacted rapidly and indicated that global tissue perfusion was insufficient even when systemic circulation was restored after a period of systemic hypoperfusion (study II). However, we also demonstrated that during severe, inadequate systemic circulation, StO_2 remained high until muscle blood flow had decreased to about 50%, after which StO_2 almost linearly followed the fall in muscle blood flow (study III). The finding that muscle blood flow decreases markedly before StO_2 decreases suggests that StO_2 cannot be used as a single measurement method for evaluation of regional or systemic circulation. Further studies are needed to investigate the precise mechanism behind the high StO_2 value even when muscle tissue perfusion is severely reduced.

7.1. FUTURE PERSPECTIVES

The anaesthesiologist's every day clinical scenario is the evaluation of adequate tissue oxygenation in patients undergoing acute and elective surgery or admitted to the emergency and intensive departments. However, the well-established systemic parameters are global assessments, but are insufficient and do not ensure adequate regional tissue perfusion. Optimising diagnosis of insufficient tissue perfusion during low blood flow conditions, there is an increasing interest in non-invasive measurement of tissue microcirculatory changes, such as NIRS. In cardiac surgery, neurological complications are still common, and hypoperfusion appears to be central to their pathogenesis. As NIRS is non-invasive and easy to use, it is already used in cardiac surgery. However, the clinical benefit obtained from NIRS monitoring has so far failed to definitively demonstrate that interventions to correct cerebral desaturations improve neurological outcomes, and therefore rSO_2 is not used routinely in cardiac surgery. New research is looking into other patient groups. Studies in septic patients using rSO_2 combined with systemic parameters (central

venous oxygen saturation and lactate) have revealed a new approach to identify septic patients with a high risk of mortality (135). Another interesting finding was recently made in a physiological study in which apnoea divers were used to stimulate apnoea with the aim of mimicking the missing ventilation during clinical scenarios such as difficult intubation "cannot ventilate cannot intubate", laryngospasm, or cardiopulmonary resuscitation. During a maximal breath hold manoeuvre, the study showed that rSO_2 and SaO_2 were correlated and both decreased significantly, but more importantly that the rSO_2 returned faster to baseline after apnoea, suggesting that it could be useful during pulseless resuscitation or difficult airway management (136).

Studies have shown that the distal extremities exhibit an early vasoconstrictor response in models of hypovolemia (91–92). Therefore, monitoring of muscle tissue oxygenation may be beneficial for detecting early signs of hypovolemia in patients. In our studies, rapid response in StO_2 was seen but only during severe CPB blood flow reductions, and under these conditions, a sustained lower level of StO_2 after re-established blood flow was also seen, indicating a telltale function of StO_2. New research is being done using sublingual microcirculatory perfusion with side-stream dark field imaging that visualises the movement of single red blood cells, and the latest research shows that this technique could be used to evaluate fluid responseness (137).

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APPENDICES

Appendix A. Paper I

Perfusion

Perfusion
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Should blood flow during cardiopulmonary bypass be individualized more than to body surface area?

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Abstract

Blood flow during cardiopulmonary bypass (CPB) is calculated on body surface area (BSA). Increasing comorbidity, age and weight of today's cardiac patients question this calculation as it may not reflect individual metabolic requirement. The hypothesis was that a measured cardiac index (CI) prior to normothermic CPB is a better estimate. A cross-over study, with random allocation to CPB blood flow for 20 minutes based on either a calculation (2.4 L/min/m^2) or on CI, with a switch to the opposite flow for another 20 minutes, was performed. Twenty-two elective cardiac surgery patients with normal ventricular function were included. Effect parameters were cerebral oxygenation, mixed venous saturation and arterial lactate. CI varied from 1.9 to 3.1 L/min/m^2 (median 2.4 L/min/m^2). No differences in effect parameters were seen. In conclusion, a CPB blood flow based on an individual estimate did not improve cerebral and systemic oxygenation compared to a blood flow based on BSA.

Keywords

Introduction

Cardiopulmonary bypass (CPB) has been used to secure adequate systemic oxygenation and perfusion during surgical procedures since 1953[1]. The systemic oxygen uptake was calculated by Fick´s principle and the pump flow, in litres per minute, was based on the product of body surface area (BSA) and a constant of 2.4 during normothermic CPB[1,2]. The rationale for using BSA to calculate CPB blood flow is that, on average, each square metre of the body mass has the same metabolic rate[3,4]. The calculation of BSA is based on a hundred-year-old formula of Dubois and Dubois[3]. Increasing comorbidity, the age and the weight of patients scheduled for today's cardiac surgery question this calculation of BSA. Blood flow during CPB is standardized worldwide[5]; i.e. 2.2-2.5 L/min/m^2 which, therefore, might not reflect the individual metabolic requirement. In the anaesthetic and surgical field, there is an increasing tendency towards a more individual circulatory treatment. In fact, individualized goal-directed therapy has been shown to reduce postoperative complications and mortality in high risk surgery[6].

We hypothesized that an individual measured cardiac index (CI) prior to CPB is a better estimate of the blood flow needed for an optimal cerebral and systemic oxygenation during normothermic CPB than a calculated blood flow (2.4 L/min/m^2) in patients undergoing cardiac surgery.

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Patients and methods

Patients

Twenty-two patients scheduled for elective cardiac surgery involving CPB were included after giving informed consent, according to the Helsinki II declaration. The study was approved by the local Ethics Committee. Patients were recruited between December 2008 and March 2009. Two patients were excluded due to cancellation of their scheduled operations. Exclusion criteria were: Left ventricular ejection fraction (LVEF) ≤ 50%, history of cerebral insult or trauma and verified stenosis of the carotid arteries.

Methods

The study was registered at a clinical trial database (ClinicalTrials.gov N-20080035) before inclusion of the first patient. The study was a randomised cross-over study where the patients served as their own controls. Patients were randomly allocated, based on computer-generated codes, to start with the calculated blood flow (2.4 L/min/m^2) or a blood flow based on an individual mean CI measured prior to CPB.

All regimes were standardized according to the institutional protocols. Patients were premedicated with midazolam 7.5 mg and paracetamol 2 g together with their usually beta-blocker treatment. Diabetic patients had an infusion of isotonic glucose mixed with 16 international units (IU) of insulin and 10 mmol of potassium chloride per 500 mL. Near-infrared spectroscopy (NIRS) was used for cerebral oximetry monitoring, based on the absorption of infrared light by biological tissue. NIRS (INVOS 4100; Somanetics Corp., Troy, MI, USA) is a non-invasive technique that uses the different absorption properties of haemoglobin to evaluate tissue oxygenation. Oxygenated and deoxygenated haemoglobin absorbs light equally at 810 nm whereas, at 730 nm, absorption is primarily from deoxygenated haemoglobin. Therefore, monitoring these two wavelengths provides an index of deoxygenating and, thereby, a measure for perfusion of the regional cerebral tissue[7]. Two Soma sensors® (Somanetics Corp., Troy, MI, USA) were placed bilaterally on the patient's forehead prior to introduction of anaesthesia. Monitoring of the regional cerebral oxygenation (rSO_2) was initiated and the cerebral oximeter readings were recorded and stored on a floppy disk with a 6 second update for the duration of the intraoperative period. Anaesthesia was induced with 5-10 mg midazolam, 15-25 µg/kg fentanyl and 0.6 mg/kg rocuronium and maintained with inhalation of servoflurane 0-3 %, also during CPB, where the evaporator was attached to the CPB circuit. All patients were monitored with radial and pulmonary artery catheters (CCOmbo catheter, Edwards Lifesciences, Irvine, CA, USA), a 5-lead electrocardiogram, bladder and peripheral temperature probes, pulse oximeter, multigas analyzer and capnography (Primus, Drägerwerk AG &Co. KGaA, Lübeck, Germany). Before CPB, the individual CI was registered by the cardiothoracic anaesthetist in charge and the specific value noted and used for the setting of the CPB blood flow.

CPB was established after standard median sternotomy. Routine aortic cannulation was used and venous cannulation was preformed via the right atrium (n = 19) or the groin (n = 1). Routine surgical techniques were performed. CO_2 flow in the field was not used. Normothermic non-pulsative CBP was performed with a heart-lung machine (HL 30, Maquet GmbH & Co KG, Rastatt, Germany) and a fully tip-to-tip coated (X coating™, Terumo, Tokyo, Japan) open system with a hollow-fibre oxygenator with a 4000 mL hard-shell reservoir (CAPIOX® CXRX25, Terumo®, Japan) and a 40-µm arterial filter line (CAPIOX® AL8X, Terumo® , Tokyo, Japan). Activated coagulation time (ACT) (Hemochron; International Technidyne Corporation, Edison, NJ) was maintained above 480 seconds by administration of heparin and reversed with protamine after CPB. Myocardial protection was achieved using intermittent cold (4°C) blood cardioplegia (Buckberg solution for cold induction, Vno. 836551, Aalborg Hospital Pharmacy, Aalborg Hospital, Denmark). Mean arterial blood pressure (MAP) was maintained between 50-90 mmHg by administration of bolus injections of norepinephrine and the haemotocrit > 20% by transfusion of allergenic packed red blood cells (PRBC). Arterial oxygen tension (PaO_2) was kept at 12-20 kPa, arterial carbon dioxide tension ($PaCO_2$) at 4.5-6 kPa (Alpha-stat strategy) and mixed venous oxygen saturation (SvO_2) above 60%.

CPB was initiated with a blood flow of 2.4 L/min/m^2 or the individual blood flow according to randomising for 10 minutes to secure a condition of steady state. Thereafter, the study protocol was started; a study period of 20 minutes with a blood flow of either 2.4 L/min/m^2 or equivalent to the individual measured CI, dependent of the randomisation; a change to the opposite blood flow for 5 minutes to secure the new steady state, followed by another study period of 20 minutes. Arterial blood samples were withdrawn at 0, 5, 10, 15, and 20 minutes in each study period. Arterial bloods were analysed for pH, PaO_2, oxygen saturation (SaO_2), $PaCO_2$, base excess (BE), hydrogen bicarbonate (HCO_3), haemoglobin (Hb), sodium (Na), potassium (K), chloride (Cl), glucose (glu), and lactate (Lac) (Radiometer, Brønshøj, Denmark). The measured regional left cerebral saturation (rSO_2left), regional right cerebral saturation (rSO_2right), MAP, SvO_2, and CI were noted. The CPB blood flow was increased by 0.1 L/min/m^2 every minute in both study groups if the SvO_2 decreased to values below 60%, according to the study protocol.

After finishing the second study period, the CPB blood flow was adjusted to the standardized 2.4 L/min/m^2.

Statistical analysis

The purpose of the study was to compare the effect of two treatments, C (calculated blood flow) and I (individual measured blood flow), on a number of biomarkers. The primary outcome was rSO_2, SvO_2 and Lac. The experiment was laid out as a 2 x 2 cross-over design with 5 replicates in each treatment period. Linear mixed effects models (LME) that included fixed and random effect were used to examine the change of biomarkers over time in the two treatment groups (C and I). With a p-value ≤ 0.05 and a power of 0.8, with an estimated difference to be detected between the two CPB blood flows of 5 % and a standard derivation of 7.5 %, 20 patients had to be included. All data were tested for normal distribution. Non-normally distributed data were log-transformed to achieve normality before further statistical analysis. Parametric statistics were used for normally distributed data and the data are shown as mean +/- standard deviation. P-values less than 0.05 were considered statistically significant. The following outcome variables were used; SvO_2, rSO_2left, rSO_2right, pH, $PaCO_2$, PaO_2, BE, HCO_3, SaO_2, Hb, Na, Cl, glu, Lac, MAP and CI. The fixed effects included in the model are treatment at two levels, C and I; period at two levels, 1 and 2; time points tested under period at 5 levels, 1, 2, 3, 4 and 5; and treatment sequence at two levels, "CI" and "IC". The random effect in the model was the individual patient's levels assumed to follow a mean zero normal distribution with unstructured covariance matrix. The residual error terms were assumed to follow a mean zero normal distribution with an autoregressive covariance structure within each time course of the subjects. A linear model for trend was included within each time period of the patient. The fitted model was used to estimate 95% confidence intervals for the treatment effect and standard errors of the subject and residual variation. Finally, tests for treatment sequence and period effects were carried out. The model was implemented and analysed with statistical software package R, Version 2.9.1[8] (using the contributed package nlme (non-linear mixed effects) for mixed effects modelling).

Results

The patients underwent the following operations: aortic valve replacement (n = 6), aortic valve replacement and coronary artery bypass grafting (CABG) (n = 5), mitral valve repair (n = 6), mitral valve repair, CABG and pulmonary vein isolation using a bipolar radiofrequency (RF) ablation devise (AtriCure Inc., Cincinnati, OH) (n = 1), mitral valve repair and a Cox maze IV procedure using both a bipolar RF devise and a AtriCure Isolator II bipolar ablation device (AtriCure, Inc.) (n = 1) and one patient had a composite graft inserted. Patients and operative characteristics are presented in Table 1. All patients had uneventful intraoperative courses. Two patients had an extended length of stay at the intensive care unit (ICU), defined as >24 hours; one patient due to postoperative bleeding with no need of re-exploration (discharged after 48 hours); one patient due to postoperative respiratory failure (discharged after 92 hours). Furthermore, one patient suffered a cardiac arrest and died in the hospital ward due to low cardiac output failure 48 hours after the operation. However, the ICU stay for this patient was uneventful and the patient was discharged within 24 hours due to standard protocol. This patient underwent mitral valve repair, CABG and pulmonary vein isolation using a bipolar radiofrequency (RF) ablation devise. The intraoperative course was normal, with ischaemic and CPB times of 132 minutes and 182 minutes, respectively. The individual blood flow (measured prior to the CPB) versus the calculated blood flow was 2.1 L/min/m^2 and 2.4 L/min/m^2. In both study periods, the SvO_2 was above 60%, BE at 3-4, and Lac less than 2.0.

Table 1. Preoperative and intraoperative characteristics (Number, mean ± standard deviation)

Male/ female	18/2
Age (y)	69 ± 9.5
BMI (kg/m^2)	27.0 ± 4.3
LVEF (%)	59 ± 9.1
EuroSCORE	4.5 ± 1.6
Preoperative haemoglobin (g/dL)	13.5 ± 1.3
Preoperative creatinine (µmol/L)	93 ± 27
Risk factors (n)	
Diabetes	4
Hypertension	11
Patients treated with statins	8
Perifereral vascular disease	8
Smoking	5
Intraoperative data	
CPB time (min)	121 ± 35
CPB clamp time (min)	90 ± 28
Bladder temperature (°C)	35.6 ± 0.9

BMI: body mass index; LVEF: left ventricular ejection fraction; n: number

In all except one patient, the individual blood flow during CPB was maintained. In this patient, during the initial 5 minutes (individual study period) when the individual blood flow of 1.6 L/min/m^2 resulted in decreasing SvO_2 from 70 to 61%, we decided to raise the blood flow before reaching the protocol value of 60% to prevent any harm. The blood flow was stepwise raised to 1.9 L/min/m^2 and a stable SvO_2 at 65%. No increase in serum lactate was detected. This patient had an uneventful perioperative course and was discharged from ICU the day after surgery. Another patient had a pre-CPB CI of 3.5 L/min/m^2; it was only possible to run a blood flow

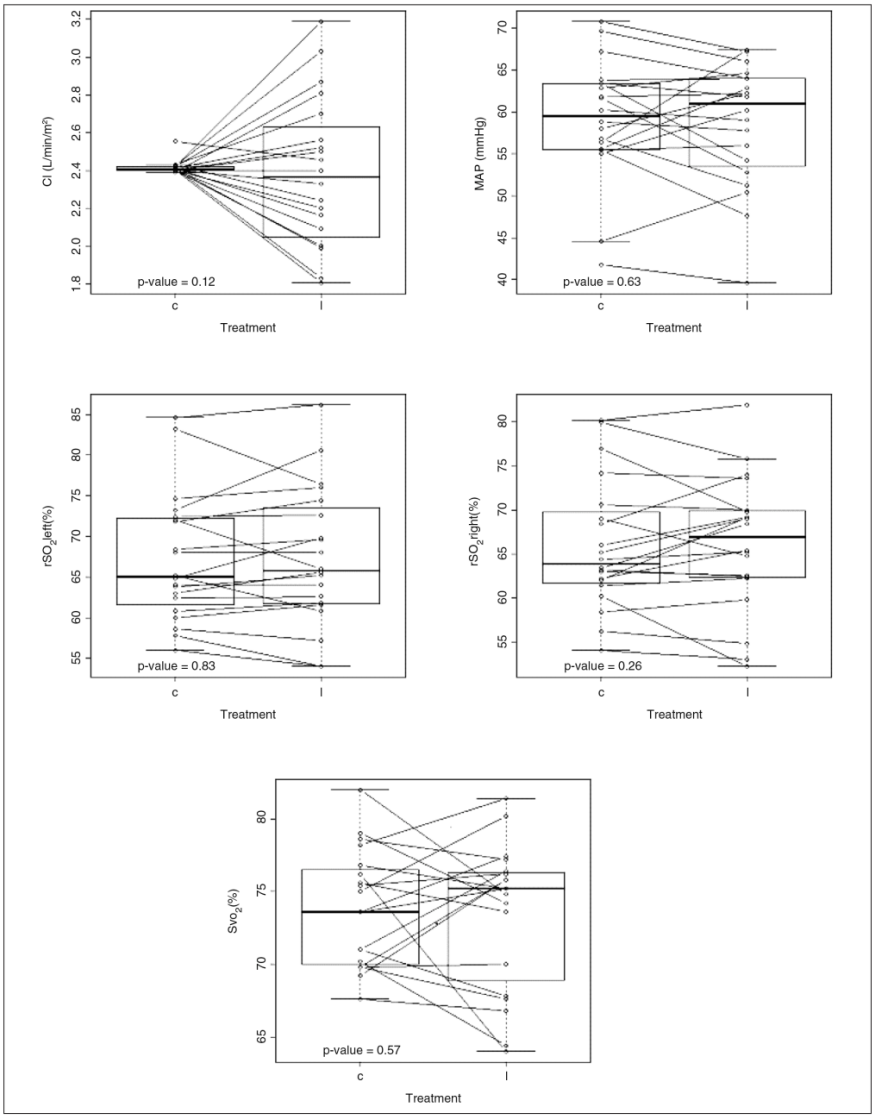

Figure 1. Box plot showing the median, 25-75 quartiles, maximum and minimum of CI, MAP, rSO_2 (left), rSO_2 (right) and SvO_2 in the two treatments: C (calculated blood flow) and I (individual measured blood flow).

at 3.1 L/min/m^2 because of arterial cannulation of the groin without exceeding the limitations on the setup. The calculated blood flow of 2.4 L/min/m^2 was maintained in all patients apart from one whose blood flow inexplicably drifted to 2.9 L/min/m^2 in the last 5 minutes of the study period. The patient had an uneventful preoperative course and was discharged from ICU the day after surgery. There were no differences between the groups regarding CI, MAP, rSO_2left, rSO_2right or SvO_2 (Figure 1). Furthermore, there were no differences in pH, PaO_2, SaO_2, $PaCO_2$, HCO_3, minimum Hb, and minimum glu between the two blood flow periods during CPB (Table 2).

Table 2. Arterial blood gas variables (median and range)

	Calculated blood flow	Individual blood flow	P-value
pH	7.41 (7.35–7.53)	7.42 (7.30–7.42)	0.83
$PaCO_2$ (kPa)	5.23 (4.08–6.10)	5.22 (4.43–5.87)	0.52
PaO_2 (kPa)	20.9 (6.3–53.8)	20.2 (13.9–36.8)	0.91
HCO_3 (mmol/L)	24.8 (20.7–30.7)	24.7 (19.7–31.1)	0.08
SaO_2 (%)	0.99 (0.82–1.00)	0.99 (0.98–1.00)	0.81
Hb minimum (g/dL)	8.9 (5.8–11.3)	8.9 (5.8–11.0)	0.08
Glucose (mmol/L)	9.5 (5.5–13.4)	9.1 (5.3–13.0)	0.98
Lactate (mmol/L)	0.9 (0.4–1.9)	0.9 (0.4–1.8)	0.73

$PaCO_2$: arterial partial pressure of oxygen; $PaCO_2$: arterial partial pressure of carbon dioxide; HCO_3: bicarbonate; SaO_2: saturation of oxygen; Hb: haemoglobin.

Discussion

The present study demonstrates that a blood flow during CPB based on an individual's measured CI prior to CPB does not improve cerebral and systemic oxygenation during short-time normothermic CPB. To our knowledge, the individually measured CI has not previously been used as an estimate for blood flow during CPB. The study supports the fact that patients scheduled for cardiac surgery can maintain cerebral and systemic oxygenation over a wide range of blood flows (1.9-3.1 L/min/m^2) during a short intervention period.

Use of a fixed blood flow during CPB has been questionable since the early days of cardiac surgery as it may not secure the individual's oxygen consumption[9]. A fixed blood flow during CPB calculated by using BSA was shown to be safe as it resulted in relative over-perfusion during hypothermic CPB[10]. Today, both invasive and non-invasive monitoring devices exist to allow a more individual perfusion strategy during CPB to optimize and secure oxygen delivery (DO_2). However, monitoring of SvO_2 during CPB does not guarantee adequate perfusion because the tissue beds of organs may be functionally removed from the circulation[11]. In fact, measuring of serum lactate concentration during CPB is a more sensitive and adequate marker of tissue hypoxia and circulatory failure than SvO_2[12]. An elevated serum lactate level is commonly observed during and after CPB and is associated with morbidity and mortality[13] just as a rapid recovery of hyperlactataemia is associated with an improved outcome[14]. No hyperlactataemia was seen in the present study. Continuous monitoring of DO_2 /CO_2 production during CPB may indicate inadequate oxygen supply earlier than serum lactate[12].

Postoperative cognitive impairment is frequent after cardiac surgery, with the highest incidence at discharge from hospital (50-80%) and is still frequently observed six months after surgery (10-30%), whereas the incidence of cerebral insults is lower (1-6%)[15]. We used NIRS as a non-invasive continuous method of monitoring the regional saturation in the cerebral cortex. The method is independent of pulsatile flow, therefore, suitable for cerebral monitoring during CPB[16]. Indeed, using NIRS monitoring during CPB to secure optimal cerebral conditions has been associated with a shorter length of stay in ICU and a significant reduction in major organ morbidity and mortality[16]. Several publications suggest various thresholds in which rSO_2 desaturation becomes significant[16-17]. A recent study showed that no patients with no desaturation periods below 50% had important incidence of postoperative cognitive impairment[17]. In our study all patients had rSO_2 above 50 % during the intraoperative course.

Tissue oxygenation is not only determined by blood flow during CPB, but also by haemoglobin concentration and oxygen saturation of the blood. DO_2 was not calculated during the study. The generally used blood flow during CPB (2.2-2.5 L/min/m^2) approximates the CI of a normothermic anaesthetized patient[5]. One study in normothermic animals has shown that brain and kidney maintained their regional perfusion over a wide range of different blood flows during CPB (1.7 to 2.3 L/min/m^2)[18]. However, blood flow during hypothermic CPB as low as 1.2 L/min/m^2 has been used with good clinical outcome[19]. In the present study, during normothermia, a CPB flow of 1.6 L/min/m^2 was insufficient to secure tissue perfusion, indicated by a marked decrease in SvO_2, with no sign of tissue hypoxaemia after an increase of blood flow to 1.9 L/min/m^2.

CPB is a dynamic process, with different needs of blood flow, depending on the use of hypothermia, anaesthesia, haemodilution and the overall metabolic demands[20]. It is mandatory to use the most adequate blood flow during CPB as an insufficient blood flow may lead to hypoperfusion and organ damage and, as a high blood flow increases the potential for air embolism, the need of blood transfusion and a systemic inflammatory response[21].

This present study indicates that blood flow during normothermic CPB is safe at a wider range of blood flows than 2.2-2.5 L/min/m^2. In fact, during elective heart surgery in patients with normal LVEFs, individual CPB flow of 1.9 L/min/m^2 to 3.1 L/min/m^2 had similar cerebral and global oxygenation as CPB flow based on BSA. Further studies are needed to identify the optimal blood flow during CPB for the individual patient.

The present study has some important limitations, for which reason the results may not be applicable to the whole population of patients scheduled for today's cardiac surgery. First, CI was measured with a pulmonary artery catheter by heating the blood in a pseudorandom stochastic fashion[22]. If the CI changes rapidly over time, the measurements are inaccurate[22]. However, we measured CI under stable conditions prior to CPB. Second, the study periods were limited to 20 minutes. Third, only patients with preoperative normal left ventricular function were included. Fourth, the number of patients was limited, which is important due to the variation in the measured parameters.

Conclusion

In this study, the use of an individual measured CI prior to CPB resulted in equal cerebral and systemic oxygenation compared with a calculated blood flow of 2.4 L/min/m^2 during short-term normothermic CPB. Further studies are needed to identify the most favourable flow rate during CPB in order to ensure optimal organ perfusion and improve clinical outcome.

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Appendix B. Paper II

Original Paper

Perfusion

Perfusion
1–8

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Regional muscle tissue saturation is an indicator of global inadequate circulation during cardiopulmonary bypass: a randomized porcine study using muscle, intestinal and brain tissue metabolomics

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Abstract

Background: Muscle tissue saturation (StO_2) measured with near-infrared spectroscopy has generally been considered a measurement of the tissue microcirculatory condition. However, we hypothesized that StO_2 could be more regarded as a fast and reliable measure of global than of regional circulatory adequacy and tested this with muscle, intestinal and brain metabolomics at normal and two levels of low cardiopulmonary bypass blood flow rates in a porcine model.

Methods: Twelve 80 kg pigs were connected to normothermic cardiopulmonary bypass with a blood flow of 60 mL/kg/min for one hour, reduced randomly to 47.5 mL/kg/min (Group I) or 35 mL/kg/min (Group II) for one hour followed by one hour of 60 mL/kg/min in both groups. Regional StO_2 was measured continuously above the musculus gracilis (non-cannulated leg). Metabolomics were obtained by brain tissue oxygen monitoring system (Licox) measurements of the brain and microdialysis perfusate from the muscle, intestinal mucosa and brain. A non-parametric statistical method was used.

Results: The systemic parameters showed profound systemic ischaemia during low CPB blood flow. StO_2 did not change markedly in Group I, but in Group II, StO_2 decreased immediately when blood flow was reduced and, furthermore, was not restored despite blood flow being normalized. Changes in the metabolomics from the muscle, colon and brain followed the changes in StO_2.

Conclusion: We found, in this experimental cardiopulmonary bypass model, that StO_2 reacted rapidly when the systemic circulation became inadequate and, furthermore, reliably indicate insufficient global tissue perfusion even when the systemic circulation was restored after a period of systemic hypoperfusion.

Keywords

animal study; experimental pig model; cardiopulmonary bypass; oxygen demand; blood flow; metabolomics; regional metabolism; near-infrared spectroscopy; microdialysis; Licox

Background

Shock resuscitation involves interventions targeting systemic oxygenation, haemodynamic variables and arterial lactate in order to achieve adequate organ perfusion.[1] However, these resuscitation aims are global and, to better pinpoint insufficient tissue perfusion during low blood flow conditions, there is an increasing interest in non-invasive measurement of tissue microcirculatory changes.[2–4] The most common method is continuous measurement of striated muscle tissue oxygen saturation (StO_2) by near-infrared spectroscopy (NIRS) that calculates both oxy- and

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deoxyhemoglobin in the tissue vascular bed. In fact, decreases in StO_2 predicted the need of massive transfusion, organ dysfunction and mortality during early resuscitation of trauma patients[5,6] and decreases in StO_2 preceded changes in base deficit and lactate during cardiopulmonary bypass (CPB) in cardiac surgery patients.[4] Additionally, animal studies have shown StO_2 to be a reliable parameter during haemorrhagic shock and during resuscitation.[7–9] In this context, it is important to emphasize that, since muscle tissue has a low metabolic rate in the resting condition and, therefore, has a high tolerance to ischaemia, a low StO_2 may not indicate that the muscle is metabolically compromised. It shows only that the local oxygen delivery to the muscle tissue is low. Furthermore, StO_2 measures microcirculatory derangement only in the superficial muscle tissue and not in deeper located tissues or organs.[10] However, importantly, muscular perfusion is strongly centrally regulated via vasoconstrictor nerves as well as humoral factors and will be markedly reduced in haemodynamic shock. In fact, in these situations, muscle blood flow may become less than 0.1% of its maximum.[11] This implies that muscle is one of first tissues/organs where perfusion is down-regulated at insufficient systemic circulation (in order to redistribute circulation to vital organs) and, in addition, the last one where perfusion is re-established after adequate systemic circulation has been restored. This can explain the findings that StO_2 could act as an early warning of hypoperfusion.[4,5,12,13] Moreover, a low StO_2 might indicate, after a period of global hypoperfusion, that the systemic circulation is still inadequate despite cardiac output (CO) and mixed venous saturation (SvO_2) being normalized. If this notion is true, StO_2 could be a useful measurement during CPB, where the perfusionist governs the systemic blood flow.

Our hypotheses were that StO_2 1) is a fast-reacting, indirect indicator of global hypoperfusion during CPB and 2) follows the changes in systemic acid/base balance and in metabolism in remote organs. Therefore, we aimed to evaluate in this porcine study with muscle, intestinal and brain metabolomics at normal and two levels of low CPB blood flow rates whether the measurement of regional muscle tissue StO_2 with NIRS can quickly detect global hypoperfusion and, furthermore, indicate tissue hypoxia in vital organs despite restored systemic circulation.

Methods

The study was conducted in 12 female Danish Landrace pigs (80 kg). The study was approved by the Danish Animal Experiments Inspectorate, Nº 2012-15-2934-00566 and followed the Upstein recommendation for uniformity in animal experiments.[14]

Anaesthesia

The animals were premedicated with midazolam 50 mg and azaperone 40 mg, given intramuscularly. Intravenous anaesthesia was used throughout the study, initiated with a bolus injection of etomidate 40 mg and maintained with a continuous infusion of midazolam 50-100 mg/h and fentanyl 500-1000 µg/h supplemented with bolus injections of pentobarbital and pancuronium. The trachea was intubated (Portex, ID 7.5 mm, Smiths Medical, London, UK) and the lungs were mechanically ventilated (Datex-Ohmeda S5 Advance, Helsinki, Finland) with a tidal volume of 6-8 mL/kg, a respiratory rate of 14-16 breaths per minute, a positive end-expiratory pressure of 5 cmH_2O, and a fraction of inspired oxygen of 0.6 to achieve normoventilation with an arterial pH of 7.4. The respiratory rate was reduced to 4 breaths per minute during CPB. The partial pressure of arterial carbon dioxide ($PaCO_2$) was kept within normal values during CPB and the partial pressure of arterial oxygen (PaO_2) above 150 mmHg. The pigs were placed in the supine position for the whole study period.

Haemodynamic monitoring

A left carotid artery branch was cannulated (7-Fr, Radifocus® introducer II, Leuven, Belgium) for continuous measurement of arterial blood pressure. Through the left internal jugular vein, a pulmonary artery catheter was placed for continuous measurements of CO, SvO_2 and core temperature (CCO MBO CCO/SvO_2 7.5 F and Vigilance, Edwards Lifesciences, Irvine, CA, USA). Heart rate and rhythm were monitored with a 5-lead ECG.

Arterial and venous blood were sampled every 20 minutes for biochemical analyses of pH, PaO_2, arterial oxygen saturation (SaO_2), SvO_2, $PaCO_2$, base excess (BE), lactate and glucose (ABL 710, Radiometer, Brønshøj, Denmark). Systemic calculated oxygen delivery (DO_2) and oxygen consumption (VO_2) were calculated based on the parameters obtained.[15]

Muscle StO_2 monitoring

A soma sensor® adult (Somanetics Corporation, Troy, MI, USA) was placed on the skin above the musculus gracilis of the left back leg for measuring regional StO_2 every 6 second (INVOS 5100; Somanetics Corporation). The depth of the subcutaneous fat layer at the place of the sensor had a maximum of 1 cm and was visualised by ultrasound scan to secure that StO_2 was an expression of the muscle tissue oxygenation.

Regional metabolism of muscle, intestinal and brain tissue microdialysis was used to express regional tissue metabolism. Catheters (CMA 60, CMA Microdialysis

AB, Solna, Sweden) were inserted into the left musculus gracilis and into the intramural tissue along the intestinal wall of the colon via a mini-laparotomy. A catheter (CMA 70 brain, CMA Microdialysis AB) and a probe for brain tissue oxygen tension ($PbtO_2$) (Licox CMP Monitoring System Integra Neurosciences, Plainsboro, NJ, USA) were inserted into the brain tissue through a hole drilled in the skull.

All three microdialysis catheters were perfused using a physiological solution (CMA perfusion fluid) with a pump flow rate of 1.0 µL/min (CMA 102, CMA Microdialysis AB) with a one-hour steady state period for all tissues; these dialysates were discarded. During the study period, dialysates were repeatedly collected in vials every 20 minutes, kept on ice and finally stored at -80°C until analysis. All dialysates were analysed for lactate, pyruvate and glycerol (CMA 600 Microdialysis Analyzer, CMA Microdialysis AB) in accordance with the manufacturer's instructions, using original (manufacturer-supplied) reagent kits and calibrators. Lactate/pyruvate ratio (LPR) was calculated, being a marker of tissue ischaemia.

Study protocol for low blood flow during CPB

CPB cannulation was performed with a 29-French three-stage venous catheter (Medtronic Inc., Minneapolis, Minnesota, USA) placed in the upper caval vein and a 19-French arterial catheter placed in the right femoral artery (Medtronic Inc., Tolochenaz, Switzerland). The CPB heparin-coated circuit was primed with isotonic saline and included a centrifugal pump (Rotaflow, Maquet Cardiopulmonary AG, Hirrlingen, Germany), an oxygenator and a heat exchanger (Jostra Quadrox D Diffusion Membrane, Maquet Cardiopulmonary AG). Normothermic CPB was used throughout the study, being a central temperature of 38-39°C for an adult pig. Heparin was administered to achieve an activated clotting time (ACT) above 500 seconds.

In order to avoid hyperperfusion during CPB, a blood flow of 60 mL/kg/min was chosen as the initial CPB blood as earlier studies have shown that anaesthetised adult female pigs have a CO of 5-6 L/min (62.5-75 mL/kg/min).[16] To secure a fully controlled blood flow during CPB, contribution from a beating heart was abounded by the institution of ventricular fibrillation via a pacewire. The study period started with a blood flow of 60 mL/kg/min for one hour followed by a randomisation to a blood flow of either 47.5 mL/kg/min (Group I) or 35 mL/kg/min (Group II) for another hour and finalised with one hour of reperfusion with a blood flow of 60 mL/kg/min (Figure 1). At the end of the study, all the animals were sacrificed with an intravenous overdose of pentobarbital.

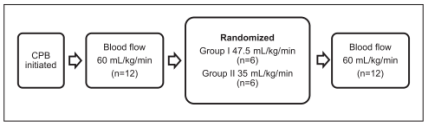

Figure 1. Flow chart.

Statistical analysis

Non-parametric statistical methods were used because all data were not normally distributed. Wilcoxon-Mann Whitney was used to compare baseline values between groups and Friedman tests were performed on outcome variables within each group to test for differences during the study period. For each outcome variable, the differences between baseline and values obtained during the period of low blood flow were calculated and analysed in each group. Similarly, the differences between baseline and values obtained during the period of re-established blood flow were analysed. Intergroup comparison of the repetitive values (differences at 20, 40 and 60 min.) were not constant over time, therefore, a Wilcoxon-Mann Whitney test was performed of the values obtained at 40-60 minutes for inter-group comparisons and for intra-group comparisons between the values at 40-60 minutes of low blood flow and 40-60 minutes after re-establishing blood flow. Spearman correlation was used to analyse the relationship between variables. Statistical analyses were performed using Stata® (version 11.2), and the level of significance was set at $p<0.05$.

Results

One pig from Group I was excluded after randomisation due to continuous and severe bleeding from both CPB cannulation sites and all data were withdrawn from the analyses.

Muscle StO_2 and metabolomics

StO_2 did not change markedly in Group I during the different blood flow rates (Figure 2). However, in Group II, StO_2 decreased immediately when the blood flow was reduced and, furthermore, was not restored despite blood flow being normalized (Table 1). A significant correlation was seen between lactate and StO_2 (Spearman; r:-0.35, $p<0.001$) and between SvO_2 and StO_2 (Spearman; r:0.29, $p<0.005$) (Figure 3).

Changes in the metabolites in muscle tissue followed muscle StO_2 values; in Group II, significant changes were seen for muscle pyruvate and muscle glycerol while no changes occurred in Group I (Table 2),

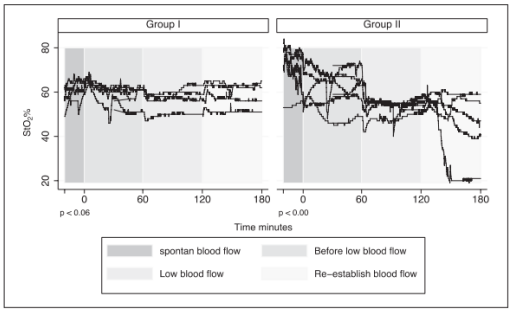

Figure 2. The two figures show the time course of StO_2 values in percentage at the y-axis for the two groups and the blood flows of 60 mL/kg/min, low blood flow 47.5 mL/kg/min (Group I, left figure) and 35 mL/kg/min (Group II, right figure) and 60 mL/kg/min at the x-axis.

Table 1. Comparison of systemic parameters during the two levels of low blood flow and during reperfusion.

	Systemic parameter Lowest blood flow period			Systemic parameter Reperfusion period		
	47.5 mL/kg/min (Group I)	35 mL/kg/min (Group II)	p-value	60 mL/kg/min (Group I)	60 mL/kg/min (Group II)	p-value
SvO_2	40 (39–42)	33 (31–33)	0.01	46 (46–50)	48 (42–58)	0.87
BE	−6.5 (−7.9–(−0.7))	−9.5 (−10.8–(−9.4))	0.01	−5.7 (−8.2–(−0.3))	−8.8 (−12.1–(−8.4))	0.01
Lac	7.4 (2.8–9.8)	9.8 (9.1–11.7)	0.01	6.3 (2.1–9.1)	10.1 (8.8–11.3)	0.01
DO_2	471 (459–501)	407 (394–415)	0.00	581 (572–586)	540 (528–673)	0.03
VO_2	288 (237–305)	273 (243–302)	0.14	311 (239–326)	311 (275–347)	0.87
MAP	55 (48–70)	41 (40–51)	0.01	64 (55–93)	65 (52–65)	0.04

Arterial blood samples taken at 60 minutes within each blood flow and p-values at 60 minutes comparing Group I and Group II during low blood flow and during re-established blood flow are presented. SvO_2: mixed venous oxygenation (%); BE: base excess (mmol/L); Lac: lactate (mmol/L); DO_2: oxygen delivery (mL/min); VO_2: oxygen consumption (mL/min); MAP: mean arterial pressure (mmHg). Data presented as median (25-75 percentiles).

but no correlation could be shown between lactate and the LPR of muscle.

Systemic variables and intestinal and brain metabolomics

Mean arterial blood pressure (MAP) was, as expected, significantly lower during the low blood flow stage in Group II compared with Group I, with a normalisation in both groups during reperfusion (Table 1). SvO_2 and DO_2 changed significantly in both groups (Figure 4), but were more reduced in Group II at the low flow stage. However, in Group II, SvO_2 increased and was almost normalised during reperfusion. VO_2 was unaltered in Group I ($p<0.24$), but decreased significantly at the low flow stage in Group II ($p<0.001$). Blood lactate followed the changes in StO_2. Likewise, changes in intestinal and brain tissue metabolomics followed muscle StO_2, with severe deterioration in Group II (Table 2), but no correlation could be shown between lactate and the LPR of intestinal and brain. However, $PbtO_2$ did not change significantly.

Discussion

In this study in a CPB porcine model, we found that StO_2 reacted rapidly when the systemic circulation became inadequate and, furthermore, reliably indicated insufficient global tissue perfusion, even when the systemic circulation and SvO_2 were restored after a period of systemic hypoperfusion.

To our knowledge, only a few studies[17–19] have investigated the continuous measurement of StO_2 by NIRS to detect critical low blood flow and hypoperfusion during normothermic CPB. However, StO_2 may, at best, indicate desaturation in a small local region of a muscle. Desaturation occurs when oxygen delivery in relation to muscle metabolism is too low, i.e., anaemia, arterial

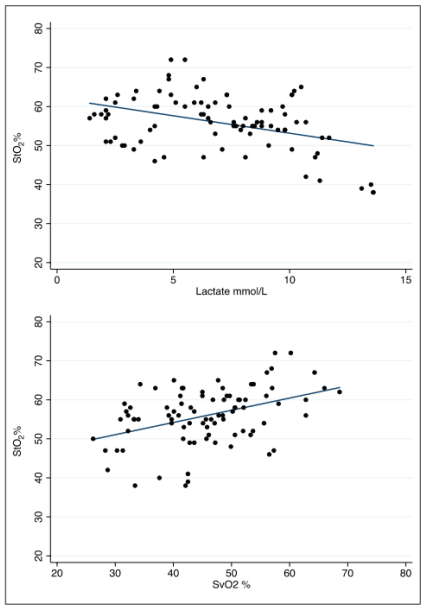

Figure 3. The two figures show the correlation between StO_2 values in percentage at the y-axis and lactate in mmol/L (top figure) and SvO_2 in percentage (bottom figure) at the x-axis.

desaturation and increased muscle metabolism, e.g., shivering, as well as insufficient perfusion caused by vasoconstriction or/and low cardiac output. Indeed, StO_2 desaturations at cardiac surgery prior to CPB are common and probably caused by factors other than reduced blood flow.[4] This variability in StO_2 has also been demonstrated in a previous animal study in trauma settings.[9] Indeed, the NIRS signal may also be affected by sensor location and the subcutaneous layer above the muscle.[20] Thus, a low StO_2 is not similar to, but may indicate impaired muscle cell metabolism. We are going a step further and speculate that a low StO_2 is not mainly a sign of a local metabolic dysfunction, it is more a sign of an insufficient systemic circulation inducing a central vasomotor response, increasing sympathetic outflow causing vasoconstriction of the muscle vessels in order to centralise blood volume. Nevertheless, if this low flow condition is extended, it will certainly induce metabolic dysfunction, not only in the muscles, but also in vital organs. In this study we compared the changes in StO_2 with changes in commonly used measures of adequacy of systemic circulation and global metabolism. In addition, muscle and remote organ metabolomics were assessed by microdialysis. Microdialysis measures the extracellular concentrations of biomarkers which reflect intracellular concentrations of substances that diffuse across cell membranes, such as lactate and pyruvate.[21] Low blood flow leads to decreased oxygen cellular supply and the accumulation of toxic metabolites which may cause cell membrane disintegration with the leakage of cell components into the interstitium.[21] Lactate accumulation and pyruvate depletion in the cytoplasm during ischaemia lead to an elevation of LPR, which reflects persistent reduced mitochondrial function or hypoxia.[21] We used microdialysis to evaluate the metabolomics of the muscle, colon and the brain. The latter two organs were chosen because of the colon's well-known vulnerability to ischaemia following cardiac surgery[22] and the brain's ability to maintain its own perfusion, at least during short periods of insufficient systemic circulation; contrary to the muscle vessels, the brain vessels have very few vasoconstrictor nerves and are, therefore, minimally affected by increased central sympathetic activity.[11] As an additional measurement of the brain's metabolism and local oxygenation, we used $PbtO_2$. $PbtO_2$ has been shown to rapidly detect cerebral hypoxia.[23]

We found that StO_2 reacted immediately and almost as fast as SvO_2 when the systemic blood flow became insufficient (Figure 2). However, in contrast to SvO_2, StO_2 did not decrease at subnormal systemic blood flow (47.5 mL/kg/min), but only at profoundly reduced systemic blood flow (35 mL/kg/min). The normal StO_2 at the subnormal flow level was associated with normal metabolomics in the muscle, colon and brain as well as no systemic lactataemia, showing that the CPB flow was sufficient, despite the decreased SvO_2. On the other hand, at a CPB flow of 35 mL/kg/min, StO_2 decreased and muscle, remote organ and global metabolomics were compromised, indicating that systemic circulation was not sufficient. Interestingly, SvO_2 increased directly after systemic blood flow was restored to normal, but StO_2 was still low, indicating hypoperfusion, corroborated both by high systemic lactate and impaired metabolomics in brain and colon as well as in muscle tissue. Thus, StO_2 seems to be a better marker of global hypoperfusion than SvO_2 which, in this study, gave non-conclusive or spurious results.

The immediate decrease in StO_2 when the CPB blood flow was reduced to 35 mL/kg/min, we believe, was mainly caused by a centrally induced vasoconstriction of the muscle arterioles and not a sign of serious muscle tissue or remote organ hypoxia.[11] However, the extended low CPB flow caused, as expected, a metabolic derangement also in the muscle cells, which was shown by increases in muscle lactate, glycerol and LPR ratio. Despite the change in metabolomics in Group II, with the lowest blood flow, no correlation could be

Table 2. Biomarkers in microdialysate obtained from brain, intestinal, muscle tissue and StO_2.

	Group I				Group II			
	60 mL/kg/min	47.5 mL/kg/min	60 mL/kg/min	p-value	60 mL/kg/min	35 mL/kg/min	60 mL/kg/min	p-value
Muscle								
Pyruvate	0.09	0.10	0.09	0.25	0.09	0.16	0.13	0.03
(µmol/L)	0.04–0.19	0.05–0.14	0.03–0.17		0.07–0.11	0.12–0.24	0.04–0.2	
Lactate	1.4	2.8	1.6	0.44	2.4	4.0	3.7	0.07
(mmol/L)	1.0–3.0	1.0–4.4	1.3–3.2		1.9–3.5	2.6–4.4	2.8–5.5	
Glycerol	65	91	73	0.45	211	474	516	0.01
(mmol/L)	35–106	61–203	36–371		157–327	361–617	475–805	
LPR	15.5	19.9	18.9	0.25	21.4	18.7	27.5	0.11
	11.5–17.3	12.0–47.9	10.5–19.1		17.5–21.9	17.5–23	24.5–88.3	
Intestinal								
Pyruvate	0.06	0.09	0.09	0.95	0.13	0.16	0.16	0.51
(µmol/L)	0.01–0.12	0.01–0.12	0.01–0.11		0.06–0.16	0.05–0.2	0.07–0.22	
Lactate	1.5	1.8	1.6	0.45	2.7	4.1	4.8	0.04
(mmol/L)	0.6–1.9	0.6–2.8	0.7–3.1		1.7–4.1	3.4–5	3.2–6.4	
Glycerol	84	56	125	0.81	177	344	385	0.03
(mmol/L)	60–135	36–315	26–543		83–253	260–384	303–634	
LPR	17.4	23.4	28.4	0.81	22.1	35	34.3	0.00
	16–29.3	15.9–30	14.5–29		21.6–24.7	29.9–37.5	27.4–62.1	
Brain								
Pyruvate	0.07	0.07	0.20	0.16	0.08	0.08	0.07	0.74
(µmol/L)	0.05–0.21	0.04–0.22	0.05–0.20		0.03–0.12	0.01–0.07	0.03–0.16	
Lactate	1.7	1.4	3.2	0.04	2.3	4.3	4.4	0.51
(mmol/L)	1.1–2	1.1–2.9	1.8–4.1		1.2–5.6	2.6–6.4	3.2–5.2	
Glycerol	38	57	98	0.16	96	204	225	0.85
(mmol/L)	28–61	23–89	25–114		66–236	86–335	214–536	
LPR	13.3	26,5	28	0.82	27.6	59.4	112.6	0.07
	7.4–26	25.8–40.1	7.9–30.6		8.6–70.9	12–296.4	6.6–190.9	
Brain $PbtO_2$	25.7	26.5	26.5	0.82	26.5	22.1	37.4	0.14
(mmHg)	16.3–41.4	16.3–31.3	12.4–32.0		15.2–62.7	1.3–65.8	2.4–76.6	
Muscle StO_2	61	59	61	0.06	60	55	53	0.00
(%)	60–63	54–60	55–63		54–65	47–55	40–56	

Brain $PbtO_2$ and biomarkers of collected microdialysate during the last 20 minutes of each blood flow (data are presented as median (25-75 percentiles)). Brain $PbtO_2$: brain tissue oxygen tension; Muscle StO_2: muscle tissue saturation; LPR: lactate-pyruvate ratio.

demonstrated between systemic parameters and the regional metabolomics, which has previously been demonstrated repeatedly[24] as well as in a recent study using sublingual microcirculatory changes with non-invasively Sidestream Dark Field technology.[25] The lack of improvements in these variables after the systemic blood flow was restored could be explained by that blood being distributed to other regions with higher basal metabolism that had, therefore, accumulated high amounts of vasodilatory metabolic products, e.g., lactate, potassium, ADP, etc., during the low flow state.[11,26] Thus, although vasoconstriction may have waned off in the muscles, there was probably "flow-steal" by these excessively vasodilated regions, causing insufficient perfusion of the muscle tissue and, therefore, a low StO_2, even with a "normal" systemic blood flow. In fact, a reperfusion flow of 60 mL/kg/min after 35 mL/kg/min was insufficient for the whole body metabolic demand as assessed by the findings of elevated blood lactate levels and compromised metabolomics in both the colon and the brain. These findings also suggest that the systemic blood flow should have pushed up further to satisfy the metabolic demand and repayment of the oxygen debt in different tissues and organs. Our results agree with a previous study where an organ-specific hierarchy of oxygen delivery has been demonstrated using fluorescent microspheres.[27]

In our study, the NIRS sensor was placed above the larger musculus gracilis to be absolutely sure that the NIRS sensor, whose location was confirmed by ultrasound, measured muscle tissue saturation. Advantageously, the musculus gracilis is only covered

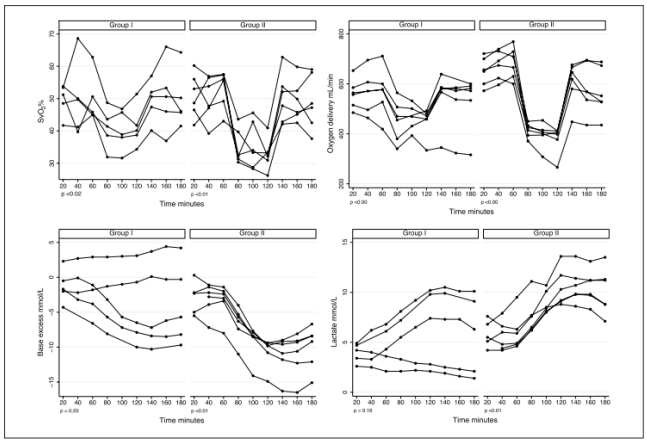

Figure 4. Time course of SvO_2, BE, lactate and DO_2 measured every 20 minutes at each blood flow. Blood flow of 60 mL/kg/min from 0-60 minutes, low blood flow from 80-120 minutes and re-established blood flow of 60 mL/kg/min from 140-180 minutes.

with a thin layer of subcutaneous tissue in pigs, thereby, minimizing contamination. The musculus gracilis represents a more central monitoring site than is commonly used in humans.[22] However, a human study by Ostadal et al.[28] demonstrated that StO_2 measured over the calf muscle could be used to estimate global haemodynamic in patients with cardiogenic shock and individuals undergoing venoarterial extracorporeal membrane oxygenation. Our results support these finding at an even more central monitoring site for the NIRS sensor in a porcine model.

As it is an animal study, clinical implications cannot be drawn directly, but StO_2 may be a tell-tale of the adequacy of the global circulation during CPB, i.e., that so long as StO_2 is normal, the systemic flow is probably adequate. However, if StO_2 drops suddenly, it is a sign of imminent failure of global circulation that, if prolonged, will cause vital organ dysfunction and, therefore, systemic flow needs to be immediately corrected. Likewise, if StO_2 continues to be low after systemic blood flow and SvO_2 have been normalized, one should consider increasing systemic blood flow further. Our results provide additional information to the knowledge regarding the use of StO_2 as a continuous non-invasive surrogate marker for insufficient blow flow states in humans. However, the results have to be confirmed in human studies.

The study has several limitations. First, it is an experimental study with a relatively small number of animals and the results can only cautiously be transferred to patients. Secondly, NIRS signals might have been affected by the cannulation of the opposite right femoral artery as the tip of the catheters are positioned in the abdominal aorta, thus, potentially limiting the flow to other limb. No measurement was done to verify the blood flow to the non-cannulated leg. Thirdly, brain saturation was not measured with NIRS as the adult pig skull is approximate 4 cm. Fourthly, steady state values of biomarkers were not the target of our study because the maximum duration of CPB was set to three hours to mirror the clinical scenario. Fifthly, no intervention was undertaken to secure adequate perfusion of the cannulated leg, which may have led to a potential spill-over of anaerobic metabolites to the systemic circulation.

Conclusion

This experimental porcine study confirms that StO_2 reacts quickly to changes in systemic circulation and that, during CPB, protracted low StO_2 is associated with compromised metabolism in muscle and remote organs as well as metabolic signs of global hypoperfusion. Our results suggest that, during CPB, StO_2 can be seen as an indicator of the adequacy of the systemic circulation, but further studies are warranted before any conclusion about the clinical implication can be drawn.

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