# Cerebral energy metabolism during haemorrhagic shock and the concept of permissive hypotension

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PhD Thesis

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# Preface

For as long as I can remember, I have wanted to be a doctor. My parents could tell you stories about how I operated my teddy's protruding ears and how I practised suturing on oranges. I wanted to help people. So, I kept pursuing my dream, and after many years of study, I finally finished medical school in 2013.

When I, together with my good friend and now a consultant in Anaesthesia and intensive care, Anders Mølgaard Rasmussen, first contacted Professor Palle Toft to begin our work with our master's dissertation, I had no idea that I now eight years later would finish my PhD. During our work, it became clear how interesting and inspiring the world of research were. However, I also learned that I had several shortcomings regarding methodical and statistical knowledge, which irritated me. Therefore, I decided that if I ever had the chance of doing a PhD, I would take it.

In December of 2016, I was enrolled as a PhD student at the Department of Clinical Medicine, University of Southern Denmark. I had just finished my introductory year in Anaesthesia and Intensive Care, but not all was bliss. During 2008-2019, Danish legislation demanded that junior doctors start specialising within 4-6 years of completing medical school if they wanted to specialise in Denmark.

In December 2016, all of my friends from medical school were starting their specialist training and the thought of me not having started my specialist training started stressing me to a point where my stomach hurt. So, I contacted my supervisor Palle Toft, and then the Head of the Department of Anaesthesia and Intensive Care at Odense University Hospital, Bjarne Dahler-Eriksen. They guided me to combine research and specialist training and encouraged me to apply for specialist training the following year.

Luckily, the application board and the senior educational consultants from Kolding Hospital, Mette Pedersen and Odense University Hospital Michael Due Nielsen accepted that I was to work on my PhD simultaneously with my specialist training. Therefore, together with the educational consultants and supervisor, we decided I should work with specialist training for one year, followed by one year as a full-time PhD student, alternating back and forth.

Looking back, it has indeed been a journey. It has been a privilege to be able to combine treatment of patients, specialist training, and research. I have learnt many things during the past years. Not only how to be critical, but also how to lead a study, apply for funding and do the statistics. I am proud of what I have achieved, but looking back, I also realise that I would have done several things differently now that I have gotten further insight into the world of research.

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There is a long list of persons who have helped during the years, of whom I am very much grateful.

First, my supervisor Professor Palle Toft who was kind enough to take me under his wings and made it possible to do my Compulsory Military Service at the Biomedical Laboratory at the University of Southern Denmark, secondly my co-supervisor Troels Halfeld Nielsen, a skilled and friendly vascular neurosurgeon whom I many times has asked for advice. Troels also taught me how to work in a laboratory and how to be systematic. Carl-Henrik Nordström is a retired consultant in neurosurgery at Lund University Hospital, Sweden, and adjunct professor of neurosurgery at the University of Southern Denmark, Odense. Carl-Henrik is an expert in cerebral physiology and the field of cerebral metabolism and microdialysis. Carl-Henrik has many times contributed with important new insight into the result obtained in my studies. I am deeply grateful for the guidance and help I have received from Carl-Henrik. Asger Grandfield MD PhD, DMSc taught me his porcine model of haemorrhagic shock and to be thorough and meticulous in the laboratory. Elisabeth Charlotte Hansen, then a medical student and now a junior doctor who worked with me as a part of her master's dissertation. It was a pleasure working with such a curious and talented colleague. My fellow PhD students Simon Mølstrøm and Jimmy Højberg Holm for their support and friendship.

Also, I want to give a special thanks to Jytte Thode, Research secretary at the Department of Anaesthesiology and Intensive care at Odense University Hospital.

Last but not least, I want to thank all of the staff at the Biomedical Laboratory at the University of Southern Denmark. Without their help and expertise, it wasn't possible to conduct experimental animal studies. Specially I would like to thank the large animal team: Diana Bianca Hansen, Denise Lerche Heilskov, Pernille Simonsen, Kristoffer Kjølby Augustesen, Louise Langhorn, and Charlotte Laurfelt Munch Rasmussen. And finally, the Service team: Gitte Sveistrup and Karin Haugaard Stubkjær.

Nobody has been more important to me in the pursuit of this project than my family. I would like to thank my parents, whose love and guidance are with me in whatever I pursue. Most importantly, I wish to thank my loving and supportive wife, Gitte, and my three wonderful children, Marius, Nora and Lauge, who provide unending inspiration.

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Department of Anaesthesiology and Intensive care at Odense University Hospital

# List of original articles

The current thesis is based on the three following original research articles. Each article represents a different sub study and will in the following thesis be referred to as study I-III

Study I: Jakobsen R, Halfeld Nielsen T, Granfeldt A, Toft P, Nordström CH. A technique for continuous bedside monitoring of global cerebral energy state. Intensive Care Med Exp. 2016 Dec;4(1):3.

Study II Jakobsen RP, Nielsen TH, Mølstrøm S, Nordström CH, Granfeldt A, Toft P. Moderately prolonged permissive hypotension results in reversible metabolic perturbation evaluated by intracerebral microdialysis - an experimental animal study. Intensive Care Med Exp. 2019 Dec 4;7(1):67.

Study III Jakobsen RP, Hansen EC, Nielsen TH, Nordström CH, Toft P. (2022). "Effects of norepinephrine infusion on cerebral energy metabolism during experimental haemorrhagic shock." Intensive Care Med Exp 10(1): 4.

Each of the articles above can be found in their full-length at the end of this thesis

Publications by the PhD student related to the thesis

1. Nordström CH, Jakobsen R, Mølstrøm S, Nielsen TH. Cerebral venous blood is not drained via the internal jugular vein in the pig. Resuscitation. 2021;162:437-438.
2. Nordström CH, Jakobsen R, Mølstrøm S, Nielsen TH. Cerebral microdialysis after cardiac arrest - Misinterpretations based on a misconception. Resuscitation. 2021;169:227-228.

# List of abbreviations

| | |
|---|---|
| AKI | Acute Kidney Injury |
| ATP | Adenosine triphosphate |
| CBF | Cerebral blood flow |
| CPC | Cerebral Performance Category |
| CPP | Cerebral perfusion pressure |
| DCR | Damage Control Resuscitation |
| DCS | Damage Control Surgery |
| $FAD^+$/FADH | Flavin adenine dinucleotide in its oxidised and reduced form, respectively |
| ICP | Intracranial Pressure |
| JBM | Jugular Bulb Microdialysis |
| LP ratio | Lactate to pyruvate ratio |
| MAP | Mean Arterial Pressure |
| MD | Microdialysis |
| $NAD^+$/NADH | Nicotinamide adenine dinucleotide in its oxidised and reduced form, respectively |
| NE | Norepinephrine, noradrenaline |
| NIRS | Near-infrared spectroscopy |
| $PbtO_2$ | Brain tissue oxygenation pressure |
| TBI | Traumatic Brain Injury |
| TCA | tricarboxylic acid cycle |

# Summary

Haemorrhagic shock continues to be the leading cause of preventable deaths from trauma. Trauma results in both tissue damage and haemorrhage, and if severe enough – haemorrhagic shock. Throughout recent years, Damage Control Resuscitation has become widely accepted as the primary strategy in treating haemorrhagic shock. Damage Control Resuscitation incorporates a wide range of techniques, including permissive hypotension. The clinician allows the systemic blood pressure to decrease to below normal limits in permissive hypotension to minimise the bleeding. However, the apparent danger of permissive hypotension is that blood supply to the organs, and especially the brain, is at risk of being compromised. The use of norepinephrine in the case of life-threatening haemorrhagic shock is still controversial and not widely accepted. No clear evidence exists whether the use of norepinephrine results in a survival benefit nor if norepinephrine results in cerebral vasoconstriction and compromised cerebral metabolism. With the use of the in-vivo technique of microdialysis, it is possible to examine the metabolic redox state of the brain via the changes in the relationship between lactate and pyruvate. The thesis aims at defining the concept of permissive hypotension based on the variables related to cerebral energy metabolism obtained by microdialysis and routine biochemical analyses (lactate, pyruvate, glucose, glutamate, glycerol):

To answer the research questions, we conducted three exploratory animal studies. The first describing the biochemical pattern when the decrease in MAP causes pronounced ischemia (depletion of oxygen and substrate) resulting in irreversible cell damage. The second study describing the biochemical pattern when the decrease in MAP causes a moderate reduction of CBF (pronounced decrease in oxygen at continued supply of substrate) compatible with a normalization at re-transfusion. And the third the biochemical pattern when pronounced hypotension due to haemorrhagic shock is treated with norepinephrine to normalize MAP. Lastly, we examined if it is possible to use microdialysis of the draining venous cerebral blood to evaluate perturbation of global cerebral energy metabolism during haemorrhagic shock.

In summary, the thesis demonstrates that the level of MAP and the duration of arterial hypotension during haemorrhagic shock are not sufficient criteria to determine whether irreversible brain damage will occur or not. From a clinical perspective, group data from large studies a used to establish general recommendations. These recommendations are not necessarily adequate for the individual patient and recent guidelines emphasize that individual considerations should be taken. However, there is presently no clinical routine technique available to determine the individual limits.

We have shown that intracerebral microdialysis may be used to determine the biochemical pattern when haemorrhagic shock will cause irreversible tissue damage. The pattern is different from that obtained during a moderate reduction in cerebral blood flow compatible with a normalization of energy metabolism after re-transfusion. Further, we have shown that early treatment with norepinephrine (i.e., before the biochemical variables indicate pronounced ischemia with depletion of oxygen and substrate) may normalize energy metabolism before re-transfusion. As intracerebral microdialysis will hardly be an option during severe haemorrhagic shock under clinical conditions, we have examined whether microdialysis of the draining venous blood may be used as a surrogate marker during a dangerous deterioration of cerebral energy metabolism.

# Dansk resumé

Et traume resulterer i både vævsskade og blødning, og hvis det er alvorligt nok – hæmoragisk shock. Hæmoragisk shock er fortsat den hyppigst dødsårsag som følge af tilskadekomst. Gennem de seneste år er Damage Control Surgery og Damage Control Resuscitation blevet bredt accepteret som den primære strategi til behandling af hæmoragisk shock. Damage Control Resuscitation indeholder en bred vifte af teknikker, herunder permissiv hypotension. Ved permissiv hypotension tillader klinikeren det systemiske blodtryk at falde til under det normalt accepterede blodtryk. Herved minimeres blødningen og behovet for behandling med væske intravenøst. Faren ved permissiv hypotension er imidlertid, at blodtilførslen til organerne og især hjernen risikerer at blive kompromitteret. Anvendelsen af noradrenalin i tilfælde af livstruende hæmoragisk shock er stadig kontroversiel og ikke bredt accepteret verden over. Der findes ingen klare beviser for, om brugen af noradrenalin resulterer i en overlevelsesfordel, og heller ikke om noradrenalin resulterer i cerebral vasokonstriktion, og deraf kompromitteret cerebral metabolisme. Ved anvendelse af mikrodialyse in-vivo er det muligt at undersøge hjernens metaboliske redox-tilstand via ændringer i forholdet mellem laktat og pyruvat. Formålet med denne afhandlingen er at definere begrebet permissiv hypotension baseret på variabler relateret til cerebral energimetabolisme opnået ved mikrodialyse og rutinemæssige biokemiske analyser (laktat, pyruvat, glukose, glutamat, glycerol):

For at besvare forskningsspørgsmålene gennemførte vi tre dyreforsøg. Det første beskriver det biokemiske mønster, når faldet i MAP forårsager udtalt iskæmi (total mangel på ilt og substrat), hvilket resulterer i irreversibel celleskade. Det andet studie, beskriver det biokemiske mønster, når faldet i MAP forårsager en moderat reduktion af cerebralt blod flow (udtalt fald i tilbuddet af ilt, men samtidig fortsat tilførsel af substrat). Det tredje studie beskriver det biokemiske mønster, når hypotension som følge af hæmoragisk shock behandles med noradrenalin for at normalisere MAP. Sideløbende har vi undersøgt om det er muligt at bruge mikrodialyse af det drænende venøse cerebrale blod til at evaluere forstyrrelse af global cerebral energimetabolisme under hæmoragisk shock.

Sammenfattet viser afhandlingen, at niveauet af MAP og varigheden af arteriel hypotension under hæmoragisk shock ikke er tilstrækkeligt til at afgøre, om der vil opstå irreversibel hjerneskade eller ej. Fra et klinisk perspektiv bruges data fra store undersøgelser til at etablere generelle anbefalinger niveauet af acceptabelt MAP under permissiv hypotension. Disse anbefalinger er ikke nødvendigvis tilstrækkelige for den enkelte patient, og nyere retningslinjer understreger, at der skal tages individuelle hensyn. Imidlertid er der på nuværende tidspunkt ingen klinisk rutinemæssig undersøgelse eller teknik tilgængelig til at bestemme de individuelle grænser for MAP. En sikker grænser for MAP kan kun opnås ved bedside-evaluering af cerebral energimetabolisme.

Vi har vist, at intracerebral mikrodialyse kan bruges til at bestemme det biokemiske mønster, når hæmoragisk shock forårsager irreversibel vævsskade. Mønsteret er forskelligt fra det, der opnås under en moderat reduktion i cerebralt blod flow. Yderligere har vi vist, at tidlig behandling med noradrenalin (dvs. før de biokemiske variabler indikerer udtalt iskæmi med udtømning af ilt og substrat) kan normalisere energimetabolismen før re-transfusion. Da intracerebral mikrodialyse næppe vil være en mulighed ved alvorligt hæmoragisk shock under kliniske forhold, har vi undersøgt, om mikrodialyse af det drænende cerebrale venøse blod kan bruges som surrogatmarkør under kompromitteret cerebralt blod flow.

# Introduction

Although many advances throughout the recent year's, trauma remains a leading cause of death. The leading course of preventable deaths from trauma is haemorrhage. (1-3) When exposed to trauma, many factors contribute to disrupting the normal homeostasis. Age, genetics, and comorbidities are pre-existing factors that might negatively influence how the organism can cope with trauma. Trauma results in both tissue damage and haemorrhage, and if severe enough – haemorrhagic shock. During this phase, a vicious spiral of systemic endotheliopathy starts, including platelet activation and dysfunction, inflammation, reduced clotting factor activity, hyperfibrinolysis, sympathoadrenal activation and glycocalyx shedding – all of which might contribute to further haemorrhage. (4-7)

The concepts of Damage Control Surgery (DCS) and Damage Control Resuscitation (DCR) have become the standard strategy in treating patients with major haemorrhages. (8-13)

Damage Control Resuscitation incorporates a wide range of strategies to minimise bleeding and ensure homeostasis. Examples of DCR are the use of balanced blood transfusions with equal amounts of erythrocytes, plasma, and platelets, avoidance of crystalloids, use of antifibrinolytic drugs such as tranexamic acid, and permissive hypotension. Permissive hypotension, also called hypotensive resuscitation, or controlled hypotension, is a strategy where the clinician allows the blood pressure to decrease to levels below normal. The goal is not low blood pressure itself, but to minimise bleeding and loss of erythrocytes, coagulation factors and platelets, and at the same time ensure adequate perfusion of vital organs. (14, 15) The safety of permissive hypotension has not been thoroughly investigated in human clinical trials. Three systematic reviews exist, one on animal models and two in humans in clinical setting trials. Two of the three reviews have focused on the required volume of resuscitation fluid given to patients in haemorrhagic shock. (16, 17) The last focuses on permissive hypotension and concludes that the use of permissive hypotension is associated with low mortality compared with an aggressive approach to fluid resuscitation. However, the review also concludes that even though studies were conducted with moderate to strong quality, there was evidence of poor methodology and concealment procedure. (18)
Shock is defined as insufficient circulation to maintain adequate oxygen and nutrients delivery to peripheral tissue and end organs. All organs and tissues are affected, but not equally as some organs are spared compared to others. (4, 19) The risk of using permissive hypotension is that the blood pressure decreases to a level with compromised end-organ perfusion, leading to depletion of energy sources, apoptosis, and cell death. In each case, the clinician must determine which blood pressure they target. Intuitively there is also an undefined time limit where the body can compensate for a decrease in blood pressure and ensure cerebral perfusion pressure and cerebral blood flow.

In the average human adult, the brain only represents about 2% of the total body weight. Oddly, despite its relatively small size, the brain accounts for about 20% of the oxygen consumed by the body. In other words, the brain is appropriately the organ most sensitive to deprivation of oxygen and nutrients. (20, 21) During a haemorrhage, the systemic blood pressure will eventually decrease, resulting in compromised end-organ perfusion. As mentioned, the brain has a high metabolic rate and does not have any nutritional and oxidative reserve if perfusion becomes jeopardised. Autoregulatory mechanisms ensure stable cerebral blood flow (CBF), doing various increases or decreases in systemic blood pressure. This mechanism for autoregulation is not clearly understood but has been described as both myogenic, metabolic, and neurogenic. (22) These mechanisms were first observed in 1890 by Roy and Sherrington but without any direct quantification of CBF. Nearly 70 years later, in 1959, Niels A. Lassen published his classical paper entitled "Cerebral Blood Flow and Oxygen Consumption in Man" His results with intraarterial injections of Krypton-85 and Xenon-133 demonstrated different ranges in CBF during different chances in systemic

blood pressure. Data suggested a range of systemic blood pressure from mean arterial blood pressure of 50-150mmHg, which resulted in a relatively constant CBF (55mL/100g brain tissue per minute). (23) Lassen's classic autoregulation curve is often misinterpreted to mean that CBF is always kept constant. However, this is only true for relatively gradual changes in blood pressure. CBF becomes incrementally more unstable for progressively faster changes in blood pressure, i.e., seconds to minutes, and may show considerable alterations. (24, 25) If circulation to the brain is completely abrupted, and the supply of oxygen and glucose is ceased. Metabolism is effectively halted, and energy reserves are depleted and eventually leads to neuronal death. Consciousness is lost within 6-7 sec. (26) If, however, the systemic arterial pressure is gradually decreased, there will be a continuous supply of glucose. The supply of oxygen may or may not also be sufficient for some degree of normal metabolism. When CBF is decreased below the autoregulatory limit, physiological compensation is possible since brain tissue may extract a more significant fraction of the oxygen in arterial blood. At a normal haemoglobin concentration of 150 g x $l^{-1}$ and 95% saturation, arterial blood contains about 9 µmol of oxygen per mL, and arterio-venous oxygen difference is about 3 µmol x $mL^{-1}$. Thus, if CBF is reduced to about 1/3, theoretically, all oxygen will be extracted. For glucose, the following approximate levels have been described: arterial concentration 5.1 mmol x $mL^{-1}$; venous concentration 4.6 mmol x $mL^{-1}$; arterio-venous glucose difference 0.5 mmol x $mL^{-1}$. Accordingly, during a gradual decrease in CBF, oxygen supply to the brain will be insufficient before the supply of glucose is seriously jeopardised. (27) Continued blood loss, will eventually lead to reduced CBF and the delivery of substrates, resulting in cerebral ischemia, hypoxia, and ultimately, cell death. (28)

## Vasopressors during haemorrhagic shock

European guidelines on the management of major bleeding recommend the use of vasopressors in the face of life-threatening bleeding. (29) They recommend using norepinephrine as it has been used in treating other types of shock for many years. However, the use of norepinephrine is limited to use transiently. At the same time, efforts are being made to correct the hypovolemia and underlying course even when treatment with fluids is in progress and normovolemia hasn't yet been reached. Studies with an intact blood-brain barrier have shown that the use of norepinephrine has a minor decreasing effect on cerebral blood flow (5-10%). (30, 31) However, in a situation where the cardiac output cannot increase due to severe hypovolemia, the cerebral blood flow might be further compromised by administering norepinephrine. Animal models have also shown that norepinephrine is associated with increased cerebral vasoconstriction through alpha receptors in vessels ranging from nonsignificant and up to 20% constriction compared to baseline. (32-38) However, treatment with norepinephrine has been reported to result in an increase as well as a decrease in cerebral blood flow. (39-42) The net effect on cerebral metabolism is, however, not investigated thoroughly. The use and safety of vasoactive drugs during haemorrhagic shock is widely discussed and still controversial. The administration of norepinephrine is not advocated in North American trauma systems due to the belief that vasopressors will increase both morbidity and mortality. (43) In a North American single-centre study by Sperry *et al.*, mortality rates were compared between patients who did and did not receive early vasopressor therapy (defined as therapy within 12 hours of injury). The use of early vasopressors was associated with an increased mortality risk at 12h (hazard ratio 1.81) and 24 hours (hazard ratio 2.15). (44) In a Japanese single centre study, 40 patients with haemorrhagic shock were included in a retrospective study to evaluate the impact of vasopressor use. The study reported that high dose use and early treatment with vasopressors (primarily norepinephrine) were associated with increased mortality. Vasopressors use results in an Odds ratio of 21.32 (3.17-121.6) and vasopressor use < 1 hour after admission in an Odds Ratio of 10.56 (1.90-58.5) (95% CI) (45) European guidelines recommend using norepinephrine in haemorrhagic shock where it is not possible to keep up with transfusion. (29) Although the authors concluded that vasopressor use during haemorrhage are

frequent, the level of recommendation is still controversial. (46) Treatment with vasopressors may be beneficial in the late stages of shock where hormone production might be decreased, furthermore it might be required to maintain an adequate mean arterial pressure until arrival at hospital where the bleeding can be controlled. (18, 47-49) As hypothesised by Beloncle *et al.*, the difference in recommendations in the use of vasopressors between European and North American trauma systems may be because of a vast difference in the population of patients with haemorrhagic shock. North American trauma systems often encounter single penetrating lesions which resemble most animal models of haemorrhagic shock well. In contrast, European studies mainly encounter multi-traumatised patients with considerable tissue damage and, accordingly, inflammatory response. (46)

## Metabolites of respiration

Under normal circumstances, glucose is the preferred energy source for maintaining normal cerebral metabolism. Glucose is transported across the blood-brain barrier and into the cell's cytoplasm via facilitated transport down the concentration gradient. Via the enzymatic process of glycolysis, glucose is converted to pyruvate. One molecule of glucose yields two molecules of the reduced form of Nicotinamide adenine dinucleotide ($NAD^+$) and two molecules of Adenosine triphosphate (ATP) through this process. Next, pyruvate is converted to acetyl coenzyme A which enters the mitochondria and the tricarboxylic acid cycle. Each turn in the cycle converts one pyruvate molecule into $CO_2$, generating 3 NADH, 1 $FADH_2$, and 1 ATP.

NADH functions as the primary substrate in the electron transport chain generating ATP and the by-products carbon dioxide and water in the inner membrane of the mitochondria. The combined process of glycolysis, tricarboxylic acid cycle and electron transport chain yields a theoretical total of 38 molecules of ATP. (50)

Most of the pyruvates produced by glycolysis enter the tricarboxylic acid cycle. Under normal circumstances, about 5% of the pyruvate is converted to lactate in the cytoplasm. The calculated LP ratio describes the balance between cytoplasmatic lactate and pyruvate that is the cytoplasmatic redox state (Eq1). (27, 51-55)

$$\frac{[Lactate]}{[Pyruvate]} \times K_{LDH} = \frac{[NADH]\,[H^+]}{[NAD^+]}$$

The latter is primarily determined by tissue oxygenation and mitochondrial function.

When the blood supply to the brain becomes critical, oxygen is the first substrate that becomes inadequate. During insufficient oxygen supply, the lactate to pyruvate ratio (LP ratio) increases instantaneously, and if oxygenation is rapidly restored, it returns to a normal or near-normal level. (56) When blood supply deteriorates further, glucose concentration decreases which efficiently holts the tricarboxylic acid cycle and electron transport chain resulting in depletion of ATP and a lasting increase in LP ratio. However, the compromised cerebral oxidative metabolism can also be following mitochondrial dysfunction where the supply of glucose and oxygen is abundant, increasing lactate simultaneous with a lesser or non-existing increase in pyruvate. (53, 57, 58)

There are several ways of examining the state of the cerebrum, e.g., near-infrared spectroscopy (NIRS), PET-CT, invasive flow measurements of the carotid artery, non-invasive transcranial doppler sonography, etc. All the techniques mentioned above have their strengths and limitations. (59, 60) Only a few of the mentioned techniques examine the cerebrum on a cellular level, whereas most of them only give information about cerebral function in indirect measures.

## Microdialysis technique

The present studies use the well-established microdialysis technique to examine the effect of haemorrhagic shock on cerebral metabolism. Microdialysis has been used for research since the mid-1970s. Microdialysis catheters are, in their simplicity, a biosensor that transports samples of molecules from the interstitial space out of the body for analysis. A microdialysis catheter consists of a double lumen catheter divided by a semipermeable membrane at its distal end. A standard perfusion fluid is pumped through the catheter at a specific rate, and the semipermeable membrane allows for the diffusion of molecules from the interstitial space to the dialysate. The recovery of a particular substance is defined as the concentration in the dialysate expressed as a per cent of the concentration in the interstitial fluid. Depending on the membrane length and flow rate, recovery can range from 70-100% of the interstitial concentrations. In the human brain, the typical perfusion flow is 0.3 µl/min, and the membrane length is usually 10 mm. Under these conditions, the recovery has been estimated to be approximately 70%. (61) Commercial products are available to analyse the dialysate employing enzymatic reagents and colourimetric measurements immediately. Microdialysis has become a standard technique for analysing both products of metabolism and larger molecules in animal studies. (62-64) During the last 20 years, it has become a clinically useful tool for evaluating cerebral and peripheral tissue metabolism. (65, 66) Monitoring with microdialysis techniques is considered good clinical practice as a supplement to ICP and $PbtO_2$ monitoring, especially in neurotrauma and intracerebral haemorrhages. (61-63)

However, measuring metabolites by microdialysis requires a small burr hole and the insertion of a foreign object into brain tissue. Although the procedure itself is minor, there may arise complications in the form of intracranial bleeding, infection, and trauma. Furthermore, the microdialysis probe only reflects the cellular metabolism in a few $mm^3$ surrounding the semipermeable membrane, making appropriate documentation about positioning in relation to a focal lesion essential to interpret the data acquired correctly. (54) During neurocritical care, information regarding the global cerebral energy state in addition to the regional information obtained from conventional intracerebral microdialysis would be valuable. Such information would also be important during critical care of other severe conditions when cerebral energy metabolism may be jeopardised without focal lesions (e.g., cardiac bypass surgery, resuscitation after cardiac arrest, septic shock). (67, 68) However, it is difficult or impossible to insert intracerebral catheters in these conditions for various reasons.

The thesis aims at defining the concept of permissive hypotension based on the variables related to cerebral energy metabolism obtained by microdialysis and routine biochemical analyses (lactate, pyruvate, glucose, glutamate, glycerol):

1. What is the biochemical pattern when the decrease in MAP causes pronounced ischemia (depletion of oxygen and substrate) resulting in irreversible cell damage?
2. What is the biochemical pattern when the decrease in MAP causes a moderate reduction of CBF (pronounced decrease in oxygen at continued supply of substrate) compatible with a normalization at re-transfusion?
3. What is the biochemical pattern when pronounced hypotension due to haemorrhagic shock is treated with norepinephrine to normalize MAP?
4. Is it possible to use microdialysis of the draining venous cerebral blood to evaluate perturbation of global cerebral energy metabolism during haemorrhagic shock?

# Method:

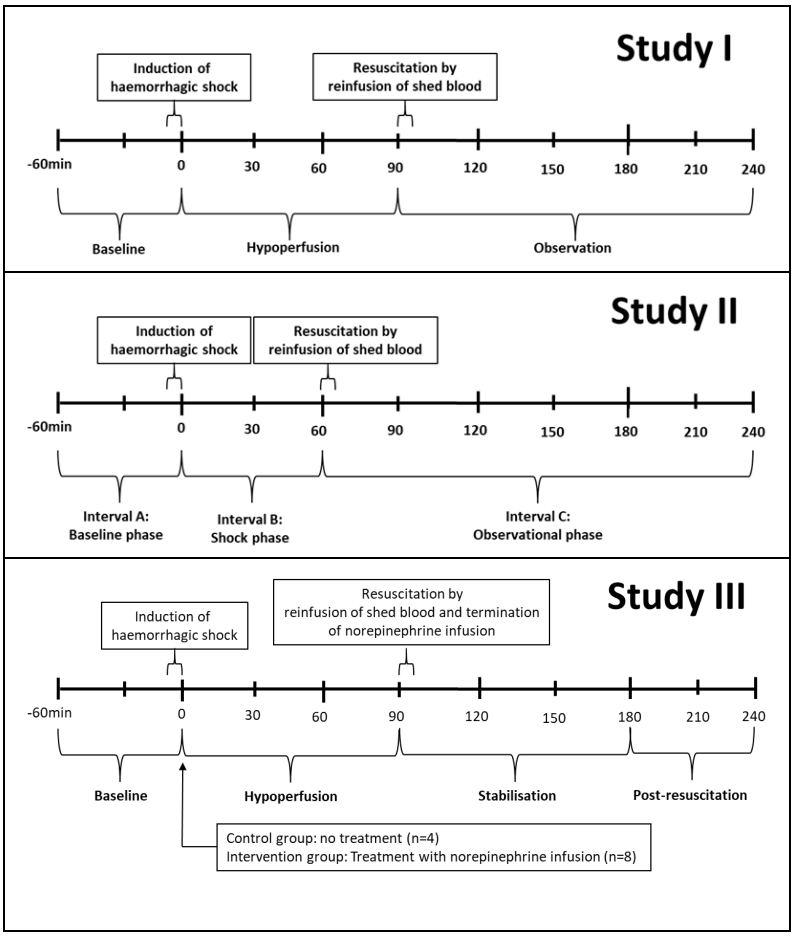
## Experimental studies

Study I, II, and III were all designed as experimental animal studies. All studies were approved by the Danish Animal Experiments Inspectorate (Dyreforsøgstilsynet). (approval no. 2020-15-0201-00478 and 2015-15-0201-00788). A comparable experimental protocol timeline for the three studies is seen in figure 1

Study I was designed to evaluate the impact of severe and prolonged haemorrhagic shock on cerebral energy metabolism, including brain tissue oxygenation and intracranial pressure. A secondary endpoint was to examine if microdialysis from cerebral venous blood could be used as a surrogate marker for global cerebral metabolism.

Study II was designed to evaluate the impact on cerebral metabolism during a moderate decrease in CBF (pronounced decrease in oxygen at continued supply of substrate). A secondary endpoint was to investigate if microdialysis on cerebral venous blood could be used as a surrogate marker for global cerebral metabolism.

*Figure 1: Overview of the differences in the experimental protocol's timeline for studies I, II, and III*




Study III was designed to evaluate treatment with the vasopressor norepinephrine compared with no treatment on cerebral energy metabolism during haemorrhagic shock. The length of prolonged hypotension was arbitrary decided to be 90min. Preliminary pilot studies had shown that the combination of MAP=40mmHg and duration of hypotension for 90min would result in pronounced ischemia (depletion

of oxygen and substrate).

## Anaesthesia, ventilation, and fluid management

Anaesthesia, ventilation, and fluid management were the same in all three studies. Female Danish Landrace Mix pigs from a local breeder (not EU registered) weighing approximately 40Kg and four months old were allowed for acclimatisation for one week before the day of their experiment. After that, they fasted overnight but with access to ad libitum water.

On the day of the experiment, sedation was achieved with an intramuscular injection of medetomidine, midazolam, and atropine. After adequate sedation was reached, the pigs were transferred to a preparation room where peripheral intravenous access was achieved by cannulation of veins in both earlobes with standard IV 20G cannulas. Anaesthesia was induced by injection of ketamine and midazolam. After sufficient anaesthesia was achieved, the animals were intubated and transferred to the operating theatre. The anaesthesia was maintained with an infusion of midazolam and fentanyl. The animals were volume-control ventilated with a tidal volume of 10mL/Kg and $FiO_2$ of 0.30. $PaCO_2$ was kept between 4 and 6 kPa, and body temperature around normal 38.5°C. Crystalloid intravenous fluid 2-4 mL/Kg/h was administered until the start of the experimental protocol.

## Surgical preparation, multimodal monitoring, and experimental protocol

### Study I

Four pigs were used to test the feasibility of the model. After the specific model was established, six animals were included in the study. The study used female Danish Landrace mix breed approximately four months old, weighing 40Kg.

The papers from studies I, II and III describe a detailed description of the methods involved. (68, 69) An intravenous sheath was placed by sonographic guidance or surgical cutdown in the right carotid artery allowing continuous measurement of invasive blood pressure (IBP) and arterial blood gas sampling. A second sheath was placed in one femoral artery to induce and maintain haemorrhagic shock.

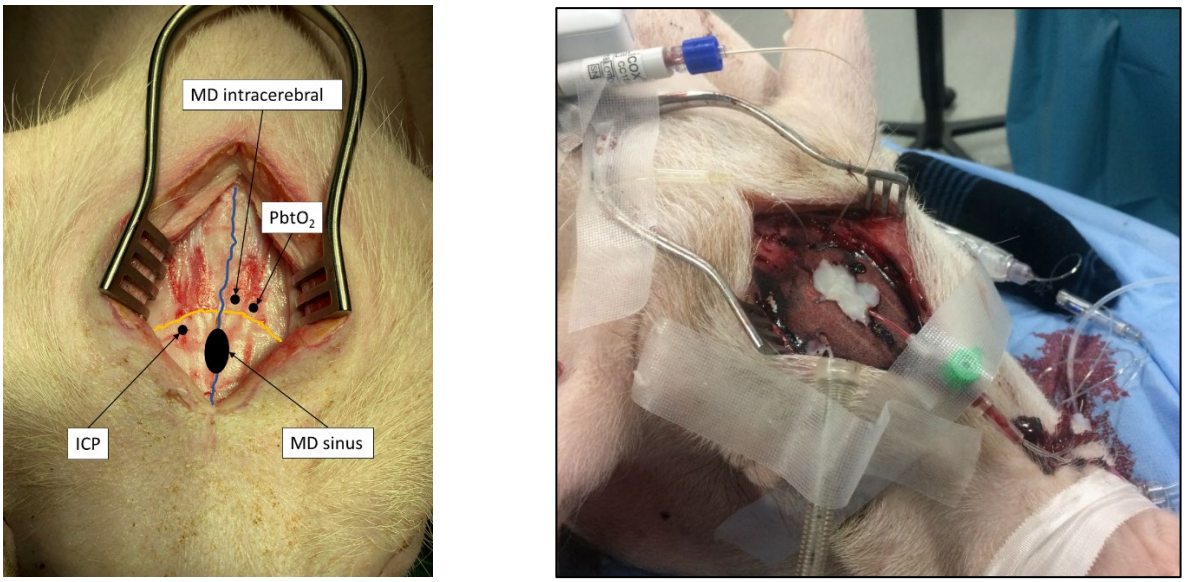
To analyse concentrations of the metabolites lactate, pyruvate, glucose, glutamate, and glycerol, a standard 18G peripheral venous catheter was placed via cutdown in the contralateral femoral artery, and a microdialysis catheter (CMA 67, MDialysis, Stockholm, Sweden) was inserted. A small craniotomy was placed in the frontal bone in the midline above the superior sagittal sinus. The sinus was cannulated with an 18G peripheral venous catheter, and one microdialysis catheter (CMA 70 Mdialysis, Stockholm, Sweden) was introduced in a posterior direction and placed in the posterior part of the superior sagittal sinus. The superior sagittal sinus was chosen for analysis of cerebral venous blood due to the anatomic characteristics of the experimental animal. Most of the cerebral blood is drained via the paraspinal venous plexus in the pig, and only a minor part passes into the internal jugular vein. (70) A third microdialysis catheter (CMA 70, Mdialysis AB, Stockholm, Sweden) was inserted 20 mm into the left parietal lobe through a small burr hole just superior and lateral from Bregma. One probe for monitoring brain tissue oxygenation ($PbtO_2$) (Licox CC1SB, Integra Neurosciences Ltd. New Jersey, USA) was introduced 15 mm into the contralateral parietal lobe. A transducer was placed in the right hemisphere to monitor intracranial pressure (ICP) (Camino, Integra Neurosciences Ltd. New Jersey, USA).

After insertion, all probes were allowed to stabilise for a minimum of 1 hour before starting baseline measurements. Before insertion, the Licox CC1SB probe was tested against atmospheric air, and after

insertion, correct placement was tested with an oxygen challenge test. Vital parameters, ICP and $PbtO_2$, were recorded every 10min, ABG samples were analysed every 30 minutes (ABL800 FLEX. Radiometer, Denmark). Microdialysis catheters were perfused with artificial CSF (Mdialysis) at a rate of 0.3µl/min. Vials were changed every 30min and immediately colourimetric analysed (IscusFlex Analyses Mdialysis).

All animals were given a baseline injection of heparin 200 IU/Kg and a supplemental dose of 100IU/Kg every hour for anticoagulation and catheter patency during the haemorrhage period.

*Figure 2: Image of insertion points of $PbtO_2$ and microdialysis catheters in the left hemisphere and ICP probe in the right hemisphere. Note the intravenous cannula inserted in the superior sagittal sinus through a small midline craniotomy (closed with bone wax after cannulation) Blue line: sagittal suture, yellow line: coronal suture, MD: microdialysis*




After stabilisation of probes, a 1-hour baseline period was started. The model of haemorrhagic shock has been described earlier. (71, 72) Haemorrhagic shock was then induced by bleeding the animals at a rate of 2.15mL/Kg/min for the first 7 min then 1.15mL/Kg/min the remaining period until a target MAP of 40mmHg was reached. During the next 90min blood was continuously withdrawn or reinfused to keep a MAP of around 40mmHg. Withdrawn blood was stored in buffered citrated glucose solution at 37°C. After 90min of hypoperfusion, the remaining withdrawn blood was reinfused at a rate of 120mL/min, mimicking arrival and resuscitation at a hospital. The animals were then observed for 150 minutes after resuscitation. At the end of the study, all animals were euthanised while under anaesthesia by an intravenous injection of pentobarbital 200mg/mL in concentrated alcohol.

### Study II

Four initial animals confirmed the feasibility of the study. This was followed by six additional female pigs approximately four months old, weighing 40Kg. The breed was Danish Landrace mix.

Anaesthesia, surgical, preparation and multimodal monitoring in study II are the same as in study I.

According to study I, after stabilisation of probes and the 1-hour baseline recordings, the haemorrhagic shock was induced. After target MAP of 40mmHg was reached, further withdrawal and reinfusion were

continued for 60min after which the pigs were resuscitated with autologous blood and observed for 180 min. At the end of the experiment, all animals were euthanised under anaesthesia with an injection of pentobarbital.

### Study III

Study III was conducted with an intervention group and a control group. Anaesthesia, surgical preparation, and multimodal monitoring were the same in the two groups. Eight animals were included in the intervention group and 4 in the control group. The study used female Danish Landrace mix approximately four months old, weighing 40Kg.

Anaesthesia, surgical preparation, and multimodal monitoring in study III were the same as in study I.

After stabilising probes and 1-hour of baseline recordings, the haemorrhagic shock was induced according to studies I and II.

The control group experiments were the first to be conducted. Animals in the control group were kept at a MAP of about 40 mmHg by withdrawing or infusing After induction of shock and MAP=40mmHg was reached, we continued to withdraw or reinfuse shed blood to keep a MAP of 40mmHg. The shed blood was stored in a citrated glucose solution at 37°C . The amount of blood withdrawn or reinfused which was needed to maintain a MAP=40mmHg were recorded at intervals of 10min. Following 90 min of haemorrhagic shock, the animals were resuscitated by re-infusing the remaining shed blood at a rate of 120 mL/min until all blood was returned. After resuscitation the pigs were observed for 150 min (figure. 1).

When animals in the NE group after haemorrhage had reached a MAP of 40 mmHg a weight adjusted infusion with norepinephrine (0.03 mg/kg in 50ml isotonic NaCl) was started. The rate of norepinephrine infusion was titrated until a MAP of 80 mmHg was reached. During the following 90min blood was withdrawn or reinfused in the same amount and rate as determined during the control group experiments. (table 1) Following the 90 min of haemorrhagic shock in the control group, the animals were resuscitated by re-infusing the remaining shed blood at a rate of 120 mL/min until all blood was returned. After resuscitation norepinephrine was titrated down and finally terminated (figure. 1).

## Statistics

### Study I

Due to this study's exploratory nature, we did not conduct a power calculation or adjust for multiple testing. Therefore, data are given as median (interquartile range) unless otherwise noted. To test the hypothesis, the time course of the LP ratio in the sagittal sinus and femoral artery were modelled with a mixed effect model for repeated measurements. The time and location of each microdialysis prober were fixed effect parameters and each animal as random effect parameter. Data were analysed with Stata 11.1 statistical software (StataCorp, College Station, TX, USA).

### Study II

Data were divided into three predefined intervals to test the hypothesis: Baseline, hypoperfusion, and observation phase. Each animal was their control, comparing the baseline LP ratio with LP ratios obtained after resuscitation in the observational phase. The data were modelled with a mixed-effect model with interval as fixed effect and each animal as random effect. Data were reported as median (interquartile range) unless otherwise noticed. Due to this study's exploratory nature, we did not calculate a sample size

or adjust for multiple testing. All P-values are to be considered exploratory. Data analysis were conducted with Stata 15 statistical software (StataCorp, College Station, TX, USA).

### Study III

The experimental protocol was divided into four intervals to compare the LP ratio between the control and intervention group. 1) baseline before induction of haemorrhagic shock, 2) hypoperfusion period for 90min, 3) Stabilisation period, and 4) post-resuscitation period. Earlier experiences have shown the cerebral metabolism might return to its baseline values if sufficient times are allowed to return to normal haemostasis. To test the LP ratio during the experiment, longitudinal data were modelled with a mixed-effect model with treatment group as fixed effect and each animal as random effect. Non-longitudinal data were tested with the non-parametric Mann Whitney U test. Data are median (interquartile range) unless otherwise noted. Due to the study's exploratory nature, we did not calculate sample size or adjust for multiple testing. All P-values are to be considered exploratory. Data were analysed with Stata 16 statistical software (StataCorp, College Station, TX, USA).

# Results

## Study I

All six animals completed the experimental protocol. Table 1 gives the general and physiological variables from study I. The median weight of the six animals included in the study were 42 (35-45Kg). At the baseline level, all physiological and biochemical variables were within normal human limits. Blood loss was 1072 mL (964-1498 mL), and median blood loss per Kg was 31 mL/Kg (26-37 mL/Kg), corresponding to approximately 48% of the total blood volume. During induction of shock, $PbtO_2$ decreased simultaneously with MAP. After resuscitation with shed blood, MAP increased to near baseline values. $PbtO_2$ did, however, stay at very low levels throughout the observational phase. (figure 3) During the observational period, ICP increased to a maximum of 29 (24-39mmHg) which is above the normal upper threshold in humans. During the studied period systemic lactate level and diuresis recovered.

*Table 1. General physiological and biochemical variables doing haemorrhagic shock in study I. Data are expressed as median levels (interquartile range). MAP: mean arterial pressure, ICP: intracranial pressure, CPP: Cerebral perfusion pressure, $PbtO_2$: Brain tissue oxygenation. S indicates the start of bleeding to achieve a MAP of 40 mmHg. N=6*

| Elapsed Time | MAP | ICP | CPP | $PbtO_2$ | PaO2 | PaCO2 |
|---|---|---|---|---|---|---|
| min | mmHg | mmHg | mmHg | mmHg | kPa | kPa |
| S -60 | 102 (96-111) | 7 (3-11) | 95 (90-106) | 16 (14-27) | 25 (24-27) | 6.0 (5.2-6.1) |
| S | 77 (73-99) | 8 (2-12) | 72 (64-93) | 22 (16-31) | 24 (23-25) | 5.7 (5.4-6.0) |
| 0 | 40 (40-40) | 8 (6-8) | 32 (28-34) | 13 (7-18) | 24 (23-25) | 5.6 (5.5-5.6) |
| 30 | 37 (33-39) | 5 (1-9) | 29 (29-31) | 5 (2-8) | 25 (25-26 | 5.9 (5.2-6.5) |
| 60 | 34 (32-36) | 5 (0-8) | 29 (28-31) | 2 (1-3) | 24 (24-25) | 5.7 (5.2-6.0) |
| 90 | 31 (31-33) | 6 (0-13) | 32 (25-43) | 1 (1-2) | 26 (26-28) | 5.0 (4.6-7.2) |
| 120 | 59 (46-84) | 17 (13-22) | 51 (38-62) | 3 (1-4) | 25 (24-26) | 5.6 (4.5-6.3) |
| 150 | 60 (58-65) | 19 (14-28) | 46 (46-47) | 11 (1-21) | 22 (22-24) | 6.5 (5.6-7.3) |
| 180 | 63 (61-99) | 27 (20-34) | 42 (41-65) | 1 (1-9) | 23 (22-23) | 5.1 (4.6-5.7) |
| 210 | 61 (59-65) | 23 (21-31) | 42 (40-42) | 1 (1-5) | 22 (21-22) | 5.4 (4.8-5.6) |
| 240 | 73 (50-104) | 29 (24-39) | 47 (39-49) | 1 (1-1) | 22 (21-23) | 6.1 (5.4-6.4) |
| Elapsed Time | b-hemoglobin | HR | b-Glucose | b-Lactate | b-pH | Diuresis |
| min | mmol/L | bpm | mmol/L | mmol/L | | mL |
| S -60 | 5.7 (5.3-5.8) | 78 (75-84) | 6.8 (6.4-7.1) | 1.5 (1.3-1.7) | 7.43 (7.40-7.47) | 40 (4-106) |
| S | 5.6 (5.2-5.9 | 90 (78-93) | 7.2 (6.8-7.7) | 1.2 (1.0-1.4) | 7.45 (7.43-7.46) | 115 (82-160 |
| 0 | 4.9 (4.7-4.9) | 135 (107-138) | 9.7 (8.9-9.8) | 2.5 (2.0-3.0) | 7.41 (7.41-7.43) | 12 (0-23) |
| 30 | 4.6 (4.5-4.7) | 116 (111-146) | 8.6 (7.7-14.4) | 5.3 (3.7-7.4) | 7.34 (7.32-7.38) | 4 (3-5) |
| 60 | 5.2 (4.9-5.5) | 166 (129-191 | 7.9 (6.2-13.1) | 7.2 (4.7-11.1) | 7.30 (7,25-7.41) | 2 (1-5) |
| 90 | 5.2 (5.2-5.4) | 125 (114-168) | 7.0 (4.8-9.2) | 10.3 (6.0-10.3) | 7.20 (7.18-7.31) | 3 (2-10) |
| 120 | 5.1 (4.6-5.6) | 98 (93-116) | 6.2 (5.2-7.0) | 5.5 (4.4-6.5) | 7.29 (7.21-7.34) | 16 (5-31) |
| 150 | 5.2 (5.1-5.6) | 101 95-120) | 6.4 (4.9-7.6) | 4.8 (3.6-6.9) | 7.20 (7.19-7.24) | 20 (17-25) |
| 180 | 5.5 (5.2-5.8) | 110 (103-113) | 6.5 (5.7-8.5) | 2.5 (2.1-3.7) | 7.43 (7.33-7.50) | 70 (25-70) |
| 210 | 5.5 (5.3-5.7) | 122 (88-128) | 4.5 (3.9-5.4) | 2.1 (1.7-3.3) | 7.43 (7.39-7.43) | 45 (5-45) |
| 240 | 5.8 5.7-5.8) | 17 (105-121) | 5.5 (4.6-6.1) | 2.0 (1.2-3.0) | 7.36 (7.33-7.47) | 18 (5-100) |

*Statistical significant p-value <0.05

### *Microdialysis*

Table 2 gives the biochemical values obtained by microdialysis in the hemisphere, sagittal sinus, and femoral artery.

Before induction of haemorrhagic shock, the LP ratios were similar in all three compartments. After induction of haemorrhagic shock, a pronounced increase in hemisphere LP ratio was seen. The increase was due to a decrease in pyruvate, and a pronounced and lasting increase in lactate, which lasted throughout the observational period. During haemorrhagic shock, hemispheric glucose concentration decreased to very low levels, which lasted until the end of the experiment despite normal arterial concentrations. Both glutamate and glycerol increased throughout the experiment. After induction of shock, the LP ratio from the sagittal sinus increased simultaneously with the hemispheric LP ratio. However, the increase did not reach the same levels as the hemisphere LP ratio but only about 1/3. The increase in sinus LP ratio was

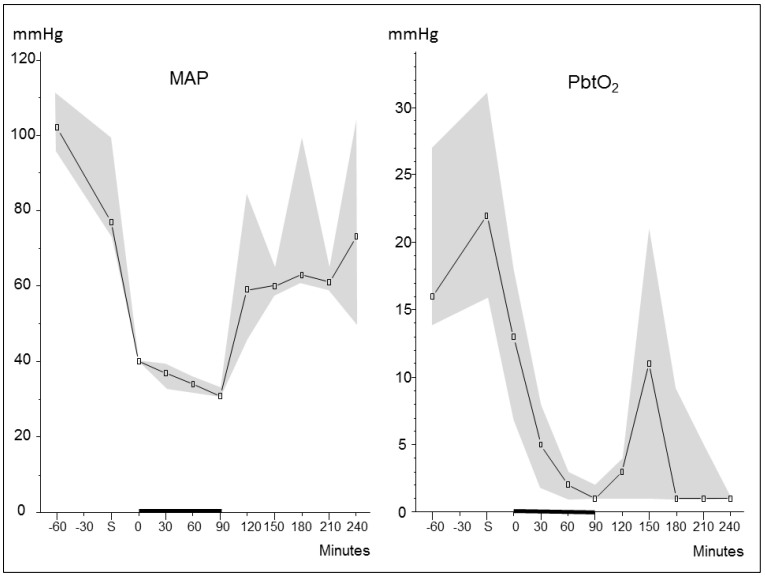



*Figure 3 Median (interquartile range) arterial pressure (MAP) and brain tissue oxygen tension ($PtbO_2$) in pigs with induced haemorrhagic shock. (study I) Note that during the period of haemorrhagic shock, the declining MAP was accompanied by a decrease in $PbtO_2$ to a very low level (< 5 mmHg). After re-infusion of autologous blood, MAP increased to close to baseline level, whereas $PbtO_2$ remained very low. S indicates the start of bleeding to achieve a MAP of 40 mmHg*

*Table 2. Biochemical variables obtained from microdialysis in study I. Data are expressed as median (interquartile range). LP: lactate/pyruvate ratio. S indicates the start of bleeding to achieve a MAP of 40 mmHg. N=6*

| Elapsed Time | | LP-ratio | | | Lactate mmol/L | | | Pyruvate µmol/L | |
|---|---|---|---|---|---|---|---|---|---|
| min | Hemisph. | Sag. Sinus | Femoral | Hemisph. | Sag. Sinus | Femoral | Hemisph. | Sag. Sinus | Femoral |
| S -60 | 15 (14-18) | 13 (9-16) | 11 (11-19) | 2,7 (1,7-3,0) | 2,1 (1,7-2,3) | 1,6 (1,1-1,9) | 131 (107-178) | 111 (106-123) | 141 (99-160) |
| S -30 | 13 (11-16) | 13 (6-16) | 12 (10-17) | 2,9 (2,4-3,5) | 2,2 (2,2-2,2) | 2 (1,7-2,6) | 191 (151-205) | 200 (193-371) | 166 (161-175) |
| S | 15 (11-18) | 14 (8-26) | 13 (10-21) | 2,4 (2,1-3,1) | 3,4 (2,6-5,2) | 2,2 (1,6-3,4) | 185 (153-210) | 220 (161-388) | 137 (131-153) |
| 0 | 20 (17-41) | 45 (32-46) | 33 (30-36) | 7,6 (7,2-8,0) | 11,1 (10,5-11,7) | 3 (2,3-4,0) | 110 (107-149) | 178 (144-208) | 181 (129-193) |
| 30 | 60 (27-117) | 36 (23-63) | 27 (26-27) | 10,3 (7,8-11,0) | 6,8 (4,6-12,1) | 3,8 (3,7-4,4) | 143 (92-163) | 222 (147-230) | 181 (111-187) |
| 60 | 169 (45-418) | 85 (36-112) | 28 (26-35) | 14,6 (11,8-17,2) | 9 (6,6-13,2) | 5,5 (5,2-6,8) | 85 (43-137) | 219 (128-272) | 214 (144-286) |
| 90 | 351 (105-774) | 78 (42-117) | 29 (25-33) | 14,9 (10,2-18,8) | 10,2 (7,0-13,6) | 7,8 (4,2-9,3) | 36 (21-49) | 175 (111-208) | 312 (256-348) |
| 120 | 704 (97-1454) | 94 (63-135) | 29 (25-35) | 12,9 (10,2-17,2) | 9,7 (8,0-12,0) | 9,7 (4,0-12,9) | 28 (9-64) | 129 (68-232) | 305 (191-348) |
| 150 | 879 (115-1238) | 123 (36-332) | 34 (24-37) | 11,2 (10,6-15,9) | 8,9 (8,0-13,8) | 6,4 (3,0-10,9) | 24 (9-210) | 80 (29-269) | 266 (162-308) |
| 180 | 724 (264-2479) | 150 (63-308) | 30 (22-34) | 12,9 (9,7-14,6) | 8,8 (6,8-13,1) | 7 (3,5-9,8) | 38 (6-158) | 90 (34-168) | 215 157-274) |
| 210 | 846 (243-1990) | 309 (103-488) | 27 (21-31) | 13,4 (11,2-13,8) | 9,6 (8,0-14,1) | 4,4 (3,1-7,7) | 8 (2-94) | 81 (15-150) | 192 (171-286) |
| Elapsed Time | | Glucose mmol/L | | | Glutamate µmol/L | | | Glycerol µmol/L | |
| min | Hemisph. | Sag. Sinus | Femoral | Hemisph. | Sag. Sinus | Femoral | Hemisph. | Sag. Sinus | Femoral |
| S -60 | 2,4 (1,2-3,7) | 3 (1,3-4,0) | 5,2 (3,1-5,3) | 5 (5-13) | 130 (75-168) | 183 (168-200) | 27 (15-50) | 31 (17-57) | 34 (28-54) |
| S -30 | 2,9 (2,4-3,7) | 2,9 (1,1-3,7) | 4,1 (2,4-7,1) | 8 (3-11) | 162 (121-177) | 199 (159-209) | 26 (14-55) | 28 (24-63) | 34 (26-35) |
| S | 3,6 (2,7-4,1) | 1,7 (1,3-3,8) | 3,7 (2,1-6,5 | 6 (4-7) | 174 (104-204) | 180 (148-209) | 23 (13-41) | 31 (23-55) | 21 (20-29) |
| 0 | 1,7 (1,5-1,9) | - | 1,2 (0,7-1,6) | - | 87 (50-121) | 202 (169-221) | 40 (40-40) | 74 (49-100) | 12 (11-14) |
| 30 | 1,9 (1,2-2,4) | 1,3 (0,6-4,2) | 6,9 (1,3-9,2) | 19 (4-55) | 158 (84-159) | 185 (163-206) | 56 (32-108) | 53 (37-112) | 21 (8-32) |
| 60 | 1,0 (0,7-1,4) | 2 (0,02-2,2) | 3,8 (1,1-8,2) | 68 (36-101) | 156 (132-204) | 181 (158-204) | 180 (133-201) | 96 (53-159) | 33 (14-117) |
| 90 | 0,4 (0,3-0,5) | 0,6 (0,02-1,5) | 5,4 (1,7-7,6) | 119 (119-119) | 225 (204-248) | 172 (149-195) | 329 (254-337) | 120 (102-190) | 89 (29-486) |
| 120 | 0,2 (1,0-0,3) | 0,3 (0,04-0,7) | 3,4 (1,2-7,3 | 245 (199-291) | 209 (209-209) | 192 (152-198) | 415 (318-455) | 192 (123-258) | 83 (23-671) |
| 150 | 0,1 (0,1-1,0) | 0,1 (0,02-0,5) | 3,1 (0,5-5,6) | 327 (231-329) | 199 (152-259) | 153 (107-177) | 403 (377-548) | 222 (151-286) | 76 (19-621) |
| 180 | 0,2 (0,1-1,3) | 0,1 (0,02-0,2) | 1,8 (0,5-5,9) | 335 (289-367) | 198 (133-256) | 173 (139-180) | 382 (333-547) | 213 (191-230) | 71 (18-314) |
| 210 | 0,3 (0,1-0,9) | 0,02 (0,02-0,7) | 5,5 (0,9-8,1) | 311 (232-322) | 257 (227-287) | 186 (184-188) | 522 (474-612) | 321 (268-331) | 101 (26-182) |

due to a lasting increase in lactate and a decrease in pyruvate. The increase in femoral lactate concentration was accompanied by a simultaneous increase in pyruvate, limiting the femoral LP ratio. During the hypoperfusion period, sagittal sinus glucose decreased to a very low level and remained low throughout the experiment. Glucose measured both by microdialysis and by measuring arterial blood gas samples were within normal limits throughout the experiment. Both glutamate and glycerol increased

during the experiment in the sagittal sinus. Glycerol also increased in the femoral artery in contrast to glutamate, which remained stable.

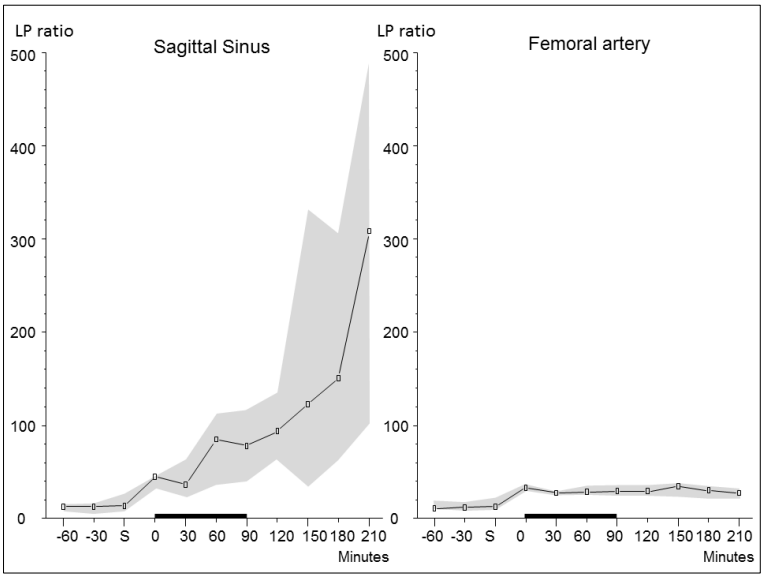



*Figure 4:* Comparison of LP ratio in sagittal sinus and femoral artery during 90min of haemorrhagic shock in pigs. Data are given as median (interquartile range). The increase in the LP ratio in the sagittal sinus was significantly higher ($p<0.001$) than in the arterial blood.

## Study II

All ten animals completed the experimental protocol. However, one animal showed signs of irreversible metabolic crisis and was excluded from the group analysis. The median weight of animals was 42 (35-45) Kg. The median volume of shed blood was 1469 (1378.6-1583.44mL), corresponding to 35mL/Kg and about 53% of total blood volume. General physiological variables from 9 of the experimental animals are shown in table 3. After initiation of haemorrhagic shock, MAP quickly decreased to approximately 40mmHg. With a moderate delay, $PbtO_2$ decreased to a low level (**Figure 5**). After resuscitation, MAP increased but did not reach baseline. In contrast, after resuscitation, $PbtO_2$ increased and exceeded baseline level (Figure 5). Blood levels of $O_2$, $CO_2$, pH and glucose were kept within physiological range throughout the experiment. ICP were within normal limits throughout the study.

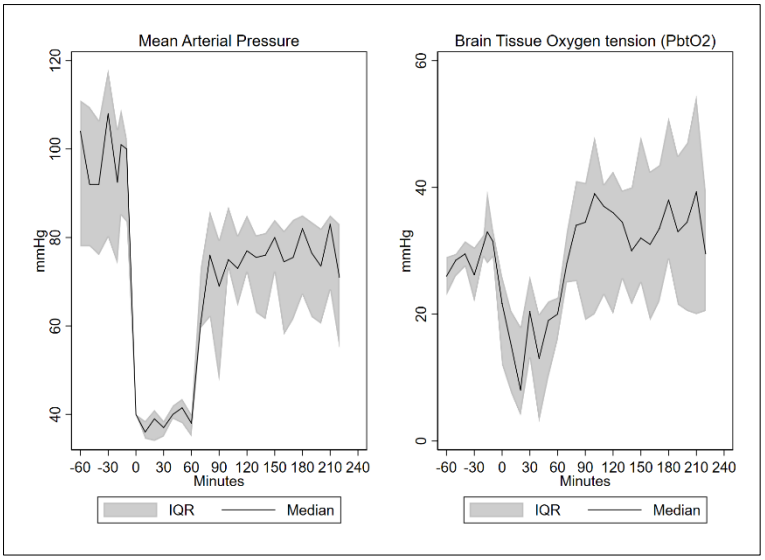



*Figure 5: Median (Interquartile range) Mean arterial pressure and PbtO2 in study II. (n = 9) Note that during the period of haemorrhagic shock the declining MAP was accompanied by a decrease in PbtO2 near critical levels (< 15 mmHg). After re-infusion of autologous blood MAP increased to close to baseline level whereas PbtO2 increased to levels above baseline. Time 0 indicates achievement of MAP equal to 40 mmHg.*

### *Microdialysis*

In three of the ten animals, the sagittal sinus catheters showed low levels of glutamate (<20 µmol/l), indicating that the catheter had dislocated into the hemisphere parenchyma. The data from these catheters was excluded from the further analysis.

*Table 3: General physiological and biochemical variables doing haemorrhagic shock in study II. N = 9. Data are expressed as median levels (interquartile range) during the experimental protocol intervals A, B, and C. MAP mean arterial pressure, ICP intracranial pressure, CPP cerebral perfusion pressure, $PbtO_2$ brain tissue oxygenation. S indicates the start of haemorrhage. Time 0 indicates achievement of MAP equal 40 mmHg. Test statistics were performed with a mixed effect model.*

| Elapsed time (min) | Baseline phase Interval A (-60) – 0 min | Shock and resuscitation phase Interval B 0 – 70 min | Observational phase Interval C 70 – 220 min | Interval A versus interval C p-value |
|---|---|---|---|---|
| MAP (mmHg) | 95 (66-107) | 39 (35-42) | 76 (67-84) | p < 0.005* |
| ICP (mmHg) | 8 (6.5-11) | 6 (5-9) | 10 (7-14) | p = 0.089 |
| CPP (mmHg) | 84 (60-97.5) | 33 (28-39) | 65 (53-79.5) | p < 0.005 * |
| $PbtO_2$ (mmHg) | 29 (24-32) | 19 (8-23) | 35 (20-45) | p < 0.005* |
| PaO2 (kPa) | 24 (22-26) | 24 (23-25) | 22 (22-24) | p = 0.002* |
| PaCO2 (kPa) | 5.4 (5,5-89) | 5.1 (4,7-5.6) | 6.1 (5.7-6.7) | p = 0.025* |
| b-hemoglobin (mmol/L) | 8.6 (5.8-9.8) | 7.9 (5.2-8.8) | 7.8 (6-9.3) | p = 0.084 |
| HR (bpm) | 80 (71-92) | 125 (82-152) | 81 (72-92) | p = 0.502 |
| b-Glucose (mmol/L) | 5.5 (4.3-7.2) | 6.3 (4.2-9.8) | 5.3 (4.1-6.9) | p = 0.010* |
| b-lactate (mmol/L) | 0.9 (0.5-1.2) | 4.3 (2.5-7.3) | 1.8 (0.9-5.1) | p = 0.009* |
| b-pH | 7.47 (7.45-7.51) | 7.44 (7.39-7.44) | 7.40 (7.32-7.46) | p = 0.249 |
| Diuresis (mL) | 55 (10-160) | 3 (0-12) | 19 (6-32) | p < 0.005* |

*Statistical significant p-value <0.05

Data for all variables obtained from three different microdialysis catheter positions are shown in Table 4. The time course for the changes in intracerebral levels of lactate, pyruvate, and glucose as well as the LP ratio are illustrated in Figure 6. A moderate increase in LP ratio started immediately after induction of haemorrhagic shock (figure 6a) due to a marked increase in lactate and a less pronounced increase in pyruvate (figure 6b). Figure 6c shows that intracerebral glucose levels remained essentially unchanged. In the microdialysis catheter positioned in the superior sagittal sinus similar changes were observed for

lactate, pyruvate, and LP ratio. The interpretation of these data is however restricted by the limited number of observations.

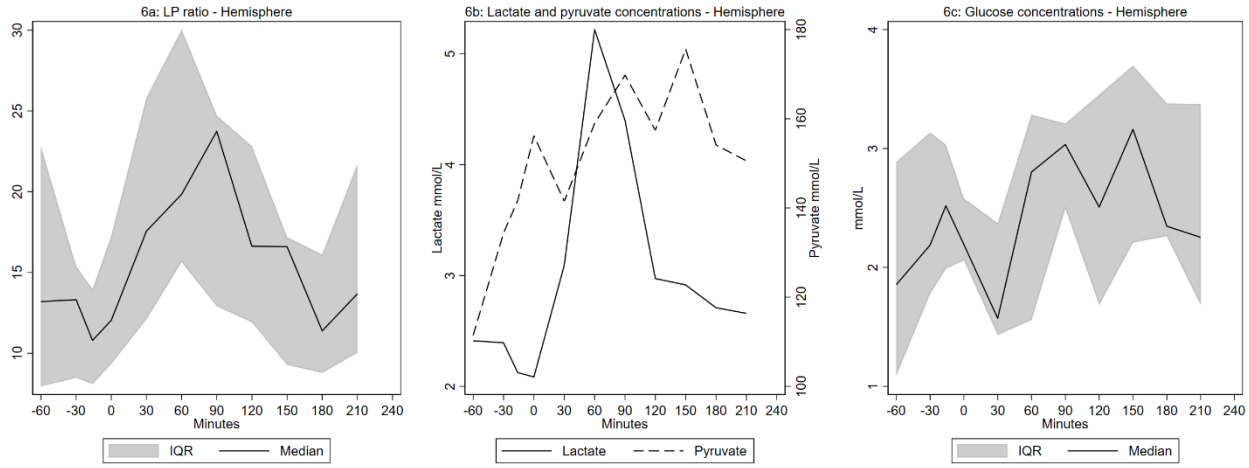



*Figure 6 Time course for the changes in intracerebral levels of lactate, pyruvate, and glucose as well as the LP ratio during study II. Data is expressed as median and (interquartile range). N=9*

*Table 4: Biochemical variables obtained from microdialysis in study II. N=9. Data expressed as median (interquartile range) during the experimental protocol interval A, B and C. LP lactate/pyruvate ratio. S indicates the start of haemorrhage. Time 0 indicates achievement of MAP equal 40 mmHg. Test statistics made with a mixed-effect model.*

| Elapsed time (min) | Location | Baseline phase Interval A (-60) – 0 min | Shock and resuscitation phase Interval B: 0 – 70 min | Observational phase Interval C: 70 – 220 min | Interval A versus interval C P-value |
|---|---|---|---|---|---|
| LP ratio | Hemisph. | 13 (8-16) | 19 (12-30) | 16 (10-23) | 0.020* |
| | Sag. Sinus | 9 (6-22) | 22 (19-26) | 17 (10-27) | 0.012* |
| | Femoral | 21 (18-24) | 28 (20-35) | 17 (15-25) | 0.467 |
| Lactate (mmol/L) | Hemisph. | 2.3 (1.3-3.0) | 3.2 (2.5-6.1) | 3 (2.3-4.5) | 0.000* |
| | Sag. Sinus | 0.9 (0.3-2.5) | 4.7 (3.4-6.3) | 2.6 (1.1-5.4) | 0.002* |
| | Femoral | 1.1 (0.8-1.8) | 4.6 (1.6-6.1) | 1.7 (0.8-3.9) | 0.009* |
| Pyruvate (mmol/L) | Hemisph. | 142 (98-203) | 147 (120-261) | 156 (127-249) | 0.000* |
| | Sag. Sinus | 109 (48-151) | 197 (187-313) | 153 (113-201) | 0.003* |
| | Femoral | 55 (44-75) | 151 (45-198) | 94 (53-154) | 0.000* |
| Glucose (mmol/L | Hemisph. | 2.2 (1.8-3) | 1.8 (1.4-3.1) | 2.5 (2-3.5) | 0.031* |
| | Sag. Sinus | 1.9 (1-3) | 3.5 (2.4-5.1) | 3.5 (2.3-4.5) | 0.000* |
| | Femoral | 4.9 (3.4-5.6) | 6 (4.9-7.3) | 5.5 (4.6-7.1) | 0.010* |
| Glutamate (µmol/L) | Hemisph. | 15 (4-22) | 12 (5-14) | 8 (3-11) | 0.050 |
| | Sag. Sinus | 121 (60-178) | 115 (94-176) | 177 (110-195) | 0.011* |
| | Femoral | 195 (172-205) | 190 (184-196) | 183 (173-231) | 0.181 |
| Glycerol (µmol/L) | Hemisph. | 38 (25-88) | 59 (35-116) | 77 (58-125) | 0.000* |
| | Sag. Sinus | 21 (7-55) | 69 (43-130) | 61 (34-123) | 0.006* |
| | Femoral | 15 (12-28) | 47 (22-174) | 46 (30-209) | 0.000* |

*Statistical significant p-value <0.05

## Study III

All animals completed the protocol. The median weight of the animals in the control group and the norepinephrine (NE) group were 43.5 (40.25-46.65Kg) and 41.9 (40.3-44.5Kg), respectively. The total amount of drained blood in the NE group was 21.36 (18.4-25.9mL/Kg) and 2655 (23.2-32.9mL/Kg) in the control group. This corresponds to approximately 33% and 41% of the total blood volume.

After induction of haemorrhagic shock, MAP decreased to very low levels in both the control and NE group. Table 5. Immediately after MAP 40mmHg was reached in the NE group, a weight-adjusted infusion with NE was started and titrated to a MAP of 80mmMg.

*Table 5: General physiological haemodynamic and systemic parameters for baseline, hypoperfusion and post-resuscitation periods in study III. NE group n=8, control group n=4. Values are medians (interquartile range). Test statistics were performed with a mixed effect model.*

| Interval | Control | NE | Control versus NE group p-value |
|---|---|---|---|
| **Baseline (-60) – 0 min** | | | |
| MAP (mmHg) | 88 (78-98) | 78 (72-95) | 0.198 |
| HR (bpm) | 56 (55-87) | 87 (74-103) | 0.016* |
| $PbtO_2$ (mmHg) | 22 (8-70) | 37 (30-40) | 0.880 |
| $PaO_2$ (kPa) | 53 (49-57) | 37 (36-40) | 0.001* |
| $PaCO_2$ (kPa) | 5,6 (5,4-6) | 6,4 (5,9-6,7) | <0.001* |
| b-hemoglobin (mmol/L) | 5,8 (5,6-6,3) | 5 (4,8-5,3) | <0.001* |
| b-Glucose (mmol/L) | 4,1 (3,7-6) | 5,8 (3,9-8,1) | 0.180 |
| b-lactate (mmol/L) ABG | 0,9 (0,5-1) | 0,5 (0,4-0,9) | 0.753 |
| b-pH ABG | 7,45 (7,44-7,48) | 7,46 (7,43-7,5) | 0.951 |
| Diuresis (mL) | 25 (0-72,5) | 0 (0-50) | 0.274 |
| **Hypoperfusion 0-90min** | | | |
| MAP (mmHg) | 41 (40-42) | 80 (75-86) | <0.001* |
| HR (bpm) | 108 (63-180) | 98 (85-115) | 0.625 |
| $PbtO_2$ (mmHg) | 8 (4-18) | 28 (23-34) | 0.160 |
| $PaO_2$ (kPa) | 52 (46,6-53,9) | 38 (35-41) | 0.019 |
| $PaCO_2$ (kPa) | 5,5 (5,2-6,3) | 6,3 (6,1-6,7) | 0.031* |
| b-hemoglobin (mmol/L) | 5,7 (5-6,1) | 5,1 (4,8-5,6) | 0.189 |
| b-Glucose (mmol/L) MD | 6 (3,8-7,0) | 4,9 (4,2-7,0) | 0.974 |
| b-lactate (mmol/L) ABG | 8,2 (2,7-13,7) | 1,1 (0,9-1,7) | 0.001* |
| b-pH ABG | 7,39 (7,23-7,4) | 7,46 (7,43-7,49) | 0.013 |
| Diuresis (mL) | 0 (0,35) | 11,5 (0-47,5) | 0.380 |
| **Post resuscitation 180-240min** | | | |
| MAP (mmHg) | 56 (47-79) | 74 (69-80) | 0.037 |
| HR (bpm) | 89 (76-107) | 90 (76-99) | 0.858 |
| $PbtO_2$ (mmHg) | 7 (2-10) | 29 (13-34) | 0.021 |
| $PaO_2$ (kPa) | 51(49-54) | 38 (37-42) | <0.001* |
| $PaCO_2$ (kPa) | 6,5 (5,3-6,8) | 6 (5,7-6,5) | 0.665 |
| b-hemoglobin (mmol/L) | 6,5 (6-6,7) | 4,6 (4,2-4,9) | <0.001* |
| b-Glucose (mmol/L) | 2,8 (0,7-3,2) | 3,6 (2,9-4,3) | 0.432 |
| b-lactate (mmol/L) ABG | 3,9 (2-8,4) | 0,5 (0,3-0,6) | 0.003* |
| b-pH ABG | 7,32 (7,29-7,39) | 7,47 (7,45-7,51) | <0.001* |
| Diuresis (mL) | 0 (0-30) | 0 (0-4) | 0.676 |

*Statistical significant p-value <0.05

The total amount of NE administered was 0.528mg (0.475-2.595mg). During induction of shock, $PbtO_2$ decreased in both groups, but the decrease was most pronounced in the control group. After resuscitation, the $PbtO_2$ increased in both groups. The increase in the NE group trending towards baseline values but not reaching them, whereas the increase in $PbtO_2$ in the control group was only momentarily. Unfortunately, due to equipment failure, ICP data from the control group were lost. The NE group data were all recovered and showed an increasing trend throughout the experiment. The ICP did not exceed normal human limits.

*Microdialysis*

As only two of the four pigs in the control group have glutamate levels indicating that the sinus catheter was correctly placed in the sagittal sinus (>20µmol/L) the biochemical analysis was restricted to data obtained from the intracerebral microdialysis catheters in the control and the NE-group. Table 6 gives the biochemical data obtained from intracerebral microdialysis. At baseline, there was no difference in LP ratio between the two groups. After induction of shock, the LP ratio in the control group increased significantly compared to the NE group. The increase in LP ratio in the control group was due to

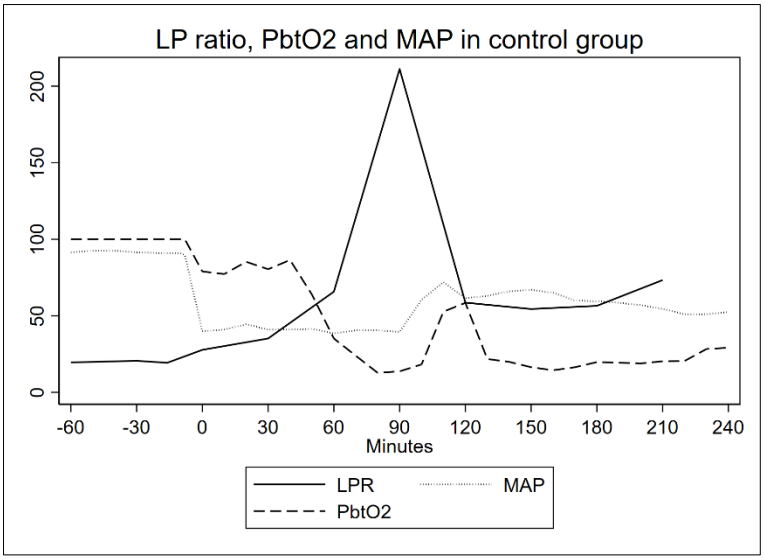



*Figure 7 Temporal comparison of intracerebral LP ratio, $PbtO_2$, and MAP in the contol group of study III. Values are median. MAP: mmHg, PbtO2= per centual change from baseline. N=4*

*Table 6: Biochemical variables obtained on microdialysis from cerebral hemisphere in study III. Values are divided into three-time intervals and expressed as median (interquartile range). Control n=4. NE group n=8. Test statistics were performed with a mixed effect model.*

| **Baseline** | **Location** | **Control** | **NE** | **Control versus NE group p-value** |
|---|---|---|---|---|
| LP ratio | Hemisph. | 20 (15-29) | 23 (18-27) | 0.834 |
| Lactate (mmol/L) | Hemisph. | 2.2 (1.4-3.8) | 2.1 (1.7-3.1) | 0.670 |
| Pyruvate (mmol/L) | Hemisph. | 100 (92-134) | 92 (73-108) | 0.643 |
| Glucose (mmol/L) | Hemisph. | 2.5 (1.8-2.8) | 2.6 (2.2-3.2) | 0.183 |
| Glutamate (µmol/L) | Hemisph. | 15 (9-72) | 12 (4-20) | 0.081 |
| Glycerol (µmol/L) | Hemisph. | 93 (58-149) | 62 (45-90) | 0.106 |
| | | | | |
| **Hypoperfusion** | **Location** | **Control** | **NE** | **Control versus NE group p-value** |
| LP ratio | Hemisph. | 66 (38-82) | 24 (19-31) | <0.001* |
| Lactate (mmol/L) | Hemisph. | 7.8 (6.2-12.3) | 2.5 (2.1-3.6) | <0.001* |
| Pyruvate (mmol/L) | Hemisph. | 104 (81-177) | 112 (91-132) | 0.670 |
| Glucose (mmol/L) | Hemisph. | 1 (0.4-1.6) | 2.9 (2.4-3.9) | <0.001* |
| Glutamate (µmol/L) | Hemisph. | 93 (6-155) | 8 (4-11) | 0.001* |
| Glycerol (µmol/L) | Hemisph. | 190 (75-270) | 59 (44-86) | <0.001* |
| | | | | |
| **Post resuscitation** | **Location** | **Control** | **NE** | **Control versus NE group p-value** |
| LP ratio | Hemisph. | 73 (29-100) | 25 (21-29) | 0.013* |
| Lactate (mmol/L) | Hemisph. | 4,9 (1-6.5) | 1.3 (0.8-1.6) | <0.001* |
| Pyruvate (mmol/L) | Hemisph. | 102 (71-185) | 108 (100-133) | 0.972 |
| Glucose (mmol/L) | Hemisph. | 0.7 (0.5-0.8) | 2.3 (1.6-2.9) | 0.003* |
| Glutamate (µmol/L) | Hemisph. | 47 (6-110) | 4 (2-7) | 0.005* |
| Glycerol (µmol/L) | Hemisph. | 461 (254-545) | 42 (23-55) | <0.001* |

*Statistical significant p-value <0.05

an increase in lactate and a concomitant decrease in pyruvate. After resuscitation LP ratio decreased but remained elevated during the rest of the experimenter. (Figure 7) This lasting increase in the LP ratio was due to a decreasing level of lactate. At the same time, pyruvate increased before once again decreasing to baseline levels. The LP ratio in the NE remained at baseline levels throughout the study.

# Discussion

The thesis aimed at defining the concept of permissive hypotension based on evaluating the pattern of variables related to cerebral energy metabolism obtained from microdialysis and routine biochemical analyses (lactate, pyruvate, glucose, glutamate, glycerol). The studies have studied the pattern in these variables obtained from intracerebral microdialysis and show

1. the pattern when the decrease in MAP caused pronounced ischemia (depletion of oxygen and substrate) resulting in irreversible cell damage
2. the pattern when the decrease in MAP caused a moderate reduction of CBF (pronounced decrease in oxygen at continued supply of substrate) compatible with a normalization at re-transfusion
3. the pattern when pronounced hypotension due to haemorrhagic shock is treated early with norepinephrine to normalize MAP
4. a possibility to use microdialysis of the draining venous cerebral blood to evaluate perturbation of global cerebral energy metabolism during pronounced ischemia

As a background we will first present some relevant clinical and experimental studies related to the concept of permissive hypotension

## Duration and degree of permissive hypotension – clinical and experimental studies

Damage Control Surgery and Damage Control Resuscitation are strategies that incorporate quick and transient measures to ensure survival. The use and especially goals of mean arterial pressure in permissive hypotension has changed during the years. The first European recommendations in 2006 were a MAP of 40mmHg in patients with uncontrollable bleeding and without TBI until bleeding is controlled. (73) During recent years, the approach in permissive hypotension have become more individualized. Hence, the current recommendation is to target systolic blood pressure of 80–90 mmHg (mean arterial pressure 50–60mmHg) until significant bleeding has been stopped in the initial phase following trauma without brain injury. Recent guidelines also state that individual considerations should be taken to determine the acceptable MAP level during permissive hypotension. Several factors may probably decrease or shorten the autoregulatory capacity of the brain during arterial hypotension. Age, genetics, and comorbidities are non-modifiable factors, but both the duration and level of hypotension constitute risks that may be modified. Most human studies behind the recommendations of permissive hypotension have been based on low-volume resuscitation and not with the primary goal of controlled hypotension in itself. (14, 15, 74) Several studies emphasize that the use of massive crystalloid infusions increases the risk of abdominal compartment syndrome, coagulopathy, multiorgan failure, nosocomial infections, and decreased survival. (75-79). Modern damage control resuscitation recommends avoidance of crystalloids and promotes the use of balanced blood transfusions. To conclude, there is currently little evidence regarding permissive hypotension and resuscitation with balanced blood products and there are no clinical studies published documenting the effects on cerebral energy metabolism. Only one study examining a high versus a low MAP is registered in clinicaltrails.gov but with unknown status (NCT00459160). Accordingly, there is a consensus that individual considerations should be taken but there is presently no technique available to determine the lower acceptable MAP or duration of permissive hypotension in the individual patient.

Several animal studies have investigated the effect of different levels of mean arterial pressure on mortality. In a study by Morgan *et al.* (80) five pigs were subjected to permissive hypotension after being resuscitated from decompensated shock, mimicking military prolonged field care. Animals were bled until MAP of 30mmHg were reached. When animals were unable to maintain MAP >30mmHg for more than

10min a resuscitation protocol was commenced. After 6 hours, a prolonged permissive hypotension strategy showed no detrimental effect on survival or neurologic outcome despite the increased ischemic burden of haemorrhage but the authors remark that significant fluid volume was required to maintain blood pressure. In a series of experimental studies Stern *et al*. have presented data indicating that mortality increases with an increased target mean arterial pressure when resuscitated with isotonic saline. (81-84) The mortality (78% in the high resuscitation endpoint group versus 22% and 11% in the middle and low resuscitation endpoint group, respectively) raised the question whether the harmful effects in the high endpoint group is due to large amounts of intravenous fluids or the increase in MAP (81).

In a study by Mongan *et al.* (85), 24 pigs had induced haemorrhagic shock, which was maintained at MAP 40mmHg until the end of the experimental protocol (240min). The primary objective of the study was to compare the effects of intravenous infusion of sodium pyruvate (0.5 g x kg-1 x $h^{-1}$) with 10% NaCl (hypertonic saline) or 0.9% NaCl (physiological saline) with matched volume and osmotic effects. The primary findings in the study showed that sodium pyruvate administered 30 min after the start of rapid severe controlled arterial haemorrhage preserved cerebral energy metabolism and prevented a major release of glutamate. The authors concluded that the study provided further evidence to support the conceptual framework for metabolic substrate manipulation of cellular metabolism to minimize injury after haemorrhagic shock.

As previously mentioned, both the duration and level of hypotension are modifiable and essential in the risk of brain injury. To examine the effect of the duration of hypotension and to verify the findings of microdialysis Study II was designed identically to Study I, with the difference that the duration of haemorrhagic shock was reduced from 90 to 60min. The results are discussed in the following paragraph. Further, two experimental studies evaluating the effect of NE-infusion on MAP and $PbtO_2$ (86) and MAP, $PbtO_2$ and cerebral energy metabolism (87) will be presented below under the paragraph of *Early treatment with norepinephrine during haemorrhagic shock*.

## Haemorrhagic shock – effects on extracranial organs

During haemorrhagic shock all tissues are at risk for ischaemic damage. This problem is outside the scope of the present thesis. However, as these injuries are clinically important, we will give a brief overview of the literature.

Several retrospective clinical studies show an association between trauma and acute kidney injury (AKI), including increased mortality. (88-90) However, many of these studies have been performed in complex trauma patients with multiple causes for AKI, i.e., haemolysis, rhabdomyolysis, hypoperfusion, direct kidney injury etc.

In an experimental shock model in mice, Yu *et al.* (91) reported that isolated haemorrhagic shock for 180min resulted in a less intensive insult in kidney function than a 60min bilateral renal artery clamping. In a study by Letson *et al*. (92) haemorrhagic shock was in pigs induced by sharp liver trauma and were assigned to receive treatment with either a hypotonic fluid comprising of Adenosine, lidocaine, and Mg2+ (ALM), or 0.9mg/mL NaCl. After 330min, animals were resuscitated with 450mL shed blood. Only 80% of pigs in the control group survived. During shock, oxygen delivery was compromised, increasing systemic lactate. However, after resuscitation with NaCl and shed blood, lactate levels returned to baseline levels. In contrast, the glycerol and lactate concentration in the brain increased to high levels, which persisted through the study period, indicating cellular damage (92). In isolated haemorrhage, the brain and other

sensitive organs as the kidney seem to be the “limiting” organs, while the rest of the body and the organs seems to recover. (93).

The brain is also identified as the limiting organ in patients resuscitated after out of hospital cardiac arrest. In those patients who survived the initial cardiac arrest, but later died during hospital care it has been reported that approximately 2/3 of deaths were from neurological injury (94).

## Comparison of MAP, $PbtO_2$ and cerebral energy metabolism between studies I, II and III

In studies I and II and in the control group of study III MAP relatively rapidly decreased to 40 mmHg after start of haemorrhage and was kept at this level for 90 min (Study I and III) or 60 min (Study II). Though the decrease in MAP were identical the three studies the caused changes in $PbtO_2$ were remarkably different (figures 3, 5, and 7). In study I $PbtO_2$ rapidly decreased to a very low level (about 15% of initial level) and in study II rapidly decreased to about 60% of initial level and remained at these levels until reinfusion of shed blood. In the control group of study III $PbtO_2$ initially decreased to about 80% followed by a relatively rapid decrease to about 10% of initial level.

Measurements of $PbtO_2$ represent the product of CBF and the cerebral arterio-venous difference in oxygen tension rather than a direct measurement of total oxygen delivery or cerebral oxygen metabolism (95). Under normal conditions, the numerical value of $PbtO_2$ obtained varies, and a definite lower acceptable limit is not possible to define (96). In the present experimental situation, this implies that $PbtO_2$ may be regarded as a qualitative reflection of CBF, and the information presented in figures 3, 5, and 7 indicates widely different levels of CBF although MAP was identical. However, as $PbtO_2$ is a qualitative indicator of CBF and as the observation is based on a limited number of experiments it is necessary to confirm the observation with a different experimental technique.

The data obtained from intracerebral microdialysis support the conclusions regarding the differences in $PbtO_2$. In study I, the profound decrease in $PbtO_2$ is reflected in a very marked increase in LP ratio (increase in lactate simultaneously with reduction in pyruvate) and a decrease in glucose to a very low level. This is the biochemical pattern of pronounced ischemia with depletion of oxygen as well as substrate. The later increase in glutamate and glycerol indicates irreversible cell damage. After re-transfusion a transitory increase in $PbtO_2$ was observed (fig. 3) followed by a second decrease and continued deterioration of energy metabolism (Table 2). The pattern is known from experimental studies of recirculation after pronounced incomplete cerebral ischemia and is caused by post-ischemic brain swelling causing a progressive decrease in CBF (97).

In study II, the moderate decrease in $PbtO_2$ corresponds to the biochemical pattern obtained: a moderate increase in lactate at preserved pyruvate resulting in a moderate increase in LP ration at a normal glucose level compatible with a normalization of all variables at re-transfusion (figure 6). After re-transfusion minor increases in lactate, pyruvate, LP ratio as well as glycerol remained. The biochemical pattern indicates that the moderate decrease in $PbtO_2$ did not cause irreversible cell damage. In the control group of Study III a very different pattern was observed. During the initial 30 min the moderate decrease in $PbtO_2$ is reflected in an equally moderate increase in LP ratio and the successive, pronounced further decrease in $PbtO_2$ causes an equally pronounced increase in LP ratio (figure 7).

Altogether, the studies demonstrate that induced haemorrhagic shock to a defined MAP may result in very different levels of CBF ($PbtO_2$) and compromised energy metabolism (Studies I and II) and that the duration of hypotensive shock may also be of importance for these variables (Study III). The results are probably

explained by the fact that cerebral pressure autoregulation could almost completely compensate for the decrease in MAP in Study II and during the initial 30 min in the control group of Study III but not at all in Study I. In the studies the experimental animals were young, healthy and from similar race. Under clinical conditions a much greater variability between patients is expected. Accordingly, though it might be possible to define a lower limit of MAP for groups of patients this limit is not necessarily correct for the individual patient. For this purpose, bedside monitoring of biochemical variables related to cerebral energy metabolism is necessary.

## Early treatment with norepinephrine during haemorrhagic shock

As discussed in the Introduction the use of vasopressor treatment in patients with haemorrhagic shock is still a matter of controversy. European guidelines recommend using norepinephrine in haemorrhagic shock where it is not possible to keep up with transfusion (29). In North American trauma systems the administration of norepinephrine is not advocated due to the belief that vasopressors will increase both morbidity and mortality (43). The controversy is also reflected in experimental studies. Some animal models have also shown that norepinephrine is associated with increased cerebral vasoconstriction ranging from nonsignificant and up to 20% constriction compared to baseline. (32-38). Treatment with norepinephrine has been reported to result in an increase as well as a decrease in cerebral blood flow (38-41) but the net effect on cerebral metabolism has not been investigated thoroughly. The results in Study III may be compared with two relatively recent experimental studies in pigs (86, 87).

In the study by Meybohm et al (86) eight anesthetized piglets were subjected to hypotension by blood withdrawal. The procedure lasted approximately 20 min or less and was terminated at the targeted MAP of 30 mm Hg. Norepinephrine was then titrated to achieve baseline mean arterial blood pressure (MAP). Then, norepinephrine was stopped, MAP was allowed to decrease again below 30 mm Hg whereupon shed blood was re-transfused. The effects on CBF and cerebral oxygenation were during the experiment evaluated from measurement of $PbtO_2$ and non-invasive techniques (doppler blood flow velocity, near infrared spectroscopy). The authors concluded that though norepinephrine increased CPP immediately, cerebral oxygenation as reflected by $PbtO_2$ could not be improved by norepinephrine, but only by re-transfusion (86).

In the study by Küchler et al (87) fourteen pigs were randomized to treatment with either NE of vasopressin (VP). Haemorrhagic shock was induced with blood withdrawal to a target MAP of 30 mmHg for 60 min without any therapy. Subsequently, the animals received a resuscitation therapy with either NE or VP given by a continuous intravenous infusion. In both groups the vasopressor dose was adjusted individually to maintain the target MAP of 60 mmHg throughout the treatment phase. The authors concluded that "*both vasopressors were effective in restoring hemodynamics and CPP and in maintaining brain oxygenation*". It is remarkable that during the prolonged period without therapy and pronounced hypotension (MAP slightly above 30 mmHg) LP ratio remained unaffected and $PbtO_2$ decreased only slightly. Further, the $PbtO_2$ levels were during the whole study period markedly different between the two experimental groups treated with NE and VP respectively.

Study III demonstrated that treatment with norepinephrine during 90min of haemorrhagic shock with an initial MAP of 40mmHg restored MAP and preserved metabolism compared with no treatment. Study III was designed to focus only on NE as the first-line treatment of haemorrhagic shock. Intentionally no other intervention such as fluid resuscitation, as the recommended standard treatment in haemorrhagic shock,

was performed to emphasise the effects of norepinephrine on cerebral metabolism. These data may be compared with the studies of Meybohm et al. (86) and Küchler et al (87).

In the latter study cerebral energy metabolism and $PbtO_2$ were only slightly affected by 60 min of profound arterial hypotension (MAP slightly above 30 mmHg). The finding underscores the fact that MAP kept at a defined low level is in itself not a reliable indicator of compromised cerebral energy metabolism. In Study III the decrease in MAP to 40 mmHg caused a minor decrease in $PbtO_2$ during the initial 30 min (fig. 7) and energy metabolism was only moderately affected. Both studies indicate that NE treatment initiated early – before major perturbations of cerebral energy metabolism have occurred – does not lead to compromised energy metabolism. Accordingly, the mentioned concerns of using vasopressors during haemorrhagic shock (32-38) appear to be unwarranted under these circumstances. The beneficial effects of NE in the present study are interpreted as caused by the increase in MAP and CBF. A direct effect of NE cerebral energy metabolism is unlikely. It has been demonstrated that the direct effect of the catecholamines adrenaline and noradrenaline result in an increase in CBF and cerebral metabolic rate at virtually unchanged levels in biochemical variables related to cerebral energy metabolism (98, 99).

From their results Meybohm et al (86) concluded: "*In this respect, normal MAP values following resuscitation with NE alone of hemorrhage-induced hypotension do not rule out compromised cerebral oxygenation, and therefore, should not be regarded as safe during pronounced fluid deficiency*". This conclusion is at variance with our results. Study III documents that normalization of MAP by NE therapy does not affect biochemical variables related to cerebral energy metabolism. Further, the level of $PbtO_2$ does not give a prober measure of cerebral oxygenation but is rather a qualitative indicator of CBF (95).

## Evaluation of global cerebral energy metabolism

Studies I and II constitute the first attempts to evaluate changes in global cerebral energy metabolism from microdialysis of the draining venous blood. A similar technique has, however, been evaluated during experimental myocardial infarct in pigs (100-103). In this series of experiments the authors concluded that intravasal microdialysis was superior to intramyocardial microdialysis in detecting local ischemia (103). To the best of our knowledge, the technique was never transferred to clinical conditions.

As in the pig the majority of cerebral venous blood drains from the transverse sinus via the sigmoid sinus into the paraspinal venous it was in this study necessary to insert the microdialysis catheter via a small craniotomy directly into the superior sagittal sinus. The vessel is delicate in small pigs and often difficult to visualize. As during normal conditions glutamate concentration is high in circulating blood compared with the intracerebral interstitial level, we verified the correct position of the catheter from glutamate analysis. The technical difficulties explain the drop out regarding intravenous data in Study III.

To document that the biochemical data obtained reflected intracerebral biochemical changes one microdialysis catheter was introduced into the femoral artery to visualize the conditions in other body tissues.

In Study I intracerebral microdialysis documented a biochemical pattern of pronounced ischemia (depletion of oxygen and substrate) which resulted in irreversible cell damage. In this situation microdialysis of the draining venous blood exhibited pronounced increase in LP ratio caused by a marked increase in lactate and a slight decrease in pyruvate. Simultaneous microdialysis of femoral arterial blood exhibited moderate increases in lactate, pyruvate, and LP ratio.

In Study II intracerebral microdialysis documented a biochemical pattern caused by a moderate reduction of CBF (pronounced decrease in oxygen at continued supply of substrate) compatible with a normalization at re-transfusion. During the shock period a minor increase in LP ratio was noted which normalized after re-transfusion. In this situation cerebral venous blood exhibited a biochemical pattern similar to that obtained from arterial blood.

In summary, under experimental conditions intravenous microdialysis can be used to identify individuals at risk for developing irreversible cerebral lesions during haemorrhagic shock. Whether the technique might be used in patients under similar conditions is discussed below.

## Experiences when conducting animal studies.

All studies whether clinical or experimental requires meticulous planning. The right equipment, skills, and experimental animal need to be at the right place at the right time. Even though everything is being done to avoid unnecessary waiting time and errors, unexpected events and mishaps always occur. As an example of the logistical challenges, there was more than 6 months waiting time from booking until the operating room and stables were available at the Biomedical Laboratory. When starting up with a new experimental model there will always be a learning curve before the final model is mastered. In studies 1 and 2, one animal died before the start of the experiment, and in in the final form of study 3, three animals.

Our model of haemorrhagic shock is demanding in terms of manpower. During haemorrhage and resuscitation one person are dedicated to ensure a continuous MAP of 40 mmHg, while another person is dedicated to monitor the anaesthesia of the animal. Hence, each experimental animal represents the work of at least two persons, beginning 7.30 in the morning and lasting until 19.00 in the evening.

When starting the collection of data presented in study 3, we encountered several problems. All animals used in studies 1-3 were from a non-registered breeder within the European Union, and animals were supplied through the Biomedical Laboratory affiliated with the University of Southern Denmark. Studies 1 and 2 utilized swine which were a mix of Landrace, Yorkshire and Duroc swine (LYD). A series of pilot experiments for study 3 also used the LYD mix (n=7). After we had finished the remaining experiments in what should have been study 3 (n=10) we became aware that the Biomedical Laboratory had changed their supplier of animals which were now Landrace/Yorkshire mix (LY). The experimental protocol proposed that haemorrhagic shock was induced by bleeding the animals with 2.15ml/kg/min during the first 7min, and subsequently 1.15ml/kg/min until a MAP of 40mmHg was reached. To avoid using a fixed volume model, we planned to stop bleeding the animals when MAP=40mmHg were reached. In the norepinephrine group a weight adjusted infusion with norepinephrine was started targeting a MAP of 70-80mmHg. After reaching MAP=40mmHg no further withdrawal of reinfusion of shed blood occurred. When MAP=40mmHg was reached in the control group no further therapy was instituted. Again, no further withdrawal or reinfusion of shed blood occurred. After 90min of hypotension all shed blood were reinfused at a rate of 120ml/min, and norepinephrine infusion were terminated in the norepinephrine group. The animals were then observed for the following 180min.

2 of the 10 pigs in the norepinephrine group died during the experimental protocol. One from groin haemorrhage and one due to ventricular fibrillation during induction of haemorrhagic shock. In the control group 1 out of 7 animals died before completing the experimental protocol.
When reviewing the remaining data, it became clear that there was a significant difference in the cardiovascular response in LYD pigs and LY pigs during haemorrhage. The LY pigs had a high incidence of

irreversible bradycardia and subsequent cardiovascular collapse due to cardiac arrest while inducing haemorrhagic shock. The LY pigs needed a significant lesser amount of blood drained to reach a MAP=40mmHg. As show in figure 8 there was a significant difference in the amount of drained blood needed to achieve a MAP=40mmHg between the LYD and LY race. The results from this experiment also showed that the LYD pigs from the control group showed a remarkable tendency to increase their blood

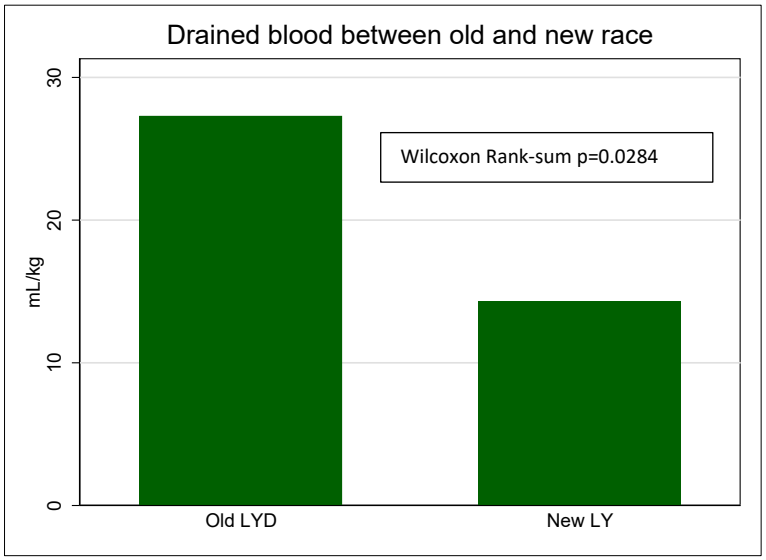



*Figure 8: Unpublished data from failed experiments. The graph illustrates that porcine from an LY breed needed a significant lesser amount of blood drained to reach a MAP of 40mmHg. LYD N=5, LY N=7 The differences were statistical significant p=0.0284*

pressure to levels >60mmHg MAP during the first 20min after the shock was induced without any treatment with fluids or vasopressors (figure 9). The same phenomenon has been observed in humans. In a randomised controlled trial by Morrison *et al. (75)* patients in haemorrhagic shock who required emergency surgery were randomised to either a low MAP group with a resuscitation endpoint of 50mmHg or a high MAP group with an endpoint of 65mmHg.

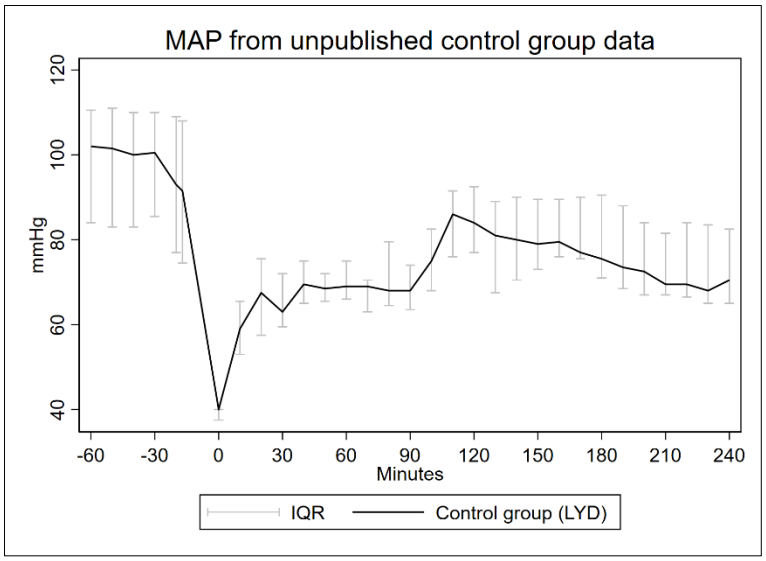



*Figure 9: Unpublished data in haemorrhagic shock in 4 female pigs. The pigs had shock induced by bleeding them at a predefined rate until MAP=40mmHg was reached (time=0). After this target MAP was reached, no further treatment occurred. Note that the*

*MAP increased spontaneously shortly after withholding further bleeding and that MAP quickly increased to near-normal levels. At time 90min the animals were resuscitated with remaining shed blood. Median (IQR)*

Even though the study was designed to evaluate the effectiveness of permissive resuscitation strategy, the patients in the low MAP groups were able to maintain a higher intraoperative blood pressure of 64.7mmHg (61.0–68.3) (mean(95%CI)) by themselves. Because of this phenomenon, the final model for study III utilised continually bleeding or reinfusion of blood during the hypoperfusion period to maintain a MAP of 40mmHg. Since the NE group were composed on both the LYD and LY race and the control group entirely on the LY this might induced a bias in the results, and thus the data were discarded. The final experimental model for study III utilised the original LYD mix supplied through the Biomedical Laboratory from their previous breeder.

## Limitations

Although 50 animal experiments were conducted, the three studies in the thesis only include a totally 28 experimental animals (pigs). Two opposing opinions had to be satisfied. First, increasing the number of research animals would make the results more robust and reduce the risk of bias. Second, considerations had to be made as the experiments were conducted on live healthy animals. During courses in Laboratory Animal Science, the importance of reduction, refinement and replacement of experimental animals was emphasized. Further, though the number of experimental animals is low the number is not unique for this kind of experiments. We have tried to identify all recent studies published under the index "hemorrhagic shock and pig and cerebral energy metabolism" utilizing PubMed and other digital repositories to identify how similar experimental studies have been conducted. We have found 10 similar experimental studies published in international peer reviewed journals including American Journal of Physiology, Resuscitation, Pediatric Research, Anesthesia & Analgesia and Intensive Care Medicine Experimental. The median number of animals (pigs) included in these studies was 10.

The $PbtO_2$ probe and the intracerebral microdialysis catheter will always provide a local estimate of their respective measurements and may thus over/underestimate global conditions. Although severe haemorrhagic shock may cause global cerebral ischemia it is well known that under some circumstances the degree of ischemia may vary in different cerebral regions (*e.g.,* water-shed areas).

It is well documented that the numerical levels obtain from $PbtO_2$ may vary considerably between individuals and regions and that a lower acceptable limit is not possible to define. The cause of the variability has not been completely elucidated.

Due to incomplete relative recovery the levels of the biochemical variables obtained during microdialysis is usually not absolutely quantitative. When the catheter is perfused at 0.3 µl/min relative recovery is approximately 70% in cerebral tissue and probably considerably higher in venous blood. In the present studies incomplete relative recovery is considered to be unimportant.

Due to the slow flow rate of the perfusion fluid, there is a considerable delay until the perfusate reaches the collecting micro-vial from the microdialysis probe. At a perfusion rate of 0.3 µl/min the delay is approximately 5-10min but may be reduced depending on the length of the tubing's. In addition, due to laminar flow of the perfusion fluid an exactly defined delay is not possible to define. The delay should be taken into consideration when comparing the data from microdialysis to the $PbtO_2$ levels in the present studies.

## Aspects on clinical relevance and future possibilities

Usually, it is not justified to transfer data regarding physiological or biochemical limits obtained in animals during experimental conditions directly to clinical conditions. The biological differences between species are usually too pronounced. In addition, the piglets used in the present studies from the same race, completely healthy and about 4 months of age – *i.e.,* very different from the usual clinical situation. Accordingly, the limits for MAP, duration of arterial hypotension and therapeutic interventions are not intended to directly reflect the clinical situation. The purpose is to give information regarding basic principles that may be further developed and applied to clinical conditions. As an example, the great difference regarding tolerable arterial hypotension between the groups of animals in the present studies illustrated that a lower MAP level defined from group data is not necessarily tolerable for the individual patient. To identify the lower acceptable limit for an individual patient it is necessary to monitor cerebral energy metabolism at the bedside.

In the present analyses we have studied $PbtO_2$ and microdialysis. $PbtO_2$ primarily reflects local CBF in the immediate surroundings of the probe, the levels obtained vary considerably between individuals and it is not possible to define the lower tolerable limit. From microdialysis it is possible to obtain quantitative data for variables related to cerebral energy metabolism. Due to variations in relative recovery the concentrations measured are not absolutely correct, but it is possible to correct for this problem. However, for both $PbtO_2$ and intracerebral microdialysis it is necessary to introduce intracerebral probes. This is hardly possible during the clinical condition of haemorrhagic shock.

Our experiments have for the first time documented that it is possible detect pronounced cerebral ischemia from microdialysis of the draining venous blood. As mentioned above, there is a need of objective variables to define the lower acceptable MAP in the individual patient during severe haemorrhagic shock. During clinical conditions it is possible to perform microdialysis of the draining cerebral venous blood by inserting a microdialysis catheter in the bulb of the internal jugular vein. The procedure may be complicated to perform under acute clinical conditions and has so far not been evaluated during haemorrhagic shock. However, during under other clinical circumstances jugular bulb microdialysis (JBM) has been evaluated.

During open heart surgery and extracorporeal circulation Mölström et al (59) have shown that LP ratio of cerebral venous blood increased significantly during cardio-pulmonary bypass, indicating compromised cerebral oxidative metabolism. In an explorative study of 18 patients resuscitated after out of hospital cardia arrest (OHCA) JBM has been tested (104). The study showed that bedside JBM was feasible and safe during post-resuscitation care. The results indicated that cerebral metabolic parameters could be distinguished from systemic parameters in patients with poor outcomes, and that it might be used in comatose OHCA patients to assess global cerebral energy metabolism. The latter question is further examined in a recently finished randomized study investigating the effect of two different levels of MAP regarding cerebral energy metabolism and clinical outcome (105).

# Conclusions

The three experimental studies have shown that the level of MAP and the duration of arterial hypotension during haemorrhagic shock are not sufficient criteria to determine whether irreversible brain damage will occur or not. From a clinical perspective, group data from large studies a used to establish general recommendations. These recommendations are not necessarily adequate for the individual patient and

recent guidelines emphasize that individual considerations should be taken. However, there is presently no clinical routine technique available to determine the individual limits. A definite decision of these limits can only be obtained from bedside evaluation of cerebral energy metabolism.

We have shown that intracerebral microdialysis may be used to determine the biochemical pattern when haemorrhagic shock will cause irreversible tissue damage. The pattern is different from that obtained during a moderate reduction in CBF compatible with a normalization of energy metabolism after re-transfusion. Further, we have shown that early treatment with NE (*i.e.,* before the biochemical variables indicate pronounced ischemia with depletion of oxygen and substrate) may normalize energy metabolism before re-transfusion. As intracerebral microdialysis will hardly be an option during severe haemorrhagic shock under clinical conditions, we have examined whether microdialysis of the draining venous blood may be used as a surrogate marker during a dangerous deterioration of cerebral energy metabolism. The technique of JBM has subsequently been tested during open heart surgery and cardiopulmonary bypass as well as after resuscitation in patients with OHCA.

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RESEARCH Open Access

# A technique for continuous bedside monitoring of global cerebral energy state

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## Abstract

**Background:** Cerebral cytoplasmatic redox state is a sensitive indicator of cerebral oxidative metabolism and is conventionally evaluated from the extracellular lactate/pyruvate (LP) ratio. In the present experimental study of global cerebral ischemia induced by hemorrhagic shock, we investigate whether the LP ratio obtained from microdialysis of cerebral venous blood may be used as a surrogate marker of global cerebral energy state.

**Methods:** Six female pigs were anesthetized and vital parameters were recorded. Microdialysis catheters were placed in the left parietal lobe, the superior sagittal sinus, and the femoral artery. Hemorrhagic shock was achieved by bleeding the animals to a mean arterial pressure (MAP) of approximately 40 mmHg and kept at a MAP of about 30–40 mmHg for 90 min. The animals were resuscitated with autologous whole blood followed by 3 h of observation.

**Results:** The LP ratio obtained from the intracerebral and intravenous catheters immediately increased during the period of hemorrhagic shock while the LP ratio in the arterial blood remained close to normal levels. At the end of the experiment, median LP ratio (interquartile range) obtained from the intracerebral, intravenous, and intra-arterial microdialysis catheters were 846 (243–1990), 309 (103–488), and 27 (21–31), respectively. There was a significant difference in the LP ratio obtained from the intravenous location and the intra-arterial location ($P < 0.001$).

**Conclusions:** During cerebral ischemia induced by severe hemorrhagic shock, intravascular microdialysis of the draining venous blood will exhibit changes of the LP ratio revealing the deterioration of global cerebral oxidative energy metabolism. In neurocritical care, this technique might be used to give information regarding global cerebral energy metabolism in addition to the regional information obtained from intracerebral microdialysis catheters. The technique might also be used to evaluate cerebral energy state in various critical care conditions when insertion of an intracerebral microdialysis catheter may be contraindicated, e.g., resuscitation after cardiac standstill, open-heart surgery, and multi-trauma.

**Keywords:** Hemorrhagic shock, Microdialysis, Cerebral energy state, Ischemia

## Background

Intracerebral microdialysis with bedside analysis and display of chemical variables related to cerebral energy metabolism, excitotoxicity, and cell membrane degradation has been available as a clinical routine technique for almost 20 years [1–3]. As the microdialysis probe reflects the biochemistry from a very narrow zone surrounding the

dialysis membrane, appropriate positioning and documentation of the position of the catheter in relation to focal lesions is necessary for a correct interpretation of the data obtained [4]. During neurocritical care, e.g., following traumatic brain injury, information regarding global cerebral energy state in addition to the regional information obtained from conventional intracerebral microdialysis would be valuable. Such information would also be of importance during critical care of other severe conditions when cerebral energy metabolism may be jeopardized without focal lesions (e.g., open-heart surgery, resuscitation after cardiac standstill, hemorrhagic or septic shock, toxic states). However, in these conditions, it is for various reasons difficult or impossible to insert intracerebral catheters. It would be of interest to develop an alternative technique that avoids the penetration of cerebral tissue by the microdialysis catheter and still gives continuous bedside information regarding cerebral energy state.

Cerebral cytoplasmatic redox state is a sensitive indicator of cerebral oxidative metabolism and is conventionally evaluated from the extracellular lactate/pyruvate (LP) ratio [5–8]. During insufficient oxygen supply (e.g., arterial hypoxia, ischemia), the LP ratio monitored by intracerebral microdialysis increases instantaneously and if oxygenation is rapidly restored, it returns to a normal or near-normal level [9]. When cerebral oxidative metabolism is compromised by mitochondrial dysfunction, a lasting increase of the LP ratio is observed [8, 10, 11]. Although lactate and pyruvate are water-soluble, they rapidly equilibrate over cell membranes as well as the blood-brain barrier [12–14]. Accordingly, from a theoretical point of view, it might be possible to evaluate changes in global cerebral redox state by monitoring the LP ratio of the cerebral venous outflow.

In the present experimental study, we explore whether cerebral venous LP ratio may be used as a surrogate marker for compromised cerebral oxidative metabolism during hemorrhagic shock. Accordingly, we test the hypothesis that the LP ratio monitored in the cerebral venous outflow reflects the cerebral energy metabolism during compromised conditions and hence is different from the LP ratio monitored in the arterial blood.

## Methods

The study was approved by the National Committee on Animal Research Ethics (2013-15-2934). The depth and duration of hemorrhagic shock necessary for producing cerebral ischemia that caused a compromised energy state and degradation of cell membranes as evaluated from biochemical variables obtained by microdialysis were based on four pilot studies. When the experimental model had been defined, six female pigs approximately 4 months old weighing 42 (35–45) kg were included in the study.

### Anesthesia, mechanical ventilation, and surgical preparation

The porcine model of hemorrhagic shock has been previously described [15]. The animals were fasted overnight with access to ad libitum water. Sedation was achieved with a standard mixture of medetomedin (0.05 mg/Kg), midazolam (0.25 mg/Kg), and atropine (0.25 mg/Kg). Anesthesia was induced with midazolam (0.625 mg/Kg) and ketamine (12.5 mg/Kg) and maintained with infusion of midazolam (5 mg/Kg/h) and fentanyl (50 μg/Kg/h). The animals were intubated and volume-controlled ventilated

(Siemens 900 Ventilator; Siemens Elema, Stockholm, Sweden) with a tidal volume of 10 mL/kg and $FiO_2$ of 0.30. $PaCO_2$ was kept between 4 and 6 kPa and body temperature around normal 38.5 °C.

### Multimodal monitoring

After establishing anesthesia, one sheath was inserted into the carotid artery for blood pressure monitoring and blood gas sampling, while the external jugular vein was cannulated for insertion of a pulmonary artery catheter (CCOmbo; Edwards Lifesciences, Irvine, CA, USA) to monitor cardiac output (CO), core temperature, and central venous pressure (CVP). Arterial blood gases ($PaCO_2$, $PaO_2$, pH), blood glucose electrolytes, and lactate levels were measured every 30 min (ABL800 FLEX, Radiometer Denmark).

One femoral artery was cannulated for withdrawing and re-infusing blood during the induced hemorrhagic shock. Another sheath was placed in the contralateral femoral artery, and a microdialysis catheter (CMA 70 Bolt, M Dialysis AB, Stockholm, Sweden) was inserted. A small craniotomy was placed in the frontal bone in the midline above the superior sagittal sinus. The sinus was cannulated by a standard 18G peripheral venous catheter, and one microdialysis catheter (CMA 70 Bolt, M Dialysis AB, Stockholm, Sweden) was introduced in the posterior direction and placed in the posterior part of the superior sagittal sinus. The superior sagittal sinus was chosen for analysis of cerebral venous blood due to the anatomic characteristics of the experimental animal. In the pig, most of cerebral blood is drained via paraspinal venous plexa and only a minor part passes into the internal jugular vein [16]. A third microdialysis catheter (CMA 70, M Dialysis AB, Stockholm, Sweden) was inserted 20 mm into the left parietal lobe and one probe for monitoring brain tissue oxygenation (PbtO2) (Licox CC1SB, Integra Neurosciences Ltd., NJ, USA) was introduced 15 mm into the contralateral parietal lobe. A transducer for monitoring intracranial pressure (ICP) (Camino, Integra Neurosciences Ltd., NJ, USA) was placed in the right hemisphere. A bladder catheter was placed for urine collection. All animals were given a baseline dose of 200 U/kg of heparin and supplemented hourly with 100 U/kg for anticoagulation during the hemorrhage period. At the end of experiment, the anesthetized animals were killed with an i.v. injection of sodium pentobarbital 200 mg/mL in concentrated ethanol.

### Experimental protocol

Following a 120-min baseline period allowing animals to stabilize, the following surgery hemorrhagic shock was achieved by bleeding the animals to a pre-defined MAP of approximately 40 mmHg at a rate of 2.15 mL/kg/min over 7 min and then 1.15 mL/kg/min over the remaining period [15]. Animals were kept at a MAP of about 30–40 mmHg by withdrawing or infusing shed blood that was stored in a citrated glucose solution at 37 °C. Following 90 min of hemorrhagic shock, the animals were resuscitated by re-infusing the shed blood at a rate of 120 mL/min until all blood was returned. The pigs were observed for 3 h after hemorrhagic shock. Microdialysis probes were perfused with artificial CSF (M Dialysis AB, Stockholm, Sweden) at a rate of 0.3 μl/min (CMA 106 MD pump, M Dialysis AB, Stockholm, Sweden). The dialysates were collected in microvials and immediately analyzed for glucose, lactate, pyruvate, glutamate, and glycerol every 30 min using an ISCUS$^{Flex}$

analyzer (M Dialysis AB, Stockholm, Sweden). After insertion, all probes were allowed a minimum of 2 h for stabilization.

$PbtO_2$ data were collected using the AC3.1 monitor (Integra Neurosciences Ltd.) and recorded every 20 s. All Licox probes were tested against atmospheric air and against each other before insertion and after removal. After insertion, appropriate function was confirmed by an oxygen challenge test.

ICP was monitored continuously and data were collected by a CAM01 monitor (Integra Neurosciences Ltd., NJ, USA) and ICP. Cerebral perfusion pressure (CPP) was calculated as MAP – ICP.

### Statistics

Data are given as median (interquartile range) unless otherwise noted. To test our hypothesis, the time course of the LP ratio in the superior sagital sinus and femoral artery was modeled utilizing a mixed-effects model for repeated measurements with time and location of microdialysis probe as random effects and each animal as fixed effect. The null hypothesis was that no difference in the LP ratio was found between the superior sagital sinus and femoral artery. A $p$ value below 0.05 was considered significant. Data analysis was performed in Stata 11.1 statistical software (StataCorp, College Station, TX, USA).

## Results

Table 1 gives the physiological and general biochemical variables monitored during the experimental period. During the period of hemorrhagic shock, CPP decreased to about 30 mmHg causing a decrease in $PbtO_2$ to a very low level (<5 mmHg). After re-infusion of autologous blood, MAP increased close to baseline levels. As ICP continued to increase during the observation period, the CPP remained low (approximately 40 mmHg). The $PbtO_2$ remained at very low levels after re-infusion of blood. The relation between MAP and $PbtO_2$ is illustrated in Fig. 1. During and after the shock period, $PaO_2$, $PaCO_2$, and b-glucose remained within normal limits. b-Lactate increased and b-pH decreased during the period of hemorrhagic shock, and both essentially normalized during the observation period. Median blood loss was 1072 mL (964–1498 mL) and median blood loss per kilogram was 31 mL/kg (26–37 mL/kg).

Table 2 gives the biochemical variables obtained from microdialysis catheters positioned in the cerebral hemisphere, in the superior sagittal sinus, and in the femoral artery, respectively. Before the induction of hemorrhagic shock, LP ratios were similar (11–18) in all three catheter positions and within normal limits [17]. In the cerebral hemisphere, hemorrhagic shock caused a marked increase of the LP ratio that increased further after re-infusion of blood. The increase of the LP ratio was caused by a pronounced increase in lactate simultaneously with a pronounced and lasting decrease in pyruvate. Intracerebral glucose decreased and remained at a very low level during the whole study period (<1 mmol/L). Extracellular intracerebral glutamate and glycerol increased during the shock period and remained at very high levels after re-infusion of blood.

In the superior sagittal sinus, the LP ratio increased during the shock period and continued to increase to a very high level (100–500) after blood transfusion. In the femoral artery, the shock period was associated with a modest increase of the LP ratio but remained close to the upper reference level (30) in normal cerebral tissue [17]. In the

**Table 1** General physiological and biochemical variables doing hemorrhagic shock

| Elapsed time (min) | MAP (mmHg) | ICP (mmHg) | CPP (mmHg) | $PbtO_2$ (kPa) | $PaO_2$ (kPa) | $PaCO_2$ (kPa) |
|---|---|---|---|---|---|---|
| S −60 | 102 (96–111) | 7 (3–11) | 95 (90–106) | 16 (14–27) | 25 (24–27) | 6.0 (5.2–6.1) |
| S | 77 (73–99) | 8 (2–12) | 72 (64–93) | 22 (16–31) | 24 (23–25) | 5.7 (5.4–6.0) |
| 0 | 40 (40–40) | 8 (6–8) | 32 (28–34) | 13 (7–18) | 24 (23–25) | 5.6 (5.5–5.6) |
| 30 | 37 (33–39) | 5 (1–9) | 29 (29–31) | 5 (2–8) | 25 (25–26) | 5.9 (5.2–6.5) |
| 60 | 34 (32–36) | 5 (0–8) | 29 (28–31) | 2 (1–3) | 24 (24–25) | 5.7 (5.2–6.0) |
| 90 | 31 (31–33) | 6 (0–13) | 32 (25–43) | 1 (1–2) | 26 (26–28) | 5.0 (4.6–7.2) |
| 120 | 59 (46–84) | 17 (13–22) | 51 (38–62) | 3 (1–4) | 25 (24–26) | 5.6 (4.5–6.3) |
| 150 | 60 (58–65) | 19 (14–28) | 46 (46–47) | 11 (1–21) | 22 (22–24) | 6.5 (5.6–7.3) |
| 180 | 63 (61–99) | 27 (20–34) | 42 (41–65) | 1 (1–9) | 23 (22–23) | 5.1 (4.6–5.7) |
| 210 | 61 (59–65) | 23 (21–31) | 42 (40–42) | 1 (1–5) | 22 (21–22) | 5.4 (4.8–5.6) |
| 240 | 73 (50–104) | 29 (24–39) | 47 (39–49) | 1 (1–1) | 22 (21–23) | 6.1 (5.4–6.4) |
| Elapsed time (min) | b-Hemoglobin (mM/L) | HR (bpm) | b-Glucose (mM/L) | b-Lactate (mM/L) | b-pH | Diuresis (mL) |
| S −60 | 5.7 (5.3–5.8) | 78 (75–84) | 6.8 (6.4–7.1) | 1.5 (1.3–1.7) | 7.43 (7.40–7.47) | 40 (4–106) |
| S | 5.6 (5.2–5.9 | 90 (78–93) | 7.2 (6.8–7.7) | 1.2 (1.0–1.4) | 7.45 (7.43–7.46) | 115 (82–160 |
| 0 | 4.9 (4.7–4.9) | 135 (107–138) | 9.7 (8.9–9.8) | 2.5 (2.0–3.0) | 7.41 (7.41–7.43) | 12 (0–23) |
| 30 | 4.6 (4.5–4.7) | 116 (111–146) | 8.6 (7.7–14.4) | 5.3 (3.7–7.4) | 7.34 (7.32–7.38) | 4 (3–5) |
| 60 | 5.2 (4.9–5.5) | 166 (129–191 | 7.9 (6.2–13.1) | 7.2 (4.7–11.1) | 7.30 (7.25–7.41) | 2 (1–5) |
| 90 | 5.2 (5.2–5.4) | 125 (114–168) | 7.0 (4.8–9.2) | 10.3 (6.0–10.3) | 7.20 (7.18–7.31) | 3 (2–10) |
| 120 | 5.1 (4.6–5.6) | 98 (93–116) | 6.2 (5.2–7.0) | 5.5 (4.4–6.5) | 7.29 (7.21–7.34) | 16 (5–31) |
| 150 | 5.2 (5.1–5.6) | 101 95–120) | 6.4 (4.9–7.6) | 4.8 (3.6–6.9) | 7.20 (7.19–7.24) | 20 (17–25) |
| 180 | 5.5 (5.2–5.8) | 110 (103–113) | 6.5 (5.7–8.5) | 2.5 (2.1–3.7) | 7.43 (7.33–7.50) | 70 (25–70) |
| 210 | 5.5 (5.3–5.7) | 122 (88–128) | 4.5 (3.9–5.4) | 2.1 (1.7–3.3) | 7.43 (7.39–7.43) | 45 (5–45) |
| 240 | 5.8 5.7–5.8) | 17 (105–121) | 5.5 (4.6–6.1) | 2.0 (1.2–3.0) | 7.36 (7.33–7.47) | 18 (5–100) |

Data are expressed as median levels (interquartile range). S indicates the start of bleeding to achieve a MAP of 40 mmHg
*MAP* mean arterial pressure, *ICP* intracranial pressure, *CPP* cerebral perfusion pressure, $PbtO_2$ brain tissue oxygenation.

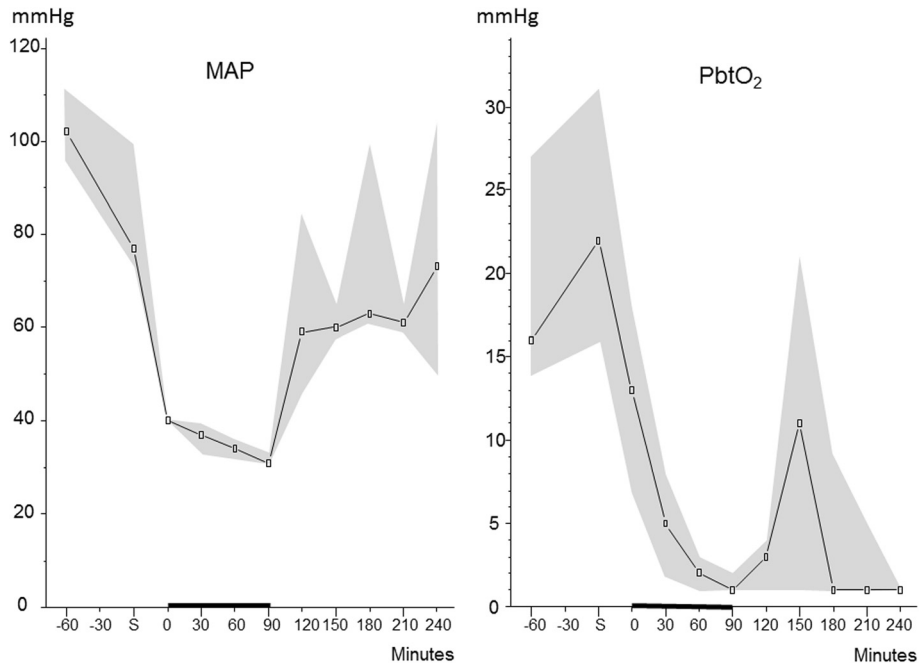


**Fig. 1** Median (interquartile range) arterial pressure (MAP) and brain tissue oxygen tension ($PbtO_2$) in pigs with induced hemorrhagic shock. Note that during the period of hemorrhagic shock, the declining MAP was accompanied by a decrease in $PbtO_2$ to a very low level (<5 mmHg). After re-infusion of autologous blood, MAP increased to close to baseline level whereas $PbtO_2$ remained very low. *S* indicates the start of bleeding to achieve a MAP of 40 mmHg. The *black bar* on the *x*-axis from 0 to 90 min indicates the shock period before resuscitation was started

superior sagittal sinus, the pronounced increase in the LP ratio was caused by a marked increase in lactate simultaneously with a marked decreased in pyruvate. In the femoral artery, the increase in lactate concentration was accompanied by a simultaneous increase in pyruvate limiting the increase in the LP ratio. The time courses of the changes in the LP ratios in the sagittal sinus and femoral artery are shown in Fig. 2. Figure 3 shows the simultaneous changes in lactate concentration in the two compartments. The difference in the LP ratio between the superior sagittal sinus and femoral artery was significant ($p < 0.001$).

After induction of hemorrhagic shock, glucose concentration decreased to extremely low levels in the superior sagittal sinus that lasted during the whole study period. In the femoral artery, glucose remained relatively constant. Already before induction of hemorrhagic shock (control conditions), glutamate concentration was very high in the superior sagittal sinus as well as in the femoral artery (100–200 μmol/L). In both positions, glutamate remained essentially unchanged during the study period. Glycerol concentration increased in the superior sagittal sinus as well as in the femoral artery during the shock period and continued to increase after re-infusion of blood.

## Discussion

Cerebral cytoplasmatic redox state is primarily determined by mitochondrial oxidative metabolism [3–7]. It is conventionally described by the ratio between the cytoplasmatic levels of lactate and pyruvate and is expressed in the ratio between lactate and pyruvate, the LP ratio.

**Table 2** Biochemical variables obtained from microdialysis

| Elapsed time (min) | LP ratio | | | Lactate (mM/L) | | | Pyruvate (μM/L) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Hemisphere | Sagittal sinus | Femoral | Hemisphere | Sagittal sinus | Femoral | Hemisphere | Sagittal sinus | Femoral |
| S −60 | 15 (14–18) | 13 (9–16) | 11 (11–19) | 20.7 (10.7–30.0) | 20.1 (10.7–20.3) | 10.6 (10.1–1.9) | 131 (107–178) | 111 (106–123) | 141 (99–160) |
| S −30 | 13 (11–16) | 13 (6–16) | 12 (10–17) | 2.9 (2.4–3.5) | 2.2 (2.2–2.2) | 2 (1.7–2.6) | 191 (151–205) | 200 (193–371) | 166 (161–175) |
| S | 15 (11–18) | 14 (8–26) | 13 (10–21) | 2.4 (2.1–3.1) | 3.4 (2.6–5.2) | 2.2 (1.6–3.4) | 185 (153–210) | 220 (161–388) | 137 (131–153) |
| 0 | 20 (17–41) | 45 (32–46) | 33 (30–36) | 7.6 (7.2–8.0) | 11.1 (10.5–11.7) | 3 (2.3–4.0) | 110 (107–149) | 178 (144–208) | 181 (129–193) |
| 30 | 60 (27–117) | 36 (23–63) | 27 (26–27) | 10.3 (7.8–11.0) | 6.8 (4.6–12.1) | 3.8 (3.7–4.4) | 143 (92–163) | 222 (147–230) | 181 (111–187) |
| 60 | 169 (45–418) | 85 (36–112) | 28 (26–35) | 14.6 (11.8–17.2) | 9 (6.6–13.2) | 5.5 (5.2–6.8) | 85 (43–137) | 219 (128–272) | 214 (144–286) |
| 90 | 351 (105–774) | 78 (42–117) | 29 (25–33) | 14.9 (10.2–18.8) | 10.2 (7.0–13.6) | 7.8 (4.2–9.3) | 36 (21–49) | 175 (111–208) | 312 (256–348) |
| 120 | 704 (97–1454) | 94 (63–135) | 29 (25–35) | 12.9 (10.2–17.2) | 9.7 (8.0–12.0) | 9.7 (4.0–12.9) | 28 (9–64) | 129 (68–232) | 305 (191–348) |
| 150 | 879 (115–1238) | 123 (36–332) | 34 (24–37) | 11.2 (10.6–15.9) | 8.9 (8.0–13.8) | 6.4 (3.0–10.9) | 24 (9–210) | 80 (29–269) | 266 (162–308) |
| 180 | 724 (264–2479) | 150 (63–308) | 30 (22–34) | 12.9 (9.7–14.6) | 8.8 (6.8–13.1) | 7 (3.5–9.8) | 38 (6–158) | 90 (34–168) | 215 157–274) |
| 210 | 846 (243–1990) | 309 (103–488) | 27 (21–31) | 13.4 (11.2–13.8) | 9.6 (8.0–14.1) | 4.4 (3.1–7.7) | 8 (2–94) | 81 (15–150) | 192 (171–286) |
| Elapsed time (h) | Glucose (mM/L) | | | Glutamate (μM/L) | | | Glycerol (μM/L) | | |
| | Hemisphere | Sagittal sinus | Femoral | Hemisphere | Sagittal sinus | Femoral | Hemisphere | Sagittal sinus | Femoral |
| S −01:00 | 2.4 (1.2–3.7) | 3 (1.3–4.0) | 5.2 (3.1–5.3) | 5 (5–13) | 130 (75–168) | 183 (168–200) | 27 (15–50) | 31 (17–57) | 34 (28–54) |
| S −00:30 | 2.9 (2.4–3.7) | 2.9 (1.1–3.7) | 4.1 (2.4–7.1) | 8 (3–11) | 162 (121–177) | 199 (159–209) | 26 (14–55) | 28 (24–63) | 34 (26–35) |
| S | 3.6 (2.7–4.1) | 1.7 (1.3–3.8) | 3.7 (2.1–6.5 | 6 (4–7) | 174 (104–204) | 180 (148–209) | 23 (13–41) | 31 (23–55) | 21 (20–29) |
| 0 | 1.7 (1.5–1.9) | – | 1.2 (0.7–1.6) | – | 87 (50–121) | 202 (169–221) | 40 (40–40) | 74 (49–100) | 12 (11–14) |
| 30 | 1.9 (1.2–2.4) | 1.3 (0.6–4.2) | 6.9 (1.3–9.2) | 19 (4–55) | 158 (84–159) | 185 (163–206) | 56 (32–108) | 53 (37–112) | 21 (8–32) |
| 60 | 1.0 (0.7–1.4) | 2 (0.02–2.2) | 3.8 (1.1–8.2) | 68 (36–101) | 156 (132–204) | 181 (158–204) | 180 (133–201) | 96 (53–159) | 33 (14–117) |
| 90 | 0.4 (0.3–0.5) | 0.6 (0.02–1.5) | 5.4 (1.7–7.6) | 119 (119–119) | 225 (204–248) | 172 (149–195) | 329 (254–337) | 120 (102–190) | 89 (29–486) |

**Table 2** Biochemical variables obtained from microdialysis *(Continued)*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 120 | 0.2 (1.0–0.3) | 0.3 (0.04–0.7) | 3.4 (1.2–7.3) | 245 (199–291) | 209 (209–209) | 192 (152–198) | 415 (318–455) | 192 (123–258) | 83 (23–671) |
| 150 | 0.1 (0.1–1.0) | 0.1 (0.02–0.5) | 3.1 (0.5–5.6) | 327 (231–329) | 199 (152–259) | 153 (107–177) | 403 (377–548) | 222 (151–286) | 76 (19–621) |
| 180 | 0.2 (0.1–1.3) | 0.1 (0.02–0.2) | 1.8 (0.5–5.9) | 335 (289–367) | 198 (133–256) | 173 (139–180) | 382 (333–547) | 213 (191–230) | 71 (18–314) |
| 210 | 0.3 (0.1–0.9) | 0.02 (0.02–0.7) | 5.5 (0.9–8.1) | 311 (232–322) | 257 (227–287) | 186 (184–188) | 522 (474–612) | 321 (268–331) | 101 (26–182) |

Data are expressed as median (interquartile range). S indicates the start of bleeding to achieve a MAP of 40 mmHg
*LP* lactate/pyruvate ratio

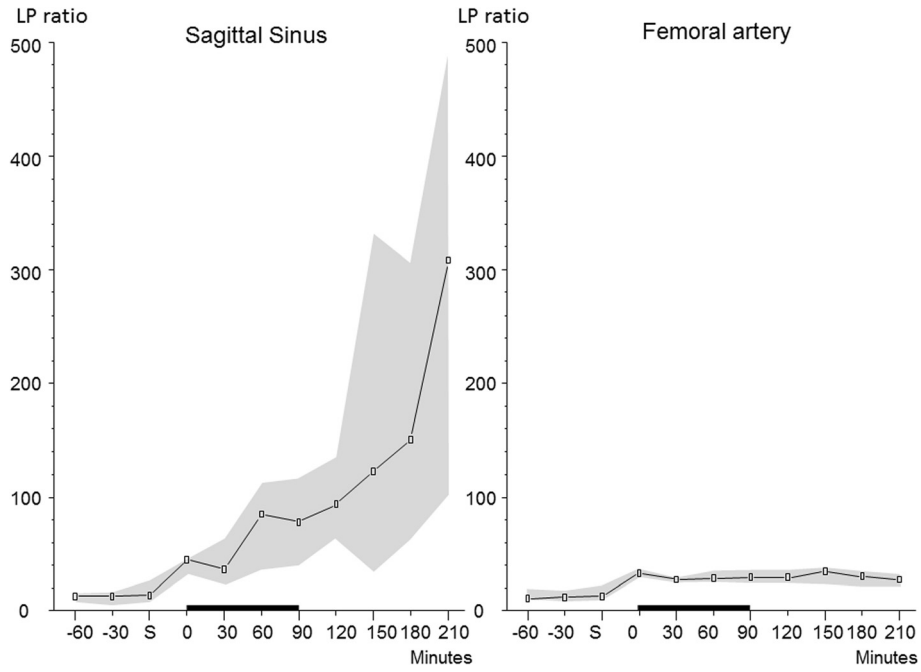


**Fig. 2** Logarithmic illustration of the LP ratios (median (interquartile range)) in the sagittal sinus and femoral artery during hemorrhagic shock in pigs. The increase in the LP ratio in the sagittal sinus was significantly higher ($p < 0.001$) than in the arterial blood. *S* indicates the start of bleeding to achieve a MAP of 40 mmHg. The *black bar* on the *x*-axis from 0 to 90 min indicates the shock period before resuscitation was started

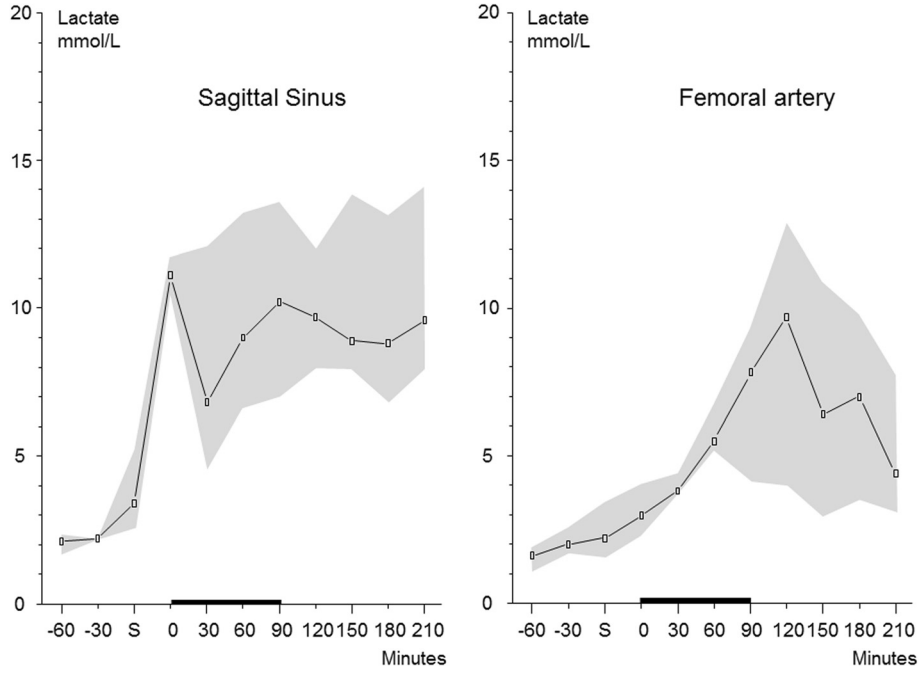


**Fig. 3** Microdialysis levels of lactate (median (interquartile range)) in the sagittal sinus and femoral artery doing hemorrhagic shock in pigs. Note that lactate levels increases in both compartments during shock. After re-infusion of blood, lactate levels in the sagittal sinus remain at a high level throughout the monitoring period. In contrast, the lactate levels in the femoral artery decline to a near-normal level. *S* indicates the start of bleeding to achieve a MAP of 40 mmHg

Lactate and pyruvate are water-soluble. However, due to monocarboxylate transporters (MCTs), they equilibrate rapidly across cellular membranes. MCTs are proton-linked membrane carriers involved in the transport of various monocarboxylates such as lactate, pyruvate, and ketone bodies [12–14, 18]. They are present in all tissues. Out of the total family of 14 members, three isoforms (MCT1, MCT2, MCT4) have been described in the brain [19]. The driving forces for the transport of the monocarboxylates are obtained from the concentration differences over the cellular membranes. The transport is consequently characterized as facilitated diffusion [20]. The MCTs appear to be very effective in transporting lactate and pyruvate. After induction of cerebral ischemia, the intracerebral microdialysis probe will detect an increase in the LP ratio instantaneously and if the circulation is rapidly restored, the LP ratio quickly returns to a normal or near-normal level [9]. Based on these conditions, the present experimental study was designed to explore whether the equilibration of lactate and pyruvate across the blood-brain barrier (BBB) was rapid enough to permit the LP ratio of the draining venous blood to be used as a surrogate marker for global cerebral redox state.

After induction of hemorrhagic shock, the intracerebral LP ratio rapidly increased to a very high level. The increase was due to a marked increase in lactate concentration simultaneously with a pronounced decrease in pyruvate (Table 2, Fig. 2). This metabolic pattern is characteristic of ischemia (i.e., a simultaneous decrease in tissue oxygenation supply and substrate/glucose) [8–10]. After re-infusion of blood, the increase in lactate and the LP ratio increased further while pyruvate concentration continued to decrease. Tissue biochemical analysis thus revealed an insufficient supply of oxygen as well as substrate during the induced hemorrhagic shock that continued after blood transfusion and normalization of MAP.

In the superior sagittal sinus, the LP ratio exhibited a parallel though less pronounced pattern (Table 2, Fig. 2). Like in the cerebral tissue, the microdialysis catheter in the sagittal sinus continued to reveal a markedly elevated LP ratio after re-infusion of blood and the pyruvate concentration decreased to a very low level. In the femoral artery, a modest increase in the LP ratio and a moderate increase in the pyruvate level were obtained during and after the induced hemorrhagic shock. During the whole experimental period, the LP ratio of the femoral arterial blood remained close to the upper reference level (≤30) of normal cerebral tissue [17]. The difference between the level of LP ratio in the superior sagittal sinus and femoral artery after induction of hemorrhagic shock was highly significant, and our null hypothesis was rejected. Accordingly, we conclude that the LP ratio monitored in cerebral venous blood reflected the pronounced global intracerebral redox shift and was not caused by affected energy metabolism in extracranial tissues.

As shown in Table 2 and Fig. 3, the lactate concentration in the superior sagittal sinus increased during and after the period of shock. However, as arterial lactate level also increased markedly, intravenous lactate monitoring alone cannot be used as a marker of compromised cerebral energy metabolism.

### $PbtO_2$ and intracerebral glucose

$PaO_2$ and b-glucose levels were kept relatively constant during the experimental period (Table 1). In spite of this fact, $PbtO_2$ and intracerebral glucose decreased to very low levels during the induced hypotensive shock and remained very low after re-infusion of

blood (Table 1, Fig. 1). This pattern is compatible with that observed during cerebral ischemia and corroborates the intracerebral microdialysis findings (Table 2). Accordingly, although MAP returned to close to the initial level after blood re-infusion (Table 1), cerebral perfusion was obviously not sufficient for restoring energy metabolism. The finding is probably explained by the fact that a progressive increase in ICP caused a decrease of CPP to a low level (40 mmHg; Table 1). The reason for the progressive increase in ICP is probably because of a global postischemic cytotoxic edema and leakage of the blood-brain barrier. Due to insufficient perfusion, arterial blood glucose was virtually completely extracted which resulted in a very low glucose level in the sagittal sinus (Table 2).

### Glutamate and glycerol

During clinical intracerebral microdialysis use, an increase in glutamate concentration is generally interpreted as insufficient astrocytic uptake of released glutamate due to energy failure [21, 22]. In the present study, intracerebral glutamate increased markedly during the hypotensive shock period and did not return to normal level after blood re-infusion (Table 2). Thus, the observed changes in intracerebral glutamate are in accordance with the interpretation above: hypotensive shock caused cerebral ischemia and energy failure that did not recover after blood transfusion.

The normal blood-brain barrier is not permeable to glutamate [21]. Under normal conditions, interstitial cerebral concentration is approximately 2 μmol/L while blood concentration is 100–200 μmol/L. Accordingly, glutamate level obtained in cerebral venous blood does not reflect the intracerebral level. In the present study, the high concentration of glutamate obtained before the start of the experiment (100–200 μmol/L; Table 2) documents that the microdialysis catheter was actually positioned in the superior sagittal sinus in each experimental animals.

Intracerebral glycerol measured by microdialysis is conventionally used as a marker of degradation of cellular membranes into free fatty acids and glycerol [23, 24]. In the present experimental situation, intracerebral glycerol increased to a very high level during hypotensive shock and remained at this high level after transfusion (Table 2). The finding supports the interpretation that induced hemorrhagic shock to MAP 30 mmHg for 90 min caused cerebral energy failure and decomposition of cellular elements. However, increase in glycerol in cerebral venous blood (Table 2) does not necessarily result from degradation of cerebral cellular elements. The intact BBB has a very low permeability for glycerol [25]. In many extracerebral tissues, triglycerides are important cellular components. During stress and increased sympathetic tonus, triglycerides are degraded, which is reflected in fat tissue and in the blood as an increase of free fatty acids and glycerol [26]. Accordingly, an increase in glycerol concentration was in the present experimental situation also obtained in the femoral arterial blood (Table 2).

### Clinical relevance of the experimental model

The study indicates that it is possible to evaluate global cerebral energy state by simultaneous monitoring of the redox state (LP ratio) in a cerebral vein. Under clinical conditions, this could be performed by placing the venous microdialysis catheter in the internal jugular vein close to the jugular bulb. In this way, it might be possible to continuously evaluate

cerebral energy state bedside without inserting an intracerebral probe. This technique would be valuable in various serious conditions in need of critical care.

After cardiac standstill and resuscitation, the possibilities of evaluating cerebral damage and prognosis are still limited [27–30]. In these patients, a bedside continuous technique might also be used to monitor the effects of various therapies (e.g., hypothermia). For this purpose, intracerebral microdialysis has been used in a few selected cases [31] but it is unlikely that this invasive technique will be used in clinical routine. In patients subjected to open-heart surgery with or without cardiopulmonary bypass, minor cerebral complications appear to be frequent [32–36]. In these patients, analysis of lactate concentration from a microdialysis catheter positioned in a central vein has been proposed [37]. Although this technique was shown to give reliable information regarding global venous lactate level, it will not give specific information regarding cerebral energy metabolism. In patients with hepatic failure (HF) leading to cerebral symptoms and coma, intracerebral microdialysis has shown that an increase in tissue LP ratio is correlated to increases in tissue glutamine and hypoxanthine [38, 39] and energy failure appears to be an important pathogenetic component of both acute and chronic HF and a potential target for therapy [40].

The technique of evaluating global cerebral energy/redox state from the LP ratio obtained from a microdialysis catheter positioned in the internal jugular vein might give important information in a multitude of severe clinical conditions when direct measurements of tissue biochemistry is difficult or impossible. However, it should be recalled that evaluation of the LP ratio in the draining vein will not give quantitative, correct information regarding cerebral extracellular LP ratio (Table 2). This is of limited importance. Under clinical routine conditions, an upper normal level for the LP ratio is utilized (usually 30 or 40) and the exact level of the LP ratio is often of secondary importance [11, 41]. During intracerebral microdialysis, the LP ratio and the concentration of pyruvate have also been used to differentiate between ischemia and mitochondrial dysfunction [8, 10, 11]. This kind of detailed analysis and interpretation may not be possible when the LP ratio is monitored in cerebral venous blood.

During neurocritical care, the cerebral interstitial levels of glutamate and glycerol are used as indicators of insufficient energy production and cellular degradation. However, if the microdialysis catheter is positioned in cerebral venous blood, these interpretations are not valid for reasons given above.

### Limitations

In experimental hemorrhagic shock, it has been described that, in contrast to the systemic macrocirculation, cerebral microcirculation may be remarkably well preserved [42]. Though the implications of this finding have been questioned, it is still an open question when and to what degree cerebral energy metabolism is compromised during hemorrhagic shock under clinical conditions [43]. In a recent experimental study utilizing multimodal monitoring with simultaneous imaging of cerebral hemodynamics and NADH signals, the authors demonstrated the temporal relationship between compromised microcirculation and compromised oxidative metabolism [44]. In this model of severe hemorrhagic shock, the oxidative metabolism was not restored after retransfusion of the extracted blood volume. From that study and the present data, it

appears that during severe hemorrhagic shock cerebral energy metabolism is severely compromised exhibiting a biochemical pattern typical of ischemia. Further, if hypotension is protracted and severe enough, cerebral energy metabolism may not be restored after transfusion. In the present experimental study, the biochemical pattern and the progressive increase of ICP indicated permanent cerebral lesions. The present experimental model was chosen because it creates reproducible severe global cerebral ischemia. However, the chosen hypotensive level of MAP around 35 mmHg is somewhat lower that the recommendations by the European Society for Intensive Care Medicine expert panel [45]. It is therefore important to stress that the present study cannot be used to determine the optimal level of MAP after hemorrhagic shock. The purpose of the present study was solely to establish a technique for "non-cranial" invasive monitoring of cerebral energy state. As shown in Table 2, there is a quantitative discrepancy in the LP ratio between the cerebral microdialysis probe and the one placed in the sinus. This suggests a "washout" effect. The degree of metabolic derangement in the present study was severe. In a clinical setting, e.g., after cardiac standstill, a less pronounced metabolic derangement will be expected. Accordingly, it might not be possible to detect minor metabolic derangements in the venous jugular bulb due to the washout effect. Future clinical studies are needed to determine this.

## Conclusions

This experimental study documents that during protracted (≈90 min) and pronounced (MAP≈35 mmHg) hemorrhagic shock, cerebral energy metabolism was severely compromised and exhibited a biochemical pattern typical of ischemia, energy failure, and cellular degradation. After re-transfusion, this pattern was even more pronounced indicating irreversible tissue damage. From intravascular microdialysis in the superior sagittal sinus, it is possible to achieve semi-quantitative information of the cerebral cytoplasmatic redox state (LP ratio) that can be separated from the biochemical alterations in extracranial tissues. Accordingly, it is possible to monitor global cerebral energy state continuously with a strictly extracerebral technique. This technique might be valuable during critical care of various severe conditions where cerebral energy metabolism may be compromised, e.g., resuscitation after cardiac standstill, open-heart surgery, multi-trauma, hemorrhagic or septic shock, and hepatic coma. Studies are in progress in these clinical conditions and will reveal whether the proposed technique for evaluating global cerebral energy state will be added to the methods to measure and monitor brain function that have evolved in recent years [46].

#### Competing interests

Rasmus Jakobsen, Troels Halfeld Nielsen, Asger Grandfeldt, and Palle Toft declare that they have no competing interests. Carl-Henrik Nordström is consulting for M Dialysis AB.

#### Authors' contributions

CHN, PT, and THN contributed to the conception of the study. RJ, AG, and THN performed the data acquisition, supervised by PT and CHN. THN performed the statistical analysis. CHN has performed the data analysis, and all authors have contributed to the interpretation. CHN has drafted the manuscript, and all authors have contributed equally to the proofreading and discussion of the final manuscript.

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RESEARCH Open Access

# Moderately prolonged permissive hypotension results in reversible metabolic perturbation evaluated by intracerebral microdialysis - an experimental animal study

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## Abstract

**Background:** Damage control resuscitation (DCR) and damage control surgery (DCS) is the main strategy in patients with uncontrollable hemorrhagic shock. One aspect of DCR is permissive hypotension. However, the duration of hypotension that can be tolerated without affecting the brain is unknown. In the present study we investigate the effect of 60 min severe hypotension on the brain's energy metabolism and seek to verify earlier findings that venous cerebral blood can be used as a marker of global cerebral energy state.

**Material and methods:** Ten pigs were anaesthetized, and vital parameters recorded. Microdialysis catheters were placed in the left parietal lobe, femoral artery, and superior sagittal sinus for analysis of lactate, pyruvate, glucose, glycerol, and glutamate. Hemorrhagic shock was induced by bleeding the animal until mean arterial pressure (MAP) of 40 mmHg was achieved. After 60 min the pigs were resuscitated with autologous blood and observed for 3 h.

**Results:** At baseline the lactate to pyruvate ratios (LP ratio) in the hemisphere, artery, and sagittal sinus were (median (interquartile range)) 13 (8–16), 21 (18–24), and 9 (6–22), respectively. After induction of hemorrhagic shock, the LP ratio from the left hemisphere in 9 pigs increased to levels indicating a reversible perturbation of cerebral energy metabolism 19 (12–30). The same pattern was seen in LP measurements from the femoral artery 28 (20–35) and sagittal sinus 22 (19–26). At the end of the experiment hemisphere, artery and sinus LP ratios were 16 (10–23), 17 (15–25), and 17 (10–27), respectively. Although hemisphere and sinus LP ratios decreased, they did not reach baseline levels ($p < 0.05$). In one pig hemisphere LP ratio increased to a level indicating irreversible metabolic perturbation (LP ratio > 200).

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**Conclusion:** During 60 min of severe hypotension intracerebral microdialysis shows signs of perturbations of cerebral energy metabolism, and these changes trend towards baseline values after resuscitation. Sagittal sinus microdialysis values followed hemisphere values but were not distinguishable from systemic arterial values. Venous (jugular bulb) microdialysis might have a place in monitoring conditions where global cerebral ischemia is a risk.

**Keywords:** Hemorrhage, Shock, Microdialysis, Cerebrum, Permissive hypotension, Lactate, Pyruvate, Venous microdialysis

## Background

Trauma remains a leading cause of mortality. The leading cause of preventable deaths in trauma patients is uncontrollable hemorrhage [1]. During recent years, damage control resuscitation (DCR) and damage control surgery (DCS) has been the main strategy in the management of major hemorrhage in trauma patients [2, 3]. Damage control resuscitation incorporates balanced blood transfusions, tranexamic acid, and permissive hypotension until surgical hemostasis or control is achieved [4–7]. The brain is the most sensitive organ in regard to hypotension, and knowledge, of the effect of permissive hypotension on the brain without traumatic brain injury is scarce [8–10]. The authors have in a previously published experimental study shown that prolonged (90 min) and severe hypotension (mean arterial pressure (MAP) of 40 mmHg) results in irreversible metabolic perturbation evaluated by intracerebral microdialysis (MD) [11]. Furthermore, the same study showed that the cerebral biochemical changes in response to severe hypotension were reflected in the cerebral venous outflow. The primary aim of the present study is to investigate the effect of moderately prolonged (60 min) severe hemorrhagic shock (MAP = 40 mmHg) on the brain's metabolism evaluated by microdialysis, and secondly to confirm our previously findings that venous microdialysis can be used as a marker of global energy metabolism. The hypothesis is that moderately prolonged and severe shock causes reversible metabolic derangement defined as a temporary increase in hemisphere lactate to pyruvate ratio (LP ratio).

## Materials and methods

The study was approved by the Danish Animal Experiments Inspectorate (2015–15–0201–00788). The depth and duration of hemorrhagic shock necessary for producing cerebral ischemia that caused compromised energy state was based on previous experiments [11] and 4 initial experiments that confirmed feasibility. This was followed by six additional female pigs approximately 4 months old. All were Danish landrace mix, Yorkshire Duroc breed. The median weight was 42 (35–45) kg (interquartile range).

### Anesthesia, mechanical ventilation, and surgical preparation

The porcine model of hemorrhagic shock has been previously described [12]. The animals fasted overnight with access to ad libitum water. Sedation was achieved with a standard combination of medetomidine (0.05 mg/kg), midazolam (0.25 mg/kg), and atropine (0.25 mg/kg). Anesthesia was induced with midazolam (0.625 mg/kg) and ketamine (12.5 mg/kg) and maintained with an infusion of midazolam (5 mg/kg/h) and fentanyl (50 μg/kg/h). The animals were intubated and volume-controlled ventilated

(Siemens 900 Ventilator; Siemens Elema, Stockholm, Sweden) with, a tidal volume of 10 mL/kg and FiO2 of 0.30. PaCO2 was kept between 4 and 6 kPa and body temperature around normal 38.5 °C. Crystalloid intravenous fluid 2–4 mL/kg/h was administered until start of the experimental protocol.

### Multimodal monitoring

After establishing anesthesia one sheath was inserted into the carotid artery for blood pressure monitoring and blood gas sampling. The external jugular vein was cannulated for the insertion of a central venous catheter to deliver anesthesia. Arterial blood gases (PaCO2, PaO2, pH), blood glucose, electrolytes, and lactate levels were measured every 30 min. (epoc vet, Alere, Waltham, MA, USA).

One femoral artery was cannulated for withdrawing and re-infusing blood during the induced hemorrhagic shock. Another sheath was placed in the contralateral femoral artery, and a microdialysis catheter (CMA 67, MDialysis, Stockholm, Sweden) was inserted through a standard 18G IV catheter. A small craniotomy was placed in the frontal bone in the midline above the superior sagittal sinus. The sinus was cannulated by an 18G peripheral venous catheter, and one microdialysis catheter (CMA 70 Bolt MDialysis, Stockholm, Sweden) was introduced in a posterior direction and placed in the posterior part of the superior sagittal sinus. The superior sagittal sinus was chosen for analysis of cerebral venous blood due to the anatomic characteristics of the experimental animal. In the pig, most of the cerebral blood is drained via paraspinal venous plexa and only a minor part passes into the internal jugular vein [13]. A third microdialysis catheter (CMA 70, MDialysis AB, Stockholm, Sweden) was inserted 20 mm into the left parietal lobe, and one probe for monitoring brain tissue oxygenation ($PbtO_2$) (Licox CC1SB, Integra Neurosciences Ltd. New Jersey, USA) was introduced 15 mm into the contralateral parietal lobe. A transducer for monitoring intracranial pressure (ICP) (Camino, Integra Neurosciences Ltd. New Jersey, USA) was placed in the right hemisphere. After insertion, all probes were allowed a minimum of 1 h for stabilization. A bladder catheter was placed for urine collection. All animals were given a baseline dose of 200 U/kg of heparin and supplemented hourly with 100 U/kg for anticoagulation during the hemorrhage period. At the end of the experiment, the anesthetized animals were killed with an intravenous injection of sodium pentobarbital 200 mg/mL in concentrated ethanol.

### Experimental protocol

Following a 60-min baseline period allowing animals to stabilize after surgery hemorrhagic shock was achieved by bleeding the animals to a pre-defined MAP of approximately 40 mm Hg at a rate of 2.15 mL/kg/min over 7 min, and then 1.15 mL/kg/min over the remaining period [12]. Animals were kept at a MAP of about 40 mmHg by withdrawing or infusing shed blood that was stored in a citrated glucose solution at 37 °C. Following 60 min of hemorrhagic shock, the animals were resuscitated by re-infusing the shed blood at a rate of 120 mL/min until all blood was returned. The pigs were observed for 3 h after hemorrhagic shock. Microdialysis probes were perfused with artificial CSF (M Dialysis AB, Stockholm, Sweden) at a rate of 0.3 μL/min (CMA 106 MD pump, MDialysis AB, Stockholm, Sweden). The dialysates were collected in

microvials and immediately analyzed for glucose, lactate, pyruvate, glutamate, and glycerol every 30 min using an Iscus Flex analyzer (M Dialysis AB, Stockholm, Sweden).

$PbtO_2$ data were collected using the AC3.1 monitor (Integra Neurosciences Ltd.) and recorded every 20 s. All Licox probes were tested against atmospheric air and against each other before insertion and after removal. After insertion, the appropriate function was confirmed by an oxygen challenge test.

ICP was monitored continuously and data were collected by a CAM01 monitor (Integra Neurosciences Ltd. New Jersey, USA) and ICP. Cerebral perfusion pressure (CPP) was calculated as MAP-ICP.

## Statistics

Data are given as median (interquartile range) unless otherwise noted. To test our hypothesis, the time course of the experimental protocol was divided into three intervals: Fig. 1 interval A which consists of the baseline prior to hypotension; interval B, during hypotension; and interval C, after resuscitation with shed blood. The end of hypotension was defined post hoc as the time when $PbtO_2$ had increased to baseline values. To test the hypothesis data were modeled utilizing a mixed effect model for repeated measurements with time interval as fixed effect and each animal as random effect. Each animal functioned as its own control. The null hypothesis was that no difference in the hemisphere LP ratio was found between interval A and C. To test our hypothesis regarding global sagittal sinus microdialysis, the time course of the LP ratio in the superior sagittal sinus and femoral artery was also modeled utilizing a mixed effect model with time and location of microdialysis probe as random effects and each animal as fixed effect. The null-hypothesis was that no difference in the LP ratio was found between the superior sagittal sinus and femoral artery. $p$ values below 0.05 was considered significant. Data analysis was performed in Stata 15 statistical software (StataCorp, College Station, Texas, USA).

## Results

All 10 animals completed the protocol. However, one animal showed signs of irreversible metabolic perturbation during the experiment indicated by very high levels of LP ratio (> 200) and low levels of glucose and brain tissue oxygenation. This animal was analyzed separately.

The sagittal sinus MD catheter of three of the animals showed low levels (< 20 mmol/L) of glutamate indicating that the catheters had dislodged into the subdural space. The data from these MD catheters were excluded.

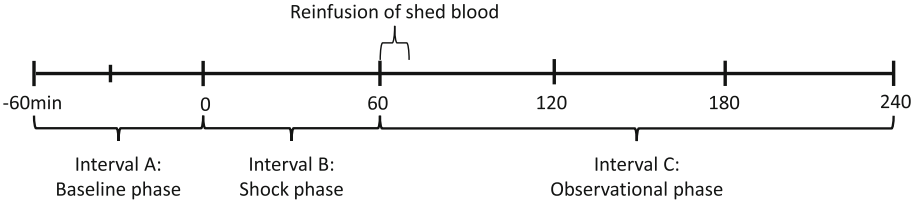


**Fig. 1** Experimental protocol timeline. Note that the experimental protocol is divided into three intervals. Interval A: baseline phase; interval B: shock phase with a mean arterial pressure of 40 mmHg; and interval C: observational phase after resuscitation with shed blood

General physiological and biological variables are listed in Table 1.

The median volume of shed blood was 1469 mL (1378.4–1583.44 mL) and median blood loss per kilogram was 35 mL/kg (31.9–36.8 mL/kg).

During the period of hemorrhagic shock, MAP decreased to a median value of 39 mmHg (35–42). Accordingly, CPP decreased to low levels of 33 mmHg (28–39). Similarly, the brain tissue oxygen tension decreased to near critical levels of 19 mmHg (8–23). After resuscitation with shed blood MAP increased to near baseline levels ($p < 0.05$). The CPP showed a similar response, normalizing, but not reaching baseline levels ($p < 0.05$). In contrast, $PbtO_2$ increased to levels exceeding baseline values and stayed at that level during the rest of the observation period ($p < 0.05$). The relationship between MAP and $PbtO_2$ can be seen in Fig. 2. Blood gases, pH, and blood glucose were all kept within normal range.

### Microdialysis values

Measurements obtained by microdialysis are listed in Table 2.

The dynamic changes in LP ratio in hemisphere, sagittal sinus, and femoral artery are shown in Fig. 3. Before induction of hemorrhagic shock, the LP ratios were similar in the hemisphere, sagittal sinus, and femoral artery. During hemorrhagic shock the LP ratio in the hemisphere increased to levels below the upper normal level for anesthetized piglets ((21 ± 9) (mean ± SD)) [14; 15]. After resuscitation, the LP ratio decreased towards baseline levels but remained elevated throughout the observation period ($p = 0.02$). The same pattern was seen for the sagittal sinus whereas the femoral artery LP ratio returned to baseline values ($p = 0.467$). LP ratio in the femoral artery reached a higher absolute level to a median of 28 (20–35) than the hemisphere or sagittal sinus.

**Table 1** General physiological and biochemical variables doing hemorrhagic shock. $N = 9$. Data are expressed as median levels (interquartile range) during the experimental protocol intervals A, B, and C. *MAP* mean arterial pressure, *ICP* intracranial pressure, *CPP* cerebral perfusion pressure, $PbtO_2$ brain tissue oxygenation. S indicates start of hemorrhage. Time 0 indicates achievement of MAP equal 40 mmHg. Test statistics made with mixed effect model

| Elapsed time (min) | Baseline phase Interval A (– 60)–0 min | Shock and resuscitation phase Interval B 0–70 min | Observational phase Interval C 70–220 min | Interval A versus interval C *p* value |
|---|---|---|---|---|
| MAP (mmHg) | 95 (66–107) | 39 (35–42) | 76 (67–84) | $p < 0.005$* |
| ICP (mmHg) | 8 (6.5–11) | 6 (5–9) | 10 (7–14) | $p = 0.089$ |
| CPP (mmHg) | 84 (60–97.5) | 33 (28–39) | 65 (53–79.5) | $p < 0.005$ * |
| PbtO2 (kPa) | 29 (24–32) | 19 (8–23) | 35 (20–45) | $p < 0.005$* |
| PaO2 (kPa) | 24 (22–26) | 24 (23–25) | 22 (22–24) | $p = 0.002$* |
| PaCO2 (kPa) | 5.4 (5,5–89) | 5.1 (4,7–5.6) | 6.1 (5.7–6.7) | $p = 0.025$* |
| b-hemoglobin (mM/L) | 8.6 (5.8–9.8) | 7.9 (5.2–8.8) | 7.8 (6–9.3) | $p = 0.084$ |
| HR (bpm) | 80 (71–92) | 125 (82–152) | 81 (72–92) | $p = 0.502$ |
| b-Glucose (mM/L) | 5.5 (4.3–7.2) | 6.3 (4.2–9.8) | 5.3 (4.1–6.9) | $p = 0.010$* |
| b-lactate (mM/L) | 0.9 (0.5–1.2) | 4.3 (2.5–7.3) | 1.8 (0.9–5.1) | $p = 0.009$* |
| b-pH | 7.47 (7.45–7.51) | 7.44 (7.39–7.44) | 7.40 (7.32–7.46) | $p = 0.249$ |
| Diuresis (mL) | 55 (10–160) | 3 (0–12) | 19 (6–32) | $p < 0.005$* |

*Statistical significant *p* value < 0.05

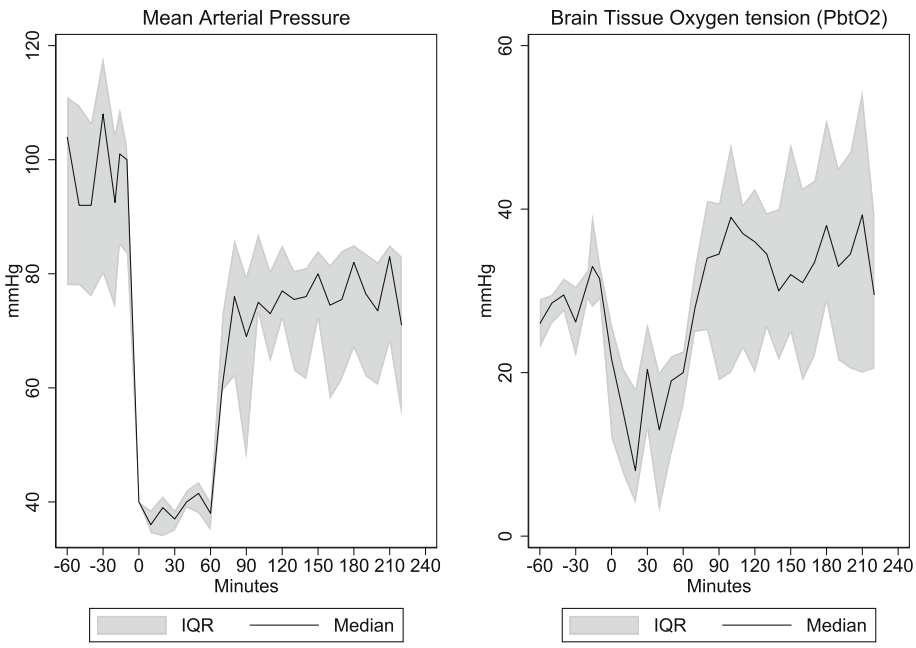


**Fig. 2** Mean arterial pressure and brain tissue oxygen tension. Median (interquartile range) arterial pressure (MAP) and brain tissue oxygen tension (PtbO2) in pigs ($n = 9$) with induced hemorrhagic shock. Note that during the period of hemorrhagic shock the declining MAP was accompanied by a decrease in $PbtO_2$ near critical levels (< 15 mmHg). After re-infusion of autologous blood MAP increased to close to baseline level whereas $PbtO_2$ increased to levels above baseline. Time 0 indicates achievement of MAP equal to 40 mmHg

However, baseline levels were also higher in the femoral artery. There was no difference in the LP ratio during the experimental protocol between the femoral artery and sagittal sinus ($p = 0.353$).

The dynamic changes of lactate, pyruvate, and glucose along with changes in LP ratio in the hemisphere are shown in Fig. 4. Lactate baseline values were near the upper normal levels ((1.8 ± 0.8) mean ± SD) [14]. During hemorrhagic shock lactate levels increased to a median value of 3.2 mmol/L (2.5–6.1 mmol/L). After resuscitation hemisphere, lactate levels fell, but not reaching baseline values in the observational period ($p < 0.05$). Pyruvate demonstrated increasing tendency during shock and throughout the observation period. Accordingly, the changes in LP ratio were mainly due to change in lactate.

Hemisphere glucose showed a small increase throughout the experimental protocol ($p = 0.031$) but remained within normal levels ((1.8 ± 0.8) mean ± SD) [14]. The same trend was seen in arterial and sagittal sinus glucose $p = 0.01$ and $p < 0.05$ respectively (Table 2).

Intracerebral glycerol increased slightly during the hypotensive and observational periods to 59 (35–116) μmol/L and 77 (58–125) μmol/L respectively (Table 2). In contrast, hemisphere glutamate fell but remained within normal values throughout the observational period (Table 2).

One animal showed signs of irreversible perturbation of cerebral energy metabolism evaluated by hemisphere microdialysis (Fig. 5). After induction of hemorrhagic shock, the LP ratio increased to very high levels (> 200). The increase was due to a pronounced and lasting elevation of lactate (> 15 mmol/L) and less pronounced but lasting

**Table 2** Biochemical variables obtained from microdialysis. $N = 9$. Data expressed as (interquartile range) during the experimental protocol intervals A, B, and C. *LP* lactate/pyruvate ratio. S indicates start of hemorrhage. Time 0 indicates achievement of MAP equal 40 mmHg. Test statistics made with mixed effect model

| Elapsed time (min) | Location | Baseline phase Interval A (− 60)–0 min | Shock and resuscitation phase Interval B 0–70 min | Observational phase Interval C 70–220 min | Interval A versus interval C *p* value |
|---|---|---|---|---|---|
| LP ratio | Hemisph. | 13 (8–16) | 19 (12–30) | 16 (10–23) | 0.020* |
| | Sag. sinus | 9 (6–22) | 22 (19–26) | 17 (10–27) | 0.012* |
| | Femoral | 21 (18–24) | 28 (20–35) | 17 (15–25) | 0.467 |
| Lactate (mM/L) | Hemisph. | 2.3 (1.3–3.0) | 3.2 (2.5–6.1) | 3 (2.3–4.5) | 0.000* |
| | Sag. sinus | 0.9 (0.3–2.5) | 4.7 (3.4–6.3) | 2.6 (1.1–5.4) | 0.002* |
| | Femoral | 1.1 (0.8–1.8) | 4.6 (1.6–6.1) | 1.7 (0.8–3.9) | 0.009* |
| Pyruvate (mM/L) | Hemisph. | 142 (98–203) | 147 (120–261) | 156 (127–249) | 0.000* |
| | Sag. sinus | 109 (48–151) | 197 (187–313) | 153 (113–201) | 0.003* |
| | Femoral | 55 (44–75) | 151 (45–198) | 94 (53–154) | 0.000* |
| Glucose (mM/L) | Hemisph. | 2.2 (1.8–3) | 1.8 (1.4–3.1) | 2.5 (2–3.5) | 0.031* |
| | Sag. sinus | 1.9 (1–3) | 3.5 (2.4–5.1) | 3.5 (2.3–4.5) | 0.000* |
| | Femoral | 4.9 (3.4–5.6) | 6 (4.9–7.3) | 5.5 (4.6–7.1) | 0.010* |
| Glutamate (μM/L) | Hemisph. | 15 (4–22) | 12 (5–14) | 8 (3–11) | 0.050 |
| | Sag. sinus | 121 (60–178) | 115 (94–176) | 177 (110–195) | 0.011* |
| | Femoral | 195 (172–205) | 190 (184–196) | 183 (173–231) | 0.181 |
| Glycerol (μM/L) | Hemisph. | 38 (25–88) | 59 (35–116) | 77 (58–125) | 0.000* |
| | Sag. sinus | 21 (7–55) | 69 (43–130) | 61 (34–123) | 0.006* |
| | Femoral | 15 (12–28) | 47 (22–174) | 46 (30–209) | 0.000* |

*Statistical significant *p* value < 0.05

decrease in pyruvate. Along with the increase in LP ratio, a dramatic decrease in glucose (< 1 mmol/L) was seen.

## Discussion

The effect of permissive hypotension on cerebral energy metabolism is not well described. The cerebral energy state is completely dependent on oxidative metabolism, which is reflected immediately in the cerebral cytoplasmic redox state. The ratio between interstitial lactate and pyruvate (LP ratio) is shown to be a robust marker of the cellular redox state.

In the present study we demonstrate that a period of 60 min severe hypotension causes a decrease in $PbtO_2$ but only a small increase in the intracerebral LP ratio. This increase along with stable values of glucose, glycerol, and glutamate indicates reversible metabolic perturbation of the cerebral redox state.

### Brain tissue oxygenation ($PbtO_2$) and LP ratio

During the induced hemorrhagic shock $PbtO_2$ decreased to levels regarded as near critical [15]. Along with the decrease in $PbtO_2$ the LP ratio increased but remained within normal levels. The elevation was due to an increase in lactate along with a less pronounced increase in pyruvate.

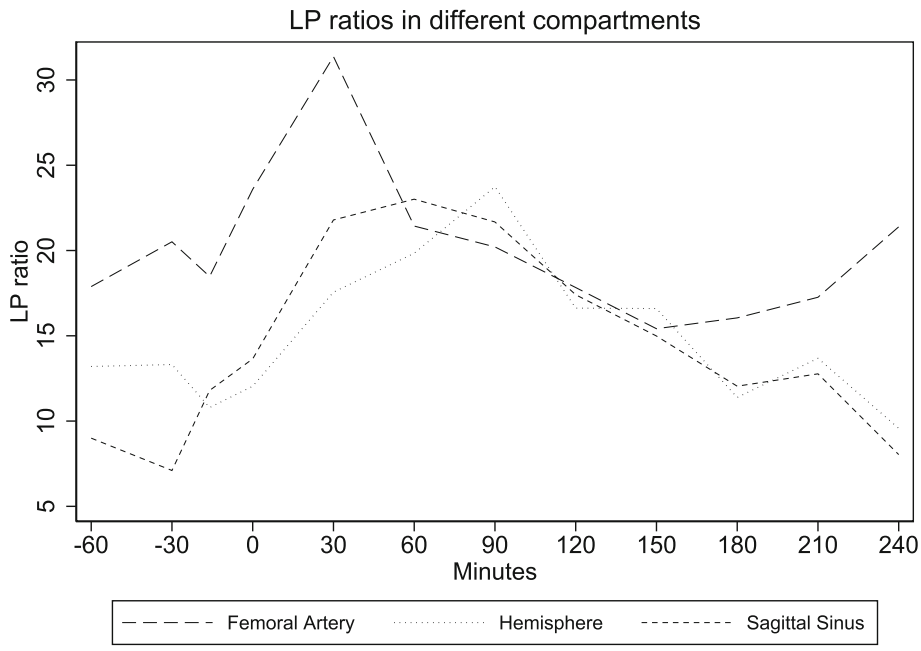


**Fig. 3** LP ratios in different compartments. LP ratio (median (interquartile range)) in brain parenchyma, venous (superior sagittal sinus), and arterial (femoral artery) blood doing hemorrhagic shock in pigs ($n = 9$). Note that changes in hemisphere LP ratio are paralleled by changes in both venous (sagittal sinus) and arterial blood. There was no difference in LP ratio between arterial and venous blood ($p = 0.353$). Time 0 indicates achievement of MAP equal to 40 mmHg

Although the metabolic perturbation is caused by a decrease blood and oxygen supply, the observed pattern is not consistent with cerebral ischemia. Cerebral ischemia is characterized by a significant decrease in $PbtO_2$ and glucose along with a significant increase in LP ratio due to an increase in lactate and decrease in pyruvate [16]. On the other hand, the observed metabolic pattern in the present study is compatible with mitochondrial dysfunction [17]. The concept of mitochondrial dysfunction should be viewed in its broadest term. Mitochondrial dysfunction will obviously exist when mitochondria are damaged, but the metabolic patterns are also seen if the metabolic

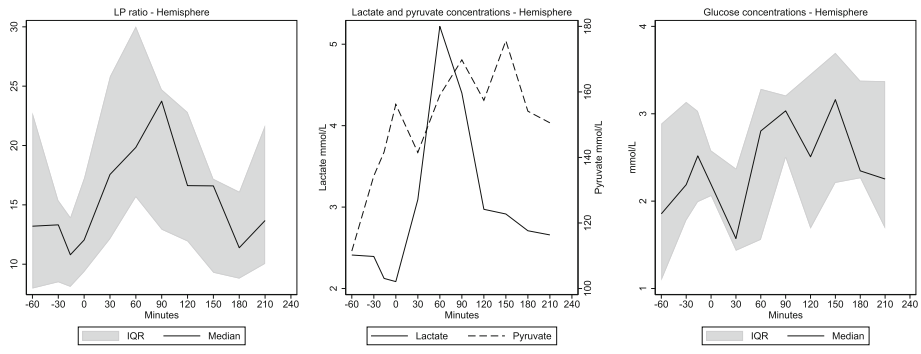


**Fig. 4** Reversible metabolic perturbation. 60 min hypoperfusion. LP ratio and microdialysis levels of lactate, pyruvate, and glucose (median (interquartile range)) in brain parenchyma doing hemorrhagic shock in pigs ($n = 9$). Note that LP-ratio increases doing hemorrhagic shock but tends to normalize after re-infusion of blood. The rise of LP ratio is due to an increase in lactate levels whereas pyruvate shows a less pronounced elevation. Time 0 indicates achievement of MAP equal to 40 mmHg

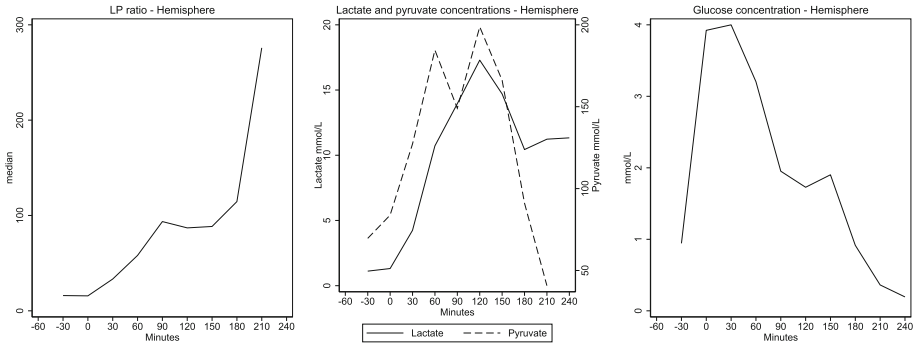


**Fig. 5** Irreversible metabolic perturbation. 60 min hypoperfusion. LP ratio and microdialysis levels of lactate, pyruvate, and glucose in brain parenchyma doing hemorrhagic shock in pig showing signs irreversible metabolic perturbations. Note that LP ratio rises almost exponentially. This increase is due to a constant elevated lactate level and a temporary rise in pyruvate level. After re-infusion of blood, a marked decrease in hemisphere glucose is seen. Time 0 indicates achievement of MAP equal to 40 mmHg

demands exceed the capacity of the oxidative metabolism, i.e., during seizures [18]. However, yet another mechanism is responsible for the observed metabolic pattern in the present study.

During hemorrhage, numerous compensatory mechanisms are activated on both the cellular, tissue, and whole-organism level. The end goal is to maintain a steady supply of oxygen to end-organ tissues as well as prevent excessive bleeding and ensure hemostasis. As bleeding continues, cardiac output, and eventually blood pressure, drops [19]. To ensure cerebral perfusion pressure and cerebral blood flow (CBF) is adequate, autoregulatory mechanism exists. This autoregulatory mechanism ensures that CPP is regulated within a narrow range. The mechanism has been described as both myogenic, metabolic, and neurogenic [20, 21]. When the blood pressure reaches a point where the delivery of oxygen to peripheral tissue is compromised, transition to anaerobic metabolism occurs. During this gradual decrease in CBF oxygen supply to the brain would be insufficient before the supply of substrate (glucose) is seriously jeopardized. This is because of the difference in concentrations and degree of tissue extraction [22]. This phase between compensated low systemic blood pressure and pronounced cerebral ischemia was characterized as hypoxic hypoxia by Siesjö in the 1970s [22], and is characterized by decreasing $PbtO_2$ and elevation of lactate along with normal values of glucose and pyruvate similar to the observed pattern in the present study. As blood pressure continues to decrease deliverance of both oxygen and glucose is further compromised resulting in a pattern of ischemia and metabolic crisis. We have previously shown in a similar animal model that hemorrhagic shock to a MAP of 40 mmHg and a duration of 90 min caused ischemia and subsequent irreversible cerebral damage evaluated by intracerebral microdialysis, ICP, and $PbtO_2$ [11]. In the present study, 60 min of hypotension resulted in an apparently reversible metabolic perturbation. Accordingly, the metabolic perturbations during hemorrhagic shock represent a continuum from normal cerebral metabolism to jeopardized but potential reversible metabolism similar to hypoxic hypoxia towards irreversible ischemia.

The pattern of pronounced cerebral ischemia was seen in one animal in the present study and was characterized by a marked increase in intracerebral LP ratio and decrease in $PbtO_2$ and glucose. The rise in LP ratio was caused by a steep increase in

lactate along with a decrease in pyruvate. This finding underlines the individual differences in each animal regarding cerebral autoregulation and ability to compensate physiological crisis, and that 60 min of severe hypotension is on the edge of how long the autoregulatory mechanisms can compensate for low CBF/systemic blood pressure.

After reinfusion of shed blood, the $PbtO_2$ level increased to levels above baseline values. As $PbtO_2$ primarily is linked to the CBF [23] this increase is likely due a post-hypoperfusion hyperemia.

Parallel to the increase in $PbtO_2$ levels during the observational period, a decrease was seen in LP ratio tending towards baseline levels but not reaching baseline values. Thus, our null hypothesis cannot be rejected. Although the LP ratio did not return to baseline values, the increase in hemisphere LP ration did not exceed normal levels at any point during the experimental protocol, thus supporting reversible metabolic perturbation. This conclusion is supported by the interstitial levels of glutamate and glycerol. Glutamate is regarded as a marker of pending energy failure [24], and glycerol is regarded as a marker of cell membrane degradation and hence cell damage [25]. In the present study glutamate remained stable and within normal values [26]. Glycerol exhibited a slight increase during the observation period but remained within normal values [26].

The microdialysis findings of the present study support the notion that not only the depth but also the duration of the hypotensive period determines if the alterations in brain redox state are reversible or irreversible.

Our findings are supported by an animal study by Wan et al. who reported that intracerebral microcirculation was unaffected by deep and long hemorrhagic hypotension [27]. These findings are also supported in two studies investigating the effect of prolonged and severe hemorrhagic hypotension in rats. The authors found that MAP 40 mmHg for 60–75 min did not cause cognitive damage to the rat, nor was it possible to detect apoptotic areas in the hippocampal area of the brain [28, 29]. However, the abovementioned studies only examined the microcirculatory flow and not the presence of oxygen and nutrients in the interstitial space. Although the authors reported that microcirculation was unaffected, and no structural brain damage was found, deliverance and uptake of oxygen and nutrients might be affected.

### Global microdialysis

Our secondary objective was to verify our earlier findings that venous (superior sagittal sinus) microdialysis can be used as a measure for the hemisphere redox state. During the experimental protocol an increase in hemisphere LP was paralleled by an increase in venous (sagittal sinus) LP ratio, but this rise is not distinguishable from the systemic LP rise. Thus, our secondary null hypothesis cannot be rejected. We have previously demonstrated that global changes in cerebral lactate and pyruvate are reflected in the cerebral venous outflow and distinguished from systemic (i.e., arterial blood) perturbations [11, 30]. However, in the present study a similar increase in lactate and pyruvate was observed in arterial blood. Less pronounced shock leads to less pronounced global metabolic crisis and thus less pronounced alterations in venous LP ratio. During hemorrhagic shock an increase in anaerobic metabolism is present on a whole-organism level. The systemic arterial values of lactate and pyruvate would increase, and thus, an increase in systemic LP ratio would be seen. This systemic increase in LP ratio

can mask an increase in global cerebral LP ratio measured in venous blood. After e.g. cardiac arrest where the systemic anaerobe metabolism has normalized to aerobic metabolism, the brain may still be suffering after a metabolic ischemic crisis. During supportive venous-arterial extracorporeal membrane oxygenation, venous microdialysis may also be able to detect unfavorable distribution of watershed line to the cerebrum which is not detected by bifrontal near-infrared spectroscopy (NIRS) [30, 31].

### Clinical implications

The present study demonstrates an increase in hemisphere LP ratio during moderately long and severe shock. By comparing present findings with earlier finding in a study with 90-min duration of shock, this indicates that there is an upper limit for how long the cerebral autoregulatory mechanisms can compensate for a low systemic blood pressure. As demonstrated by one animal which experienced severe cerebral metabolic changes, not only time but also individual differences between each animal affect how long the autoregulatory mechanisms are able to maintain an adequate cerebral perfusion pressure. During damage control resuscitation and permissive hypotension, all is being done to ensure control of hemorrhage and secure the patient's survival. Although this is being done as fast as possible it is wise to bear in mind that there might be an upper limit in which autoregulatory mechanisms exist. The rise in hemisphere LP ratio during hemorrhagic shock was followed by a rise in the sagittal sinus LP ratio, but this rise is not distinguishable from the systemic LP rise. In a clinical context, the less invasive technique with jugular bulb microdialysis might have a place in advanced monitoring where global cerebral ischemia or isolated cerebral pathologies might be present. When viewing the results from the present and earlier study [11] together this indicates that reversible metabolic crises might not be detected, but as soon as metabolic crises reach a level where the irreversible injury is imminent, it would be detected.

### Limitations

In the present study, we use a fixed-pressure experimental model of hemorrhagic shock. The advantages of this model are its reproducibility as well as the ability of maintaining of a desired target MAP. It also mimics the clinical context where ongoing blood loss is replaced by transfusion with whole blood until a certain target MAP. One disadvantage of the model is the use of heparin to maintain the patency of intravascular catheters before, during, and after hemorrhage. Some studies have shown a possible effect on microcirculation after hemorrhagic shock. However, these studies use substantial higher doses, than in the present study [32]. The use of anesthetics also depresses the animal's cardiovascular ability to compensate for a decreasing blood pressure. The anesthetized animals also mimic patients undergoing DCS, while utilizing the concepts of DCR. Many of the data collected showed a visual trend towards normalizing, and one might wonder if the values had returned to baseline values if the observation period had been longer. Obviously, the definition of each of the three intervals might affect the statistical test. The definition of each interval, when it starts, and when it ends could also influence the results. Intuitively a rise in LP ratio after a reversible metabolic crisis needs time to return to baseline values after the hypotensive period has ended. In our study we have defined the end of interval B when $PbtO_2$ has normalized

indicating a non-critical deliverance of oxygen and nutrients to the brain. Although this does not account for the time the brain's redox status to return to normal, we consider it to be safer to overestimate the effect of injury.

## Conclusion

This experimental study demonstrates the severe (40 mmHg) and moderately prolonged (60 min) hemorrhagic shock results in a reversible metabolic crisis evaluated by microdialysis. The study also demonstrates that while minor perturbations in the cerebral metabolism might be paralleled in the venous outflow through the sagittal sinus, they are indistinguishable from systemic values during systemic ischemia.

Since time apparently is an important factor in the prevention of irreversible brain damage future studies should focus on methods (e.g., vasoactive drugs) to prolong the period in which permissive hypotension is safe for the brain.

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**Authors' contributions**
RPJ, PT, and THN contributed to the conception of the study. RJ, AG, SM, and THN performed the data acquisition, supervised by PT and CHN. RPJ and THN performed the statistical analysis. RPJ has performed the data analysis, and all authors have contributed to the interpretation. RPJ has drafted the manuscript, and all authors have contributed equally to the proofreading and discussion of the final manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**
The study was approved by the Danish Animal Experiments Inspectorate (2015–15–0201–00788).

**Consent for publication**
Not applicable

**Competing interests**
Rasmus Peter Jakobsen, Troels Halfeld Nielsen, Simon Mølstrøm Asger Grandfeldt, and Palle Toft declare that they have no competing interests. Carl-Henrik Nordström is consulting for Mdialysis AB, Sweden.

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# Effects of norepinephrine infusion on cerebral energy metabolism during experimental haemorrhagic shock

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## Abstract

**Background:** The use of norepinephrine in the case of life-threatening haemorrhagic shock is well established but widely discussed. The present study was designed to compare the effects of early norepinephrine treatment vs. no treatment on cerebral energy metabolism during haemorrhagic shock.

**Methods:** Twelve pigs were subjected to haemorrhagic shock, 4 in the control group and 8 in the norepinephrine (NE) group. Following a 60 min baseline period haemorrhagic shock was achieved by bleeding all animals to a pre-defined mean arterial blood pressure (MAP) of approximately 40 mm Hg. When mean arterial pressure had decreased to 40 mmHg NE infusion started in the treatment group. After 90 min, NE infusion stopped, and all pigs were resuscitated with autologous blood and observed for 2.5 h. During the experiment cerebral tissue oxygenation ($PbtO_2$) was monitored continuously and variables reflecting cerebral energy metabolism (glucose, lactate, pyruvate, glutamate, glycerol) were measured by utilizing intracerebral microdialysis.

**Results:** All 12 pigs completed the protocol. NE infusion resulted in significantly higher MAP ($p < 0.001$). During the shock period lactate/pyruvate (LP) ratio group increased from 20 (15–29) to 66 (38–82) (median (IQR)) in the control group but remained within normal limits in the NE group. The significant increase in LP ratio in the control group remained after resuscitation. After induction of shock $PbtO_2$ decreased markedly in the control group and was significantly lower than in the NE group during the resuscitation phase.

**Conclusion:** NE infusion during haemorrhagic shock improved cerebral energy metabolism compared with no treatment.

**Keywords:** Haemorrhagic shock, Norepinephrine, Microdialysis, Cerebral metabolism, Trauma

## Background

The prevailing regimen in treating patients in haemorrhagic shock is damage control resuscitation and damage control surgery [1–5]. Damage control resuscitation incorporates different strategies as minimized use of crystalloids, tranexamic acid, balanced

blood-transfusion, and permissive hypotension [6]. During permissive hypotension the clinician allows a lower target-mean arterial pressure (MAP), to avoid adverse effects, such as dilutional coagulopathy or acceleration of haemorrhage [7–9]. A decrease in mean arterial pressure (MAP) below the lower autoregulatory limit will result in a decrease in the cerebral perfusion pressure, which could compromise cerebral energy metabolism [10]. However, the use of vasopressors in haemorrhagic shock is still controversial [11]. Some guidelines promote the use of vasoactive drugs in the face of life-threatening haemorrhage, whereas others warn against them [12–14].

The objective of this study was to investigate the effect of the vasopressor norepinephrine on MAP, cerebral energy metabolism evaluated from intracerebral microdialysis, cerebral tissue oxygen tension ($PbtO_2$) and intracranial pressure (ICP) in an experimental model of induced haemorrhagic shock. The experimental model has been used in previous studies of cerebral energy metabolism during haemorrhagic shock [15, 16].

## Materials and methods

The study was designed as a non-randomised, non-blinded experimental study using pigs 4 months of age weighing approximately 40 kg. Twelve female Danish Landrace mix pigs were allowed to acclimatise for 1 week at the biomedical laboratory. At the day of the experiment, they had fasted overnight with free access to water. Four pigs were allocated to the control group, and 8 pigs in the group treated with the vasopressor noradrenalin (NE). Approval of the study had been given from The Danish Animal Experiments Inspectorate (2020-15-0201-00478).

### Anaesthesia, mechanical ventilation, and surgical preparation

The porcine model of haemorrhagic shock and the surgical preparations have been described previously [16–18]. Instrumentation and anaesthesia were the same in the control group and NE group. Sedation was achieved with an intramuscular injection of midazolam (0.25 mg/Kg) ketamine (5 mg/Kg) medetomidine (0.03 mg/Kg), butorphanol (0.1 mg/Kg) and atropine (0.025 mg/kg). Standard 18G intravenous cannulas were placed in veins in both ears. Anaesthesia was induced with intravenous midazolam (0.625 mg/Kg) and ketamine (12.5 mg/Kg) and maintained with an infusion of midazolam (5 mg/Kg/h) and fentanyl (50 µg/Kg/h).

Arterial blood gas samples were analysed every 30 min for pH, $PaO_2$, $PaCO_2$, lactate, $Ca^{2+}$ and haemoglobin (EPOC blood analyser, Woodley Equipment Company Ltd, England).

### Cerebral monitoring

A microdialysis catheter (CMA 70 Bolt MDialysis, Stockholm, Sweden) was inserted through a small burr hole in the left hemisphere and perfused with artificial CSF (M Dialysis AB, Stockholm, Sweden) at a rate of 0.3 µL/min (CMA 106 MD pump, MDialysis AB, Stockholm, Sweden). The dialysates were collected in microvials and immediately analysed for glucose, lactate, pyruvate, glutamate, and glycerol every 30 min using an Iscus Flex analyser (M Dialysis AB, Stockholm, Sweden).

To monitor brain tissue oxygenation ($PbtO_2$), a probe (Licox CC1SB, Integra Neurosciences Ltd. NJ, USA) was introduced to the right hemisphere through a small burr hole. $PbtO_2$ data were collected using the AC3.1 monitor (Integra Neurosciences Ltd.).

A transducer for monitoring intracranial pressure (ICP) was placed in the left hemisphere (Integra Neurosciences Ltd. New Jersey, USA) ICP was monitored continuously, and data were collected by a CAM01 monitor (Integra Neurosciences Ltd. New Jersey, USA).

### Experimental protocol

The experimental protocol is illustrated in Fig. 1. After preparation and insertion of all probes, the animals were allowed a minimum of 1 h to stabilize before start of the hypoperfusion period. Vital parameters were recorded every 10 min. Following a 60 min baseline period haemorrhagic shock was achieved by bleeding the animals to a pre-defined MAP of approximately 40 mm Hg at a rate of 2.15 mL/kg/min over 7 min, and then 1.15 mL/kg/min over the remaining period [15, 16]. The depth and duration of haemorrhagic shock necessary for producing cerebral ischemia that caused compromised energy state was based on previous experiments [15]. At the end of the experimental protocol the animals were euthanatized under anaesthesia with pentobarbital 200 mg/mL in concentrated ethanol.

The control group experiments were the first to be conducted. Animals in the control group were kept at a MAP of about 40 mmHg by withdrawing or infusing After induction of shock and MAP = 40 mmHg was reached, we continued to withdraw or reinfuse shed blood to keep a MAP of 40 mmHg. The shed blood was stored in a citrated glucose solution at 37 °C. The amount of blood withdrawn or reinfused which was needed to maintain a MAP = 40 mmHg were recorded in intervals of 10 min. Following 90 min of haemorrhagic shock, the animals were resuscitated by re-infusing the remaining shed blood at a rate of 120 mL/min until all blood was returned. After resuscitation the pigs were observed for 150 min (Fig. 1). When animals in the NE group after haemorrhage had reached a MAP of 40 mmHg a weight adjusted infusion with norepinephrine (0.03 mg/kg in 50 ml isotonic NaCl) was started. The rate of norepinephrine infusion

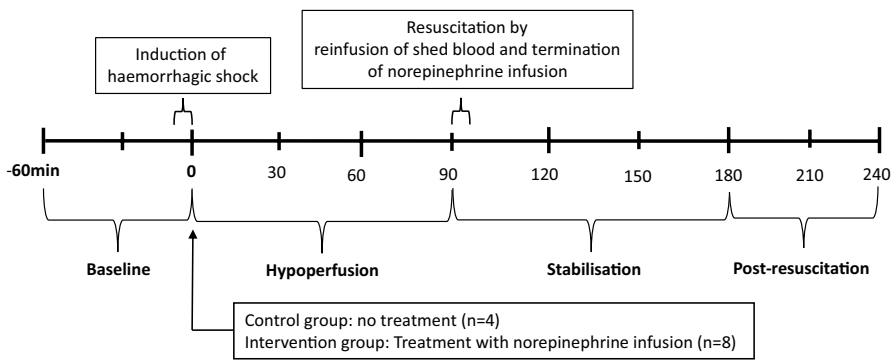


**Fig. 1** Experimental protocol timeline. Note that the experimental protocol is divided into four intervals. Interval 1: Baseline — 60–0 min. Interval 2: Hypoperfusion period 0–90 min, Interval 3: Stabilisation period 90–180 min in which physiological state might return to homeostasis. Interval 4: post-resuscitation period 180–240 min

was titrated until a MAP of 80 mmHg was reached. During the following 90 min blood was withdrawn or reinfused in the same amount and rate as determined during the control group experiments. Following the 90 min of haemorrhagic shock in the control group, the animals were resuscitated by re-infusing the remaining shed blood at a rate of 120 mL/min until all blood was returned. After resuscitation norepinephrine was titrated down and finally terminated (Fig. 1).

## Statistics

The time course of the experimental protocol was divided into four separate periods (Fig. 1). The discrimination between the hypoperfusion and resuscitation period is the time when all the shed blood has been reinfused. The post-resuscitation period was arbitrary defined as the last 60 min of the experimental protocol. Experiences from earlier studies have showed that there is a need for a stabilisation period after a period with hypoperfusion, in which the cerebral metabolism might return to normal. Data were modelled with a mixed effect model for repetitive measurements using treatment with NE as a fixed effect, and each animal as a random effect. Non-repetitive data were tested with a Mann Whitney U test. Unless otherwise noted data is reported as median and interquartile range (25–75 percentiles). We did not calculate sample size due to the explorative nature of this study. All $p$ values are to be considered exploratory, and therefore, no adjustment for generating multiple $p$ values was made. A $p$ value less than 0.05 is considered statistically significant. The data analysis and graphs were carried out in STATA 16 statistical software. (StataCorp, College station, Texas USA).

## Results

All 12 pigs completed the experimental protocol. The weight of the pigs in the control group and NE group was 43.5 kg (40.25–46.65 kg) and 41.9 kg (40.3–44.5 kg), respectively ($p=0.386$). The total amount of drained blood in the control group was 26.55 mL/Kg (23.2–32.9 ml/Kg) and in the NE group 21.36 ml/Kg (18.4–25.9 ml/Kg) ($p=0.1649$). The drained volumes corresponded to between 33 and 41% of the total blood volume [21]. The total dose of norepinephrine in the NE-group was 0.528 mg (0.475–2.595 mg).

### Systemic physiological and biochemical variables

Table 1 shows systemic physiological and biochemical variables as well as $PbtO_2$ for both experimental groups during baseline, hypoperfusion and post resuscitation. During baseline all variables were within normal ranges in both groups. After initiation of bleeding, MAP decreased to 40 mmHg in both groups (Fig. 2). Treatment with NE caused a rapid increase in MAP in the NE group which remained during the hypoperfusion period (Table 1). After reinfusion of shed blood, a rapid increase in MAP was observed in the control group. However, a significant difference in MAP persisted after resuscitation and termination of NE infusion (Table 1). The time course for the changes in MAP in both groups are shown in Fig. 2.

#### *Intracerebral variables: Cerebral energy metabolism and $PbtO_2$*

Table 2 shows median (IQR) levels for all intracerebral biochemical variables. During baseline there was no significant difference between control and NE group. During the

**Table 1** General physiological haemodynamic and systemic parameters for baseline, hypoperfusion and post resuscitation periods

| Interval | Control | NE | Control vs. NE group *p* value |
|---|---|---|---|
| Baseline (— 60)–0 min | | | |
| MAP (mmHg) | 88 (78–98) | 78 (72–95) | 0.198 |
| HR (bpm) | 56 (55–87) | 87 (74–103) | 0.016* |
| PbtO2 (mmHg) | 22 (8–70) | 37 (30–40) | 0.880 |
| PaO2 (kPa) | 53 (49–57) | 37 (36–40) | 0.001* |
| PaCO2 (kPa) | 5.6 (5.4–6) | 6.4 (5.9–6.7) | < 0.001* |
| b-Hemoglobin (mM/L) | 5.8 (5.6–6.3) | 5 (4.8–5.3) | < 0.001* |
| b-Glucose (mM/L) | 4.1 (3.7–6) | 5.8 (3.9–8.1) | 0.180 |
| b-Lactate (mM/L) ABG | 0.9 (0.5–1) | 0.5 (0.4–0.9) | 0.753 |
| b-pH ABG | 7.45 (7.44–7.48) | 7.46 (7.43–7.5) | 0.951 |
| Diuresis (mL) | 25 (0–72.5) | 0 (0–50) | 0.274 |
| Hypoperfusion 0–90 min | | | |
| MAP (mmHg) | 41 (40–42) | 80 (75–86) | < 0.001* |
| HR (bpm) | 108 (63–180) | 98 (85–115) | 0.625 |
| PbtO2 (mmHg) | 8 (4–18) | 28 (23–34) | 0.160 |
| PaO2 (kPa) | 52 (46.6–53.9) | 38 (35–41) | 0.019 |
| PaCO2 (kPa) | 5.5 (5.2–6.3) | 6.3 (6.1–6.7) | 0.031* |
| b-Hemoglobin (mM/L) | 5.7 (5–6.1) | 5.1 (4.8–5.6) | 0.189 |
| b-Glucose (mM/L) MD | 6 (3.8–7.0) | 4.9 (4.2–7.0) | 0.974 |
| b-Lactate (mM/L) ABG | 8.2 (2.7–13.7) | 1.1 (0.9–1.7) | 0.001* |
| b-pH ABG | 7.39 (7.23–7.4) | 7.46 (7.43–7.49) | 0.013 |
| Diuresis (mL) | 0 (0.35) | 11.5 (0–47.5) | 0.380 |
| Post resuscitation 180–240 min | | | |
| MAP (mmHg) | 56 (47–79) | 74 (69–80) | 0.037 |
| HR (bpm) | 89 (76–107) | 90 (76–99) | 0.858 |
| PbtO2 (mmHg) | 7 (2–10) | 29 (13–34) | 0.021 |
| PaO2 (kPa) | 51(49–54) | 38 (37–42) | < 0.001* |
| PaCO2 (kPa) | 6.5 (5.3–6.8) | 6 (5.7–6.5) | 0.665 |
| b-Hemoglobin (mM/L) | 6.5 (6–6.7) | 4.6 (4.2–4.9) | < 0.001* |
| b-Glucose (mM/L) | 2.8 (0.7–3.2) | 3.6 (2.9–4.3) | 0.432 |
| b-Lactate (mM/L) ABG | 3.9 (2–8.4) | 0.5 (0.3–0.6) | 0.003* |
| b-pH ABG | 7.32 (7.29–7.39) | 7.47 (7.45–7.51) | < 0.001* |
| Diuresis (mL) | 0 (0–30) | 0 (0–4) | 0.676 |

NE group $n = 8$, control group $n = 4$. Values are medians (interquartile range). Test statistics performed with mixed effect model

*Statistical significant *p* value < 0.05

period of hypoperfusion significant increases in lactate, glutamate, glycerol and LP ratio were obtained simultaneously with a significant decrease in glucose. These significant differences remained during the post resuscitation period.

The time courses for the changes in lactate, pyruvate and glucose during the study are illustrated in Fig. 3. During the hypoperfusion period (0–90 min) lactate increased dramatically in the control group simultaneously with a decrease in pyruvate. During the initial phase of resuscitation (90–180 min) lactate remained high with a profound increase in pyruvate. This was followed by a second decrease in pyruvate, while lactate remained elevated. In the NE group a slight increase in lactate and a moderate increase

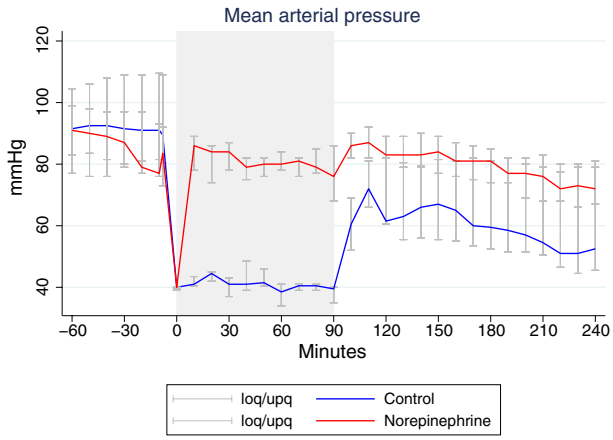


**Fig. 2** Dynamic changes of median (interquartile range) mean arterial pressure (MAP) throughout the experiment. NE group $n=8$. Control group $n=4$. Note the steep decrease in MAP in both groups at the start of hypoperfusion period. In addition, note the normalisation of MAP after start of NE infusion. Shaded area illustrates hypoperfusion period

**Table 2** Biochemical variables obtained on microdialysis from cerebral hemisphere

| **Baseline** | **Location** | **Control** | **NE** | **Control vs. NE group *p* value** |
|---|---|---|---|---|
| LP ratio | Hemisph | 20 (15–29) | 23 (18–27) | 0.834 |
| Lactate (mM/L) | Hemisph | 2.2 (1.4–3.8) | 2.1 (1.7–3.1) | 0.670 |
| Pyruvate (mM/L) | Hemisph | 100 (92–134) | 92 (73–108) | 0.643 |
| Glucose (mM/L) | Hemisph | 2.5 (1.8–2.8) | 2.6 (2.2–3.2) | 0.183 |
| Glutamate (µM/L) | Hemisph | 15 (9–72) | 12 (4–20) | 0.081 |
| Glycerol (µM/L) | Hemisph | 93 (58–149) | 62 (45–90) | 0.106 |
| **Hypoperfusion** | **Location** | **Control** | **NE** | **Control vs. NE group *p* value** |
| LP ratio | Hemisph | 66 (38–82) | 24 (19–31) | < 0.001* |
| Lactate (mM/L) | Hemisph | 7.8 (6.2–12.3) | 2.5 (2.1–3.6) | < 0.001* |
| Pyruvate (mM/L) | Hemisph | 104 (81–177) | 112 (91–132) | 0.670 |
| Glucose (mM/L) | Hemisph | 1 (0.4–1.6) | 2.9 (2.4–3.9) | < 0.001* |
| Glutamate (µM/L) | Hemisph | 93 (6–155) | 8 (4–11) | 0.001* |
| Glycerol (µM/L) | Hemisph | 190 (75–270) | 59 (44–86) | < 0.001* |
| **Post resuscitation** | **Location** | **Control** | **NE** | **Control vs. NE group *p* value** |
| LP ratio | Hemisph | 73 (29–100) | 25 (21–29) | 0.013* |
| Lactate (mM/L) | Hemisph | 4.9 (1–6.5) | 1.3 (0.8–1.6) | < 0.001* |
| Pyruvate (mM/L) | Hemisph | 102 (71–185) | 108 (100–133) | 0.972 |
| Glucose (mM/L) | Hemisph | 0.7 (0.5–0.8) | 2.3 (1.6–2.9) | 0.003* |
| Glutamate (µM/L) | Hemisph | 47 (6–110) | 4 (2–7) | 0.005* |
| Glycerol (µM/L) | Hemisph | 461 (254–545) | 42 (23–55) | < 0.001* |

Values are divided into three-time intervals and expressed as median (interquartile range). Control $n=4$. NE group $n=8$. Test statistics performed with mixed effectt model

*Statistical significant *p* value < 0.05

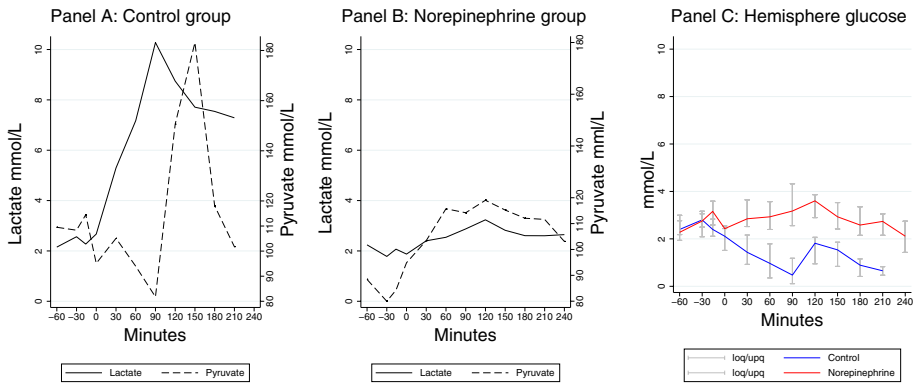


**Fig. 3** Median (interquartile range) values of lactate, pyruvate and glucose concentrations in the hemisphere in the controls and NE group during the experiment. Note that the increase in LP ratio in the control group during hypoperfusion is due to an increase in lactate simultaneously with a decrease in pyruvate. After resuscitation the lactate levels in the control group remains elevated but he pyruvate levels trends towards normalising. In addition, note the normalisation of glucose in the control group after resuscitation. Control $n = 4$. NE group $n = 8$; *loq* lower quartile, *upq* upper quartile

in pyruvate was observed during these phases (0–240 min). During hypoperfusion and resuscitation glucose decreased in the control group but remained essentially unchanged in the NE group.

During hypoperfusion and post resuscitation the levels of glutamate and glycerol increased markedly in the control group (Table 2). In the NE group these variables remained virtually unchanged.

During the baseline period median $PbtO_2$ was lower in the control than in the NE group (22 vs. 37 mmHg) but the difference did not reach statistical significance (Table 1). The decrease in median $PbtO_2$ was in the control group more pronounced during hypoperfusion (8 vs. 28 mmHg) and the difference was even more explicit and statistically significant in the post resuscitation period (7 vs. 29 mmHg) (Table 1). During the latter part of the hypotension period (40–90 min) a marked decrease in $PbtO_2$ was observed in the control group. Immediately after resuscitation a transitory increase was obtained (100–120 min) followed by a secondary marked decrease (130–240 min). In the NE group a moderate, stabile decrease in $PbtO_2$ occurred during the whole study (0–240 min).

During the experiment ICP was recorded every 10 min. In the NE group all data were recovered: baseline 3 mmHg (− 2–8 mmHg), hypoperfusion 6 mmHg (2–8 mmHg) and post resuscitation 12.5 mmHg (7–17 mmHg). Due to equipment failure ICP data from the control group were lost. Accordingly, a comparison of the two groups was impossible.

## Discussion

The use of vasopressors in haemorrhagic shock is controversial [11–14]. Widely different experiences regarding the effects of noradrenaline on cerebral blood flow (CBF) and cerebral energy metabolism have been reported [21–24]. In the present study we explore the effects on biochemical variables related to cerebral energy metabolism when

noradrenaline is used to restore MAP acutely during haemorrhagic shock for 90 min before reinfusion of the shed blood to restore the circulating volume (Fig. 1).

Measurements of $PbtO_2$ represent the product of CBF and the cerebral arterio-venous difference in oxygen tension rather than a direct measurement of total oxygen delivery or cerebral oxygen metabolism [25]. In the present experimental situation this implies that $PbtO_2$ may be regarded as a qualitative reflection of CBF. Under normal conditions the numerical value of $PbtO_2$ obtained varies and a definite lower acceptable limit is not possible to define: the limit for "cerebral ischemia" has in various publications been defined at levels from 5 to 23 mmHg and normal values between 15 and 42 mmHg have been reported in experimental studies [26]. In the present study median $PbtO_2$ was slightly lower in the control group (Table 1). As cytoplasmatic redox state evaluated by LP ratio was similar in the experimental groups tissue oxygenation was in both groups sufficient for normal energy metabolism (Table 2). Accordingly, we regard the initial non-significant difference in $PbtO_2$ as irrelevant for the interpretation of observed changes in biochemical variables.

In the control group $PbtO_2$ decreased to below 10 mmHg during the latter part of the hypoperfusion period indicating a very pronounced decrease in CBF. After resuscitation a transient increase in $PbtO_2$ indicated a temporary increase in blood flow followed by a final period of very low CBF. In the NE group $PbtO_2$ exhibited a moderate, stable decrease in $PbtO_2$ indicating relatively well preserved CBF. Our data regarding the effect of NE on $PbtO_2$ are in agreement with the study by Meybohm et al. in piglets [27]. However, these authors concluded that although NE increased $PbtO_2$ the improvement was probably not sufficient to restore cerebral energy metabolism. In the present study we can relate $PbtO_2$ to cerebral biochemical variables reflecting energy metabolism.

Cytoplasmatic red-ox state is described by the lactate/pyruvate ratio (LP ratio) [28]. In the present study LP ratio was almost identical in the two study groups during baseline (Table 2). About 30 min after start of the hypoperfusion period the LP ratio increased rapidly in the control group due to a marked increase in lactate simultaneously with a decrease in pyruvate (Fig. 3). A delay in the detection of biochemical changes is expected as the microdialysis perfusion fluid must be transported from the dialysis catheter to the collecting vial before the chemical analyses can be performed. However, the increase in LP ratio coincided with a pronounced decrease in $PbtO_2$, indicating a dramatic reduction in CBF. Accordingly, the delayed biochemical deterioration observed in the control group is in this study not a technical artifact caused by the microdialysis technique.

The biochemical and physiological variables document that in the control group the hypoperfusion period caused profound cerebral ischemia which was avoided in the NE group. The increase in glutamate in the control group indicates that cerebral energy metabolism was insufficient to cover the tissue demands and the increase in glycerol shows that irreversible degradation of cellular membranes had occurred [29–31]. In the NE group biochemical variables remained unchanged demonstrating that during induced haemorrhagic shock acute infusion of NE will not only increase MAP but also preserve $PbtO_2$ (CBF) and prevent biochemical signs of cerebral ischemia, compromised oxidative energy metabolism and signs of cellular degradation.

The results of the present study may be compared with similar experimental studies. In their study Meybohm et al. [27] observed that NE increased $PbtO_2$ but the effect on

cerebral energy metabolism was not directly investigated. When comparing the effects of either epinephrine or vasopressin during induced hypotension and resuscitation Küchler et al. [32] observed no differences between the two treatments regarding restoration of cerebral hemodynamic and energy metabolism after induced haemorrhagic shock. However, in their study the level of hypotension was not sufficient to cause biochemical signs of ischemia. The beneficial effects of NE in the present study are interpreted as caused by the increase in MAP and CBF. A direct effect of NE cerebral energy metabolism is unlikely. It has been demonstrated that the direct effect of the catecholamines adrenaline and noradrenaline result in an increase in CBF and cerebral metabolic rate at virtually unchanged levels in biochemical variables related to cerebral energy metabolism [21, 33].

The beneficial effects of NE may be of clinical importance also in other conditions with a dangerous decrease in cerebral perfusion pressure. It has been shown that following cardiac standstill and resuscitation many patients exhibit compromised cerebral energy metabolism due to ischemia/mitochondrial dysfunction up to 20 h after return of spontaneous circulation [34]. The data indicate that initial brain recirculation is often insufficient due to too low MAP/cerebral perfusion pressure. Conflicting results regarding the possible beneficial effect of vasopressor therapy in after cardiac arrest have recently been published. In a clinical study it was shown that incremental doses of norepinephrine increased blood pressure and systemic vascular resistance without affecting cardiac output or cerebral oxygenation [35]. However, an experimental study of cardiac arrest reported that adrenaline administration increased cerebral perfusion pressure and regional CBF as well as cerebral oxygenation and energy metabolism [36].

## Limitations

The $PbtO_2$ and MD probes in the hemisphere will always provide a local estimate of ischemia in the given area and may over/underestimate global conditions. To achieve equivalent measurements across all animals, it was striven to methodically place all probes at the same location in all experimental animals. In the clinical setting, it is standard to verify correct positioning in white matter by CT scans. Unfortunately, this was not possible in our laboratory. Second, we assume conditions to be equally distributed across the cerebrum and would only expect small regional differences in $PbtO_2$ and microdialysis measurements. If we had continued to monitor the animals for a longer period it is possible that some of the parameters would have normalized. However, even though a biochemical normalization would have occurred, it does not eliminate the fact that the animal had been subjected to an ischemic event which might lead to focal or cognitive deficits. MAP 40 mmHg for 90 min would probably not be accepted in a clinical situation without interventions. The experimental model is thus only limited transferable in a clinical context as we focused on treatment with norepinephrine alone without any other interventions, such as fluids or blood transfusions. The authors chose the length of hypotension, because earlier studies had showed that 90 min hypotension with a MAP of 40 mmHg resulted in an irreversible metabolic crisis. In this context we would be able to observe if norepinephrine would worsen or better cerebral metabolic state. One drawback of the present study is that MAP of the NE group exceeded what would normally be targeted during haemorrhagic resuscitation. Treatment with norepinephrine

resulted in a MAP that was comparable with measurements made at baseline. When using norepinephrine in a clinical situation, the target MAP would often be the same as in permissive hypotension, i.e., MAP of 50–60 mmHg. However, one of the concerns of using vasopressors during haemorrhagic shock is the risk of cerebral vasoconstriction and reduced CBF. (31–37) Most likely, the preserved cerebral metabolism is a result of increased MAP and thus increased CPP. However, if norepinephrine causes cerebral vasoconstriction, the larger doses needed for obtaining a higher MAP would probably have resulted in a decrease in CBF and eventually in compromised delivery of oxygen and glucose to the brain, which would have been detected by microdialysis.

## Strengths

We chose a combination of fixed pressure and fixed volume model for haemorrhagic shock. The fixed volume model does not consider intraindividual variations in total blood volume or intraindividual adaptations to blood loss. The total amount of blood withdrawn at induction of shock ranged from 701 to 1654 ml indicating that different amounts of blood withdrawn was needed to reach the same physiological endpoint of MAP 40 mmHg. A fixed pressure model relies on physiological parameters, rather than a fixed volume. In a clinical setting it is rare to address the level of haemorrhagic shock by quantifying volume of blood lost, instead clinicians evaluate severity of shock by the physiological response. However, since it was not possible to maintain a "true" MAP during treatment with norepinephrine we used a fixed volume determined by the control group during this phase of the experiment.

## Conclusion

Early treatment with NE during severe and prolonged haemorrhagic shock restored MAP and chemical variables related to cerebral energy metabolism. As evaluated from the biochemical variables, treatment with NE in hypovolemic shock did in this experimental study not cause cerebral vasoconstriction and hypoperfusion. Further studies are required before NE is routinely implemented in early treatment of haemorrhagic shock.

**Abbreviations**

CBF: Cerebral blood flow; CPP: Cerebral perfusion pressure; CSF: Cerebrospinal fluid; ICP: Intracranial pressure; LP ratio: Lactate to pyruvate ratio; MAP: Mean arterial pressure; NE: Norepinephrine; $PbtO_2$: Brain tissue oxygen pressure.

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**Authors' contributions**

RPJ, PT, and THN contributed to the conception of the study. RPJ and ECH performed the data acquisition, supervised by THN and PT. RPJ and ECH performed the statistical analysis. RPJ and CHN has performed the data analysis, and all authors have contributed to the interpretation. RPJ and ECH has drafted the manuscript, and all authors have contributed equally to the proofreading and discussion of the final manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**

The data sets used and analysed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethics approval and consent to participate**
The study was approved by the Danish Animal Experiments Inspectorate (2020-15-0201-00478).

**Consent for publication**
Not applicable.

**Competing interests**
Rasmus Peter Jakobsen, Elisabeth Charlotte Hansen, Troels Halfeld Nielsen, Carl-Henrik Nordström and Palle Toft declare that they have no competing interests.

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