Brain Tissue Oxygenation in Traumatic Brain Injury

Experimental and Clinical Studies

KARLIS PURINS
Traumatic brain injury (TBI) is a major cause of death and disability. TBI is frequently followed by cerebral ischemia which is a great contributor to secondary brain damage. The main causes of cerebral ischemia are pathophysiological changes in cerebral blood flow and metabolism. Treatment of TBI patients is currently based on intracranial pressure (ICP) and cerebral perfusion pressure (CPP) targeted treatment protocols. However, ICP and CPP alone do not provide information of the oxygen availability in the brain. Monitoring of brain tissue oxygenation (B\textsubscript{ti}pO\textsubscript{2}) may give additional and valuable information about the risk for development of ischemia in TBI patients.

The aims of this thesis were to study B\textsubscript{ti}pO\textsubscript{2} monitoring devices \textit{in-vitro} regarding accuracy and stability, to detect threshold level of cerebral ischemia \textit{in-vivo} and finally to examine the cerebral oxygen levels and cerebral metabolism in TBI patients.

The B\textsubscript{ti}pO\textsubscript{2} probes performed with high accuracy and stability at different clinically relevant oxygen concentrations.

A pig TBI model was developed by step-wise intracranial volume/pressure increase. Volume increase resulted in a gradual increased ICP, decreased CPP, intracranial compliance and B\textsubscript{ti}pO\textsubscript{2}, respectively. Brain death (BD) was confirmed by negative CPP and negligible amount of previously injected microspheres in the brain tissue. The model simulated the clinical development of BD in humans with a classical pressure-volume response and systemic cardiovascular reactions. The model should be suitable for studies of brain injury mechanisms.

From the same \textit{in-vivo} model it was also possible to detect the threshold level of cerebral ischemia in the pig, where B\textsubscript{ti}pO\textsubscript{2} below 10 mmHg and CPP below 30 mmHg was associated with an impaired cerebral metabolism (microdialysis lactate to pyruvate ratio $>30$).

B\textsubscript{ti}pO\textsubscript{2} together with cerebral microdialysis were studied in 23 severe TBI patients. We observed different patterns of changes in B\textsubscript{ti}pO\textsubscript{2} and cerebral microdialysis biomarkers in focal and diffuse TBI. Increased cerebral microdialysis levels of glutamate, glycerol or the lactate/pyruvate ratio were observed at B\textsubscript{ti}pO\textsubscript{2} $< 5$ mmHg, indicating increased vulnerability of the brain at this critical level of tissue oxygenation in TBI patients.

Keywords: Brain tissue oxygenation, Cerebral metabolism, Traumatic brain injury, Cerebral ischemia, Threshold levels, Neurovent-PTO, Microdialysis

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To my family
List of papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

**Paper I**
Karlis Purins, Per Enblad, Bo Sandhagen, Anders Lewén
*Brain tissue oxygen monitoring: a study of in vitro accuracy and stability of Neurovent-PTO and Licox sensors.*
*Acta Neurochirurgica (Wien). 2010 Apr;152(4):681-8*

**Paper II**
Karlis Purins, Amir Sedigh, Christian Molnar, Leif Jansson, Olle Korsgren, Tomas Lórant, Gunnar Tufveson, Lars Wennberg, Lars Wiklund, Anders Lewén, Per Enblad
*Standardized experimental brain death model for studies of intracranial dynamics, organ preservation, and organ transplantation in the pig.*
*Critical Care Medicine. 2011 Mar;39(3):512-517*

**Paper III**
Karlis Purins, Per Enblad, Lars Wiklund, Anders Lewén
*Brain tissue oxygenation and cerebral perfusion pressure thresholds of ischemia in a standardized pig brain death model.*
*Neurocritical Care. 2012 Jun;16(3):462-9*

**Paper IV**
Karlis Purins, Anders Lewén, Lars Hillered, Tim Howells, Per Enblad
*Brain tissue oxygenation and cerebral metabolic patterns in focal and diffuse traumatic brain injury.*
*Submitted*

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<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
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<tr>
<td>BBB</td>
<td>Blood-brain-barrier</td>
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<td>BD</td>
<td>Brain death</td>
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<td>$B_{i}pO_{2}$</td>
<td>Brain tissue oxygenation</td>
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<tr>
<td>CBF</td>
<td>Cerebral blood flow</td>
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<td>CPP</td>
<td>Cerebral perfusion pressure</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<tr>
<td>CT</td>
<td>Computed tomography scan</td>
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<tr>
<td>DAI</td>
<td>Diffuse axonal injury</td>
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<tr>
<td>FiO$_2$</td>
<td>Inspired oxygen concentration</td>
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<td>GCS</td>
<td>Glasgow coma scale</td>
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<td>HR</td>
<td>Heart rate</td>
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<td>IC</td>
<td>Intracranial compliance</td>
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<td>ICP</td>
<td>Intracranial pressure</td>
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<tr>
<td>L/P ratio</td>
<td>Lactate/Pyruvate ratio</td>
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<td>LX</td>
<td>Licox®</td>
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<td>MAP</td>
<td>Mean arterial pressure</td>
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<td>MD</td>
<td>Microdialysis</td>
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<tr>
<td>N.S.</td>
<td>Not significant</td>
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<tr>
<td>NICU</td>
<td>Neurointensive care unit</td>
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<td>NV</td>
<td>Neurovent-PTO®</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>$pCO_2$</td>
<td>Carbon dioxide partial pressure</td>
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<td>PET</td>
<td>Positron emission tomography</td>
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<td>$pO_2$</td>
<td>Oxygen partial pressure</td>
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<td>ROS</td>
<td>Reactive oxygen species</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>$S_jvO_2$</td>
<td>Jugular venous oximetry</td>
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<td>TBI</td>
<td>Traumatic brain injury</td>
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Introduction

Traumatic brain injury (TBI) is a major cause of morbidity and mortality.\textsuperscript{18,96,149,170} Significant reduction in morbidity and mortality has been achieved in TBI patients by controlling intracranial pressure (ICP) and maintenance of adequate cerebral perfusion pressure (CPP).\textsuperscript{14,94} Better understanding of the brain injury pathophysiology, improved neuromonitoring and introduction of standardized neurointensive care units (NICU) has also improved outcome of TBI patients.\textsuperscript{39} In the NICU patients are systematically monitored in order to detect abnormal physiological and intracranial parameters. However, cerebral ischemia may follow TBI by pathophysiological changes in cerebral blood flow and brain metabolism and is a great contributor to secondary brain injury which can not be detected with the standard neuromonitoring techniques. However, advanced neuromonitoring with cerebral microdialysis (MD) and brain tissue oxygenation ($B_t$PO$_2$) may provide additional information of cerebral metabolism and the oxygen availability in the brain.\textsuperscript{2,61,64,73,107,133,186} It would be of great value to identify if monitoring of cerebral oxygenation and cerebral metabolism can enable earlier detection of cerebral ischemia, further improve and refine the NICU and thereby reduce secondary insults leading to better outcome in TBI patients.
Background

Epidemiology

Traumatic brain injury is an important public health problem throughout the world. TBI remains a common cause of death or permanent disability in young adults and adolescents.\textsuperscript{17,104} In all age groups the incidence of TBI is about three times higher in males than in females. The most common causes of TBI are falls and road traffic accidents. Annually, the mean incidence rate of hospitalization due to TBI in Europe and U.S. is 235 per 100 000 and 103 per 100 000, respectively.\textsuperscript{17,87,146,171} TBI incidence rate in Scandinavia lays around 200 per 100 000 per year.\textsuperscript{143} Severe TBI is around 10\% of all cases where half of those require neurosurgical intervention. The annual incidence rate of severe TBI in the Uppsala region is around 100 cases per 1.9 million.\textsuperscript{176} The overall mortality rate is around 15-20 per 100 000 per year in U.S. and Europe.\textsuperscript{171}

Primary brain injury

TBI results from any external forces applied to the head as a direct impact, penetrating object, relative motions (acceleration/deceleration) or from blast waves. The most common cause of TBI is closed head injury with either focal injury (haematomas, contusions) or diffuse axonal brain injury. The extent of brain damage is depending on intensity, duration and nature of the impact.

Secondary brain injury

Avoidable factors

In middle 1970’s it was noticed that TBI patients who had talked at admission to the hospital later deteriorated and died in many cases.\textsuperscript{138,144} The
fact that patients at admission were able to talk indicated that the primary injury was mild or moderate. Therefore, it was suggested that the cause of death was not always associated with the primary injury alone and the fatal outcome was explained by increased vulnerability of the injured brain to secondary insults. The term avoidable factors was introduced since the secondary insults appeared to be avoidable.\textsuperscript{144} Initially, delays in treatment of intracranial haemorrhages, hypotension and hypoxia were identified as avoidable factors.\textsuperscript{138,144} Further studies confirmed that post-traumatic systemic hypotension occur frequently in TBI patients and is a contributor to poor outcome.\textsuperscript{24,98} If such processes were avoided the patients would likely survive.\textsuperscript{113,117} To minimize those avoidable factors, intracranial bleedings were removed more rapidly, hypotension was managed by intravenous fluid administrations and treatment of extracranial injuries, airway obstructions was avoided by intubation and mechanical lung ventilation.\textsuperscript{37,47,109,132} Despite all these efforts the mortality rate still remained high. This led to further advancements in monitoring techniques, standardization of treatment protocols and development of neurointensive care units (NICU) (discussed below).

**Brain injury mechanisms**

Numerous molecular, biochemical and pathophysiological events occurs following the primary injury. Several secondary brain injury mechanisms have been identified on basis of cellular level and clinical level (Figure 1).

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*Figure 1. A schematic illustration showing the concept of secondary brain injury mechanisms at cellular level and clinical level (Adapted from Sara Ekmark-Lewén, Uppsala University, 2010).*
Cellular level

After primary injury a cascade of events may follow including metabolic disturbances, oedema development, inflammation, excessive release of amino acids, free radical production, disruption of blood-brain-barrier (BBB), cell membrane dysfunction and disturbance of ion homeostasis. In addition to the primary damage these events may contribute to the evolution of the secondary cellular injury eventually leading to delayed cell death by apoptotic or necrotic mechanisms.

Clinical level

On the clinical level secondary injury mechanisms comprise more physiological events such as hypotension, hyperthermia, hypo/hyperglycaemia, seizures, increased ICP etc.

TBI patients frequently suffer from hypotension (systolic blood pressure <90 mmHg) and hypoxic (PaO₂ < 60 mmHg) events during the time after the primary injury. There is a strong association between hypotension and hypoxia/ischemia with increased mortality rate in TBI patients. Secondary ischemic brain damage is also a result of insufficient cerebral blood flow (CBF) in relation to cerebral metabolism. The CBF may decrease because of an increase in ICP or decrease in CPP (CPP = mean arterial blood pressure - ICP) which can be caused by increased mass effect due to oedema, intracranial bleeding, contusions or decreased mean arterial pressure.

TBI patients are at high risk of brain energy failure and ischemia. Cerebral ischemia is often caused by insufficient blood supply resulting in decreased amount of oxygen delivered to the brain or increased demand of oxygenated blood and substrates. The advances in neuromonitoring techniques enable measurements to detect the amount of oxygen and metabolites in the brain in order to evaluate cerebral oxidative metabolism (see below).

Neurointensive care

Patients with severe TBI are usually admitted to NICU. The main purpose of NICU is to protect the primary injured brain against secondary insults which could cause secondary brain damage and worsen patient outcome. At NICU patients are resuscitated, monitored and treated according to standardized protocols in order to avoid secondary brain damage and to improve outcome. Neurointensive care of TBI patients is currently based on
ICP and CPP targeted protocols. However, ICP and CPP alone do not provide information of the brain tissue oxygenation. Therefore, there are reasons to believe that knowledge of recently developed cerebral monitoring techniques (brain tissue oxygenation and microdialysis) would add information with the potential of improving patient outcome.

Intracranial pressure

The Monro-Kellie doctrine proposed that the cranial compartment (closed rigid skull) is incompressible and its total volume of the brain, cerebrospinal fluid (CSF) and venous blood is constant. The cranial compartment and its constituents create a state of volume equilibrium. Any increase of one of the cranial constituent needs to be compensated by the two others. Increased intracranial volume (increased brain volume and expanding mass lesions) can also be compensated to a certain extent by displacement of CSF and brain venous blood. However, the craniospinal axis is a closed system which has a limited capacity to compensate for added intracranial volume. Only a certain amount of intracranial volume can be added before ICP begins to rise (Figure 2, left). Therefore, it is crucial to monitor ICP in TBI patients in order to avoid pressure induced secondary brain injury. Monitoring of ICP was initially introduced into clinical neurosurgery practice in 1951. The basic principles for the ICP monitoring as used today in NICU was described by Nils Lundberg who measured intraventricular pressure in patients and described the frequency with which raised ICP occurs clinically.

Normal ICP in healthy humans is 2-7 mmHg and ICP above 20-25 mmHg is defined as hypertension. ICP can be measured either by an intraparenchymatous probe or by an intraventricular catheter. The intraparenchymatous probe is preferred when brain ventricles are collapsed and an intraventricular catheter can not be inserted. The intraventricular ICP measurement system allow drainage of the CSF, thereby decreasing ICP.
High ICP impairs the circulation and contributes to ischemic damage. Increased ICP occurs commonly in patients with severe head injury and is associated with poor outcome.\textsuperscript{10,23,32,85,103,114,115,165} Significant reductions in mortality and morbidity can be achieved in TBI patients by using ICP based treatment protocols.\textsuperscript{29,38,48} The treatment of intracranial hypertension is dependant of the cause of elevated ICP. Strategies to control ICP include head elevation, hyperventilation and barbiturate induced coma when there is no surgically available treatment of increased ICP.\textsuperscript{1,13} Intracranial contusions and/or haematomas can be evacuated surgically. Another option to treat incontrollable ICP is to perform decompressive haemicraniectomy. However, secondary brain injury is not always associated with pathologic changes in ICP and adequate resuscitation (normal ICP or CPP) following TBI does not prevent cerebral hypoxia.\textsuperscript{167}

**Intracranial compliance**

Intracranial compartment has the ability to compensate for added intracranial volume (increased ICP) by reducing the cerebral blood volume and/or CSF. However, when the compensatory mechanisms are exhausted, ICP increases exponentially. The compliance or volume/pressure ratio ($\Delta V/\Delta P$) is equal to the volume versus the pressure curve (Figure 2, right). By injecting and withdrawing of certain amount of liquid in the cerebrospinal fluid space it is possible to calculate the intracranial compliance. The intracranial compliance (IC) measures the ability to compensate for added intracranial volume.\textsuperscript{116} When IC is reduced the patient moves to the right on the pressure/volume curve and the slope of the pressure/volume curve may change (Figure 2, right). Knowledge about the patient location and the

\[\text{Figure 2. Intracranial pressure volume relationship (left) and compliance or volume pressure relationship (right)}\]
The individual shape of the pressure/volume curve is important for prediction of secondary insults vulnerability. Recent experimental and clinical studies suggested that decreased IC increases the vulnerability of the brain to secondary volume insults.\textsuperscript{150} Therefore, IC monitoring in NICU may add clinically valuable information.\textsuperscript{151}

Cerebral perfusion pressure

Cerebral perfusion pressure is calculated as a difference between mean arterial pressure (MAP) and ICP (CPP = MAP – ICP). CPP is the pressure gradient causing cerebral blood flow to the brain. Under normal conditions, cerebral autoregulation aims to maintain adequate cerebral blood flow during continuous changes in blood pressure and CPP.\textsuperscript{80} Cerebral autoregulation can be impaired as a result of injury causing brain perfusion be passively dependant on systemic blood pressure.\textsuperscript{31,42}

CPP assessments are used as an estimate measure of the CBF which reflects the amount of blood delivered to the brain to meet the metabolic demands.\textsuperscript{100} There is still a great debate regarding the appropriate CPP level of treatment TBI patients. European brain injury consortium suggest keeping CPP 50-70 mmHg to ensure sufficient cerebral blood flow and thereby reduce the risk of ischemia.\textsuperscript{16,94} Decreased CPP can be treated with artificially induced hypertension by using vasopressors and fluid resuscitation in order to increase MAP. However, it is also suggested that increased CPP may lead to transcapillary fluid filtration\textsuperscript{50} and interstitial oedema formation and thereby increased ICP. Therefore, a management protocol has been proposed with a consideration to reduce ICP and oedema including hypotensive treatment.\textsuperscript{5,6}

Cerebral blood flow measurements

The brain receives 20% of the cardiac output and has a high energy demand in order to maintain its function. The energy and oxygen supply to the brain is maintained through the cerebral blood flow (CBF). Under normal conditions, CBF has been estimated about 50 ml/100g/min.\textsuperscript{188} Electroencephalogram slowing and loss of consciousness occurs when CBF decrease to 23ml/100g/min. When CBF is reduced below 18 ml/100g/min, ionic homeostasis jeopardizes and neurons convert to anaerobic metabolism.\textsuperscript{77,161,164} Moreover, the risk of development of ischemic damage not only depends on degree of the CBF decrease, but also on the duration. At a CBF of 10 ml/100g/min, membrane integrity is lost and irreversible brain damage occurs.
Microdialysis

Microdialysis was introduced in early 1970s with the idea to implant an artificial blood capillary into the brain tissue in order to obtain samples of the interstitial fluid. The first MD paper on experimental cerebral ischemia was published in 1986. Since then, MD has been used to study the mechanisms of cerebral ischemia, membrane degradation, excitotoxicity and free radical damage. MD as a clinical neurochemical tool was introduced by Hillered et al. and Meyerson et al. in 1990. With MD it is possible to monitor the neurochemical composition of the brain interstitial fluid. The probe consists of a double lumen catheter with a semi permeable membrane at the tip. The probe can be inserted into the tissue and slowly perfused with artificial CSF. The molecules of the surrounding tissue will diffuse through the membrane of the tip of the probe into the artificial CSF (now called dialysate) (Figure 3C, page 18). The dialysate will then contain a certain concentration of the tissue fluid molecules and will pass through the outlet tubes where it can be collected and analysed. The changes in the neurochemical composition then can be studied and related to brain injury mechanisms. Several MD biomarkers are used in TBI research such as glucose, lactate, pyruvate, and lactate to pyruvate ratio (L/P ratio) for energy metabolism and glutamate and glycerol for cellular distress.

Lactate to Pyruvate ratio

Interstitial levels of lactate, pyruvate and glucose provide information about glucose availability and utilization, the extent of anaerobic glycolysis and information of intracellular redox state which is related to the mitochondrial function. The L/P ratio is a balance between lactate and pyruvate reflecting the state of cerebral oxidative metabolism and is known as a sensitive marker of cerebral ischemia. Increased L/P ratio together with severely decreased interstitial glucose level is a typical pattern of cerebral ischemia. An experimental animal study showed a positive correlation of increased L/P ratio and cerebral ischemia defined by positron emission tomography (PET) criteria.

Normal MD-LP ratio values have been reported previously as approximately 15-20. Prior studies have used different L/P ratio threshold levels of cerebral ischemia ranging from 25 to 40. However, in our studies an L/P ratio threshold level of 30 was derived from combining data from several clinical and experimental MD studies, in which the L/P ratio level was measured in normal human brain during neurosurgical procedures or related to: CPP decrease in the pig, various clinical events in TBI and subarachnoid hemorrhage (SAH) patients, PET
in SAH patients\textsuperscript{41} and TBI patients\textsuperscript{71}, clinical outcome in SAH patients\textsuperscript{131} and vasospasm in SAH patients\textsuperscript{162}, respectively.

**Glutamate**

Glutamate is the main excitatory transmitter of the central nervous system. After glutamate is released from the pre-synaptic cleft it binds to post-synaptic ligand-gated ion channels which lead to excitation through depolarization. In a healthy brain it is actively taken up by neurons and astrocytes after it is released in the synaptic cleft. Excitatory amino acid transporters and cell membrane bound are responsible for the removal of the glutamate from the interstitial space in order to terminate synaptic transmission and to reduce glutamate levels before it increases to neurotoxic levels. During energy failure or ischemia the transporters responsible for glutamate uptake can be impaired or even reversed and therefore can release the glutamate instead of its uptake.\textsuperscript{173} If glutamate becomes excessive the target neuron will die and such glutamate induced cell death is called excitotoxicity. Thus, increased interstitial glutamate levels can be used for detection of impaired cerebral metabolism and impending cell damage.\textsuperscript{152} Increased brain interstitial MD-glutamate has been reported in TBI studies.\textsuperscript{19,21,78,130,185} A clinical TBI study identified MD-glutamate as a sensitive and early marker of cerebral ischemia.\textsuperscript{156} A positive correlation has been demonstrated in TBI patients between high levels of MD-glutamate and increased ICP and poor outcome.\textsuperscript{20} The basal interstitial glutamate concentration in humans ranges between 5 to 15 µmol/L.\textsuperscript{140,162}

**Glycerol**

Glycerol is one of the end products in cell membrane phospholipid degradation. As a water soluble molecule it can be easily collected with MD and used as a marker of phospholipid degradation in cerebral ischemia.\textsuperscript{63} Increased glycerol levels as a response to injury have been reported in animal models during TBI and ischemia involving free radical mediated mechanisms.\textsuperscript{46,88,101} Clinical studies have shown increased glycerol levels in association with subarachnoid hemorrhage (SAH), secondary adverse clinical events, cerebral ischemia and epilepsy.\textsuperscript{63,122,140,184} Normal cerebral MD-glycerol levels have been reported previously from patients during wakefulness, anesthesia and neurosurgical procedures.\textsuperscript{140}

**Glucose**

The main energy source delivered to the brain is glucose.\textsuperscript{160} Glucose in the brain tissue is metabolized to pyruvate. Pyruvate in the presence of oxygen is further metabolized to adenosine triphosphate (ATP). This process is called
oxidative phosphorylation where glucose serves as an energy source and oxygen as for its metabolism. The main ATP production in the cell takes place in the mitochondrial respiratory chain. The presence of ATP is linked to the brain activity. In a situation of decreased oxygen amount there is a progressive failure of ATP production and pyruvate reduction to lactate.

Figure 3. An illustration shows the Neurovent-PTO probe (A, B) and basic principles of the microdialysis catheter (C).

Brain tissue oximetry

Measurement of brain tissue oxygenation may be an important contribution to the neurointensive care of head injured patients. Lack of oxygen supply to the brain is considered to play an important role in development of secondary brain damage. Standard care of TBI patients is focused on maintaining adequate brain tissue oxygenation by controlling ICP, CPP and resuscitation. However, despite standard treatment protocols periods of cerebral ischemia are common after severe head injury and their occurrence and duration is negatively correlated with patient outcome. Therefore, it would be of great value to explore if monitoring of cerebral oxygenation can enable earlier detection of cerebral ischemia, further improve and refine the NICU and thereby reduce secondary insults leading to better outcome in TBI patients. Currently, there are several techniques available to measure global and local brain oxygenation (discussed below).

Global brain oxygen monitoring

To measure global brain oxygenation a fiberoptic oxygen probe can be inserted into the jugular bulb (SjvO2). This technique provide information
related to cerebral oxygen supply, perfusion and consumption and has the ability to detect ischemic episodes of the entire brain. Normal \( S_{jv}O_2 \) levels have been reported earlier and ranges between 55% and 75%. Abnormalities that decrease oxygen delivery (hypoxia, increased ICP, hypotension) or increase oxygen consumption (seizures, fever) can decrease \( S_{jv}O_2 \). Several studies have linked decreased \( S_{jv}O_2 \) with poor outcome. A PET study showed that more than 13% of the brain needs to be ischemic before \( S_{jv}O_2 \) decreases below 50%. On the other hand, increased \( S_{jv}O_2 \) levels can be seen in cases of extensive infarctions due to decreased consumption rate. However, cerebral oxygenation may vary between different brain regions following TBI, therefore, the major disadvantage of \( S_{jv}O_2 \) monitoring is its inability to detect focal brain ischemia.

Local brain oxygen monitoring

\( B_{tp}O_2 \) monitoring allows direct measurement of local tissue oxygen tension in a specific region of the brain. It is likely that \( B_{tp}O_2 \) monitoring reflect the balance between regional oxygen supply and cellular oxygen consumption. The \( B_{tp}O_2 \) measurements are influenced by the microvascular composition and perfusion rate, diffusion distance between capillaries and cells and the dominance of arterioles and venules in the area of probe placement. Under normal conditions arterial \( pO_2 \) and venous \( pO_2 \) lays around 90 and 30 mmHg, respectively. Therefore, a broad spectrum of \( B_{tp}O_2 \) values can be apparent in the healthy brain. It is assumed that \( B_{tp}O_2 \) reflects venous \( pO_2 \) due to venule dominance (~70%) in the microvasculature.

Currently there are two different techniques available for measurements of \( B_{tp}O_2 \) i.e. Clark-type and Fiberoptic.

Clark-type technique

Monitoring of local \( B_{tp}O_2 \) was introduced in 1980’s when the Clark-type Licox® (LX) oxygen measurement device became available. The Clark-type electrode consists of an anode and cathode in contact with an electrolyte solution. The tip of the probe is covered by a semi-permeable membrane which is only permeable to gases. Oxygen molecules diffuse through the membrane to the measurement chamber where are reduced by a polarographic cathode creating an electric current which is directly proportional to oxygen concentration.

Early trials of LX \( B_{tp}O_2 \) monitors were done in an animal model to ensure the usefulness and validity of the information derived from the placement of the probe in both CSF and brain tissue. The insertion of the probe in the lateral ventricle can be easily accomplished in animals and humans. Values obtained reflect changes during manipulation of the blood
pressure and oxygenation and correlate with measurements taken in the white matter of the brain. However, measuring the oxygen in the brain tissue is obviously more appropriate. It was suggested that insertion of BtipO2 probes in the deep white matter of the brain is more valuable and stable because the oxygen consumption is the most stable in that area. Other aspects regarding the probe location in the brain is that probes placed in the penumbra or contusional areas may mirror the regional oxygenation and do not necessarily reflect the global brain situation. Results from a recent study confirmed that the more injured hemisphere had significantly lower BtipO2 levels than in the less injured brain tissue.

During the last few decades experimental and clinical research have been done regarding safety, accuracy and reliability of the Clark-type BtipO2 monitors. In animal experiments normal BtipO2 was found to be 25 to 40 mmHg and in humans undergoing neurosurgical procedures values ranged between 20 to 30 mmHg.

Measurements of BtipO2 using the Clark-type LX monitor, have been shown to positive correlate with MAP, F\textsubscript{i}O\textsubscript{2}, CPP, CBF, S\textsubscript{j}vO\textsubscript{2}, MD, arterial oxygen concentration, blood hemoglobin concentration, cerebral autoregulation and oxygen extraction fraction on PET (discussed below).

A clinical TBI study showed a correlation of decreased BtipO2 and impaired cerebral metabolism where increased levels of glutamate (cellular distress) and lactate (oxidative metabolism) where seen at BtipO2 levels of 15mmHg and 10mmHg, respectively. BtipO2 <10 mmHg was also found to be critical according to increase in MD L/P ratio levels in TBI patients. This finding was supported by Hlatky et al. who observed significant increase of L/P ratio when BtipO2 decreased far below 10 mmHg measured in peri-contusional brain tissue. Several studies have assessed the correlation of local BtipO2 with global brain oxygenation measured by S\textsubscript{j}vO\textsubscript{2}. It was shown that periods of the S\textsubscript{j}vO\textsubscript{2} <50% correlate with decreased BtipO2 values below 10 mmHg. A PET study suggested BtipO2 of 14 mmHg as a threshold at which critical oxygenation extraction occurs. Experimental studies have shown linear correlation between BtipO2 and changes in measurements of CBF or end-tidal carbon dioxide and sinusoidal correlation with MAP, suggesting that BtipO2 is influenced by factors that regulate CBF and cerebral autoregulation. It has been shown that aggressive hyperventilation leads to decrease of BtipO2 which is presumably mediated through a primary reduction of CBF due to cerebral vasoconstriction. Data varies regarding the correlation between BtipO2 and ICP; in general BtipO2 decreases with increased ICP and simultaneous decreased CPP, suggesting that BtipO2 can be related to measures of cerebral perfusion.

Several observational TBI studies suggest that monitoring of BtipO2 may help prevent hypoxic events and improve patients’ outcome.
shown that patients $B_{ti}pO_2$ levels less than 10-15 mmHg for extended periods of time have an increased rate of morbidity and mortality.\textsuperscript{7,82,97,166,177,180}

Taking all results into account, it seems that human brain has a critical threshold for $B_{ti}pO_2$, where levels below 10 mmHg (depending on duration) indicate critical brain ischemia when using Clark-type $B_{ti}pO_2$ catheters.\textsuperscript{7,34,65,84,107,108}

\textit{Fiberoptic technique}

A more recently developed $B_{ti}pO_2$ measurement technique is based on fiberoptic wavelength analysis. Oxygen molecules change the optical properties of indicator compounds by photochemical reactions. The fiberoptic technique does not consume any oxygen.

The optical based $B_{ti}pO_2$ monitoring technique (Neurotrend\textsuperscript{®} and Paratrend\textsuperscript{®}) was introduced in late 90s and was used in few studies.\textsuperscript{53,75} The $B_{ti}pO_2$ critical ischemia level was identified to be around 20 mmHg defined by decreased regional cerebral blood flow (18ml/100g/min).\textsuperscript{56} This level of ischemia seems high since it is close to normal values reported. It may be explained by the regional cerebral blood flow definition of ischemia or by the accuracy of the $B_{ti}pO_2$ probe used. However, production of Neurotrend\textsuperscript{®} and Paratrend\textsuperscript{®} $B_{ti}pO_2$ probes where discontinued since year 2004, therefore, are not commercially available anymore.\textsuperscript{54}

Recently, a new fiberoptic probe (Neurovent-PTO, NV) was introduced (Figure 3 A,B). The device has a great clinical advantage of measuring brain tissue oxygenation, ICP and brain temperature, simultaneously in a single probe. The experience of the new NV probe is not extensively reported and its potential benefits and limitations need to be explored. Especially, we need further knowledge to understand the clinical significance of low $B_{ti}pO_2$ levels.
Aims

General aim

To study brain tissue oxygenation monitoring in traumatic brain injury by using the recently developed fiberoptic Neurovent-PTO® probe.

Specific aims

- To study the Neurovent-PTO® and Licox® brain tissue oxygen tension catheters *in-vitro* regarding sensor accuracy, response time to different oxygen tensions, response to temperature changes and long term stability (Paper I).
- To develop a clinically relevant pig brain death model with the control of intracranial pressure, cerebral perfusion pressure, intracranial compliance and brain tissue oxygenation for studies of intracranial dynamics and evaluation of cerebral monitoring devices (Paper II).
- To identify threshold levels of brain tissue oxygenation and cerebral perfusion pressure leading to cerebral ischemia by using together BIPo2 and MD probes in an animal model (Paper III).
- To examine the cerebral metabolism during different cerebral oxygen levels and to explore if the type of injury and probe localization affects the measurements of cerebral MD and BIPo2 in TBI patients (Paper IV).
Bench test *in-vitro* (Paper I)

In this study two types of oxygen sensors were used based on different technologies: Fluorescent Neurovent-PTO® (NV, Raumedic, Munchberg, Germany) and electrochemical Licox® (LX, Integra Neurosciences Ltd., Hampshire, UK). For all experiments five NV and five LX sensors were used. The NV sensors do not need to be calibrated, however, the LX sensors needs calibration using an individual chip card before the experiments were initiated. The experiments were started with a preparation of an equilibrator (a liquid filled container filled with a buffer solution) and warming it up to 37.0 °C. For all experiments the temperature was kept constant except when looking at the sensor responses to temperature changes. Temperature in the equilibrator was measured by two precision temperature probes (Equilibrator Tonometer integrated probe, RNA Medical and Physitemp TH-5, Clifton, NJ, USA). The equilibrator was then connected to previously prepared high precision oxygen gas tubes (Table 1). Then the oxygen was bubbled through the buffer solution producing desired oxygen concentration in the equilibrator. Simultaneously, both types of oxygen sensors were placed into the equilibrator and the measured values recorded for each test (described in detail below).

*Table 1. Gas concentrations used.*

<table>
<thead>
<tr>
<th>Calibration gas</th>
<th>( pO_2 ) mmHg</th>
<th>% O_2</th>
<th>% CO_2</th>
<th>% N_2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>0.7</td>
<td>5.6</td>
<td>93.7</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>1.4</td>
<td>5.6</td>
<td>93.0</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>2.8</td>
<td>5.6</td>
<td>91.6</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>4.2</td>
<td>5.6</td>
<td>90.2</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>5.6</td>
<td>5.6</td>
<td>88.8</td>
</tr>
</tbody>
</table>

**Accuracy test**

To detect the accuracy of the sensors five different (5 mmHg, 10 mmHg, 20 mmHg, 30 mmHg, 40 mmHg) high precision oxygen calibration gases (AGA, Enköping, Sweden) were used (Table 1). The sensors were placed into the pre-equilibrated buffer solution at each gas concentration for 20
minutes continuous measurements of partial oxygen pressure (pO₂). The measured value after 20 minutes was recorded. The solution was kept at 37.0±0.2°C for the complete monitoring time.

**Response time**

The time for the sensors to reach a new level after a change in pO₂ gas concentration was defined as the response time. At first the sensors were kept for 20 minutes in pre-equilibrated liquid solution with 10 mmHg pO₂ gas, and then placed in solution equilibrated with 40 mmHg pO₂ gas for another 20 minutes. The time it took for the sensor to change from 10 mmHg to 90 % of 40 mmHg was recorded, denoted the 90% response time. After reaching the 90 % level (i.e. 36 mmHg) the sensor was replaced in the 10 mmHg gas solution, and the 90% response time was again recorded, i.e. the time it took the sensor to reach 11 mmHg. The temperature was kept constant at 37.0 ± 0.2°C during the experiment.

**Response to temperature changes**

To measure the sensor response to temperature changes two calibration gases were used (10 and 20 mmHg O₂). After the equilibration period the probes were left in the buffer for 20 minutes for both gases. For each gas concentration the temperature in the solution was changed starting from 37.0°C to 38.5°C and 40.0°C. At each temperature the pO₂ was measured for additional 20 minutes.

**Long term drift**

To test if there was a time dependant drift in pO₂ recordings 2 NV and 2 LX sensors were used and readings were recorded after 24, 48, 72, 96, 120, 144, 168, 192, 216 and 240 hours in pre-equilibrated gas with 10 mmHg O₂. The solution was bubbled continuously and kept at 37.0 ± 0.2°C for the complete monitoring time.

**Data collection and statistical analysis**

Data were collected using Microsoft® Excel® and presented as mean ± standard deviation. A t-test for dependant samples was run to asses the significances of any differences. Regarding the long term drift test, an overall test for the effect of time was made with an analysis of variance model, for NV and LX separately. Factors in the model were probe and time. For all the results the threshold of significance was set at a p-value of 0.05. The analyses were performed with Statistica® 8.0 (StatSoft Inc., Tulsa, OK, USA).
Animal model (Paper II and III)

Animals and anaesthesia

For paper II and III, six pigs of triple breed and of both sexes were included; age 10 to 12 weeks with a weight of 24.5±1.4kg (mean±SD). The animals had only access to water during the night before the experiment. The animals received an intramuscular injection of 2.2mg/kg xylazine (Rompun®, Bayer, Leverkusen, Germany) in combination with zolazepam 6mg/kg (Zoletil®, Virbac, Carros, France) at arrival to the laboratory. A peripheral venous catheter was inserted in an ear vein and was used for induction and maintenance of anesthesia and for fluid administration. All studies were performed under general anesthesia. Anesthesia was induced with 20mg of morphine (Morphine Meda, Solna, Sweden) and 25mg of Ketamine (Ketaminol®, Intervet AB, Boxmeer, Netherlands). Anaesthesia was maintained with continuous intravenous infusion of pentobarbital (Apoteket, Uppsala, Sweden) 8mg/kg/h, morphine 0.5mg/kg/h and pancuronium bromide 0.25mg/kg/h (Pavulon®, Organon, Oss, Netherlands). After endotracheal intubation and mechanical ventilation (Servo 900C, Siemens-Elema, Solna, Sweden), all animals were secured in the prone position. The ventilation was set to 30% oxygen in air and adapted to maintain physiological pCO₂ at a level between 4.5-5.5 kPa (34-41mmHg). The capnogram and peripheral oxygen saturation were displayed continuously (CO₂SMO Plus-8100, Novametrix, Wallingford, CT, USA).

The subclavian artery was catheterized and connected to pressure transducers for continuous measurements of arterial pressure. The jugular vein was catheterized for maintenance of anesthesia, for fluid administration and for blood sampling. Samples of arterial and jugular blood were collected for blood gas analysis (ABL 5, Radiometer, Copenhagen, Denmark). Electrocardiography was used for continuous heart rate and rhythm monitoring.

A temperature probe was inserted in the esophagus for continuous core temperature measurements. All physiological data was simultaneously collected and recorded in a PC with commercial data collection software (Biopac® systems, Goleta, CA, USA). The bladder was catheterized with a Foley (8 Fr) catheter (Willy Rusch AG, Kernen, Germany) after sharp skin and bladder incision.

All animals received fluid replacement with acetate Ringer solution (Ringer-acetet®, Fresenius Kabi, Stockholm, Sweden) as follows: 30 ml/kg during the first hour of preparation and thereafter a continuous infusion of 10 ml/kg/h.
Induction of brain death and validation of the model (Paper II)

Arterial blood pressure, ICP, CPP, IC and $B_{tp}O_2$ was recorded continuously during stepwise elevation of ICP by inflation of an epidural Foley catheter. A sagital midline skin incision was performed and the skin was retracted laterally. Four drill holes were made for introduction of (i) a multiparameter Neurovent-PTO® (NV) probe (Raumedic, Munchberg, Germany) for measurements of ICP, $B_{tp}O_2$ and brain temperature, (ii) a microdialysis catheter (CMA 70 Microdialysis catheter, Solna, Sweden), (iii) an IC balloon catheter (adapted from a Mullan Percutaneous Trigeminal Ganglion Microcompression Set, William Cook Europe, Bjaeverskov, Denmark) and (iv) an epidural balloon catheter (Foley catheter, 14 Fr (Willy Rusch AG, Kernen, Germany) (for exact placements see Figure 4).

![Schematic axial view of the pig’s skull presenting exact locations of the burr holes for the placement of the epidural Foley balloon catheter, IC balloon catheter, NV probe, MD catheter. Anterior (A), Posterior (P).](image)

**Figure 4.** Schematic axial view of the pig’s skull presenting exact locations of the burr holes for the placement of the epidural Foley balloon catheter, IC balloon catheter, NV probe, MD catheter. Anterior (A), Posterior (P).

The experimental steps are presented in Figure 5. The experiment was started by an initial recording of 30 minutes baseline values for ICP ($ICP_0$), $B_{tp}O_2$ and physiological data. Thereafter, the ICP elevation phase followed.
Figure 5. Schematic illustration of the experimental protocol used for BD model. (Paper II) After a baseline period of 30 min, the IC balloon catheter was inflated and deflated (C). Ten minutes later the epidural catheter was inflated with 1 ml saline (V).

It was started by an initial measurement of IC by inflating the micro balloon catheter with 0.5 ml saline (compliance volume, CV). The effect on ICP was recorded and when ICP reached maximal increase (ICPm) the balloon was deflated. IC was calculated by the formula:

\[ IC = \frac{CV}{(ICP_m - ICP_0)} \text{ ml/mmHg} \]

After 10 minutes the ICP was increased by inflation of the epidural Foley balloon catheter with 1ml saline. Another IC measurement was then made 10 minutes later. The same procedures were repeated in similar fashion until CPP had been below zero mm Hg for at least 60 min and at least 10 ml had been inflated in the epidural balloon. CPP was calculated from the mean arterial pressure (MAP) and ICP (CPP=MAP-ICP).

To prove the cessation of cerebral blood flow, a total of 2 x 10⁶ yellow fluorescent non-radioactive microspheres (Dye-Trak™; Triton Microspheres, San Diego, CA, USA), with a diameter of 10 µm were injected through a catheter with its tip in the left atrium to achieve sufficient mixing with the arterial circulation. 30 minutes after microspheres injection the animals were sacrificed by an overdose of potassium intravenously. Thereafter, in 5 of 6 pigs, small pieces (~3-5 g) of the cerebrum, brain stem and kidney were removed, blotted and fixed in 4% (vol/vol) formaldehyde for 2 days. After this, the samples were cut in smaller pieces (25 µg), which were weighed and placed between object slides as previously described for rat organs. The number of microspheres in the samples was then calculated manually in randomly chosen sections in a microscope equipped with both bright and fluorescent light illumination. BD was proven by no or negligible amount of microspheres in the cerebral vessels.
BtipO2 threshold levels of cerebral ischemia (Paper III)

In Paper III, microdialysis samples were collected at 20-min intervals (see below). For the MD data analysis the continuous data of BtipO2, CPP and physiological parameters were averaged for the corresponding 20-min interval to match the timing of the microdialysis. The experimental steps are shown in Figure 6. Data are presented as mean ± standard deviation (SD).

![Figure 6](image)

**Figure 6.** Schematic illustration of the experimental protocol used. (Paper III) The experiment was started by an initial recording of 20 min baseline values for CPP, BtipO2 and MD. The intracranial volume (V) elevation phase followed by increasing the intracranial volume by 1 ml every 20 minutes.

Microdialysis in animals

In five animals a microdialysis catheter (CMA 70 Microdialysis catheter, Solna, Sweden) were inserted in the brain cortex (Figure 1) and perfused (Perfusion fluid, CMA Microdialysis, Stockholm, Sweden) with a microdialysis pump (Microdialysis CMA/102, Solna, Sweden). The flow rate was set to 2.5 µl/min and samples were collected every 20 minutes using a CMA 142 microfraction collector, (CMA Microdialysis, Solna, Sweden). The microdialysis samples were analyzed (Lactate, Pyruvate, Glucose, Glutamate, Glycerol) using a CMA 600 Microdialysate analyzer (CMA Microdialysis, Solna, Sweden). The lactate/pyruvate ratio was calculated by the following equation (lactate × 1000 / pyruvate) and a ratio >30 was used as a specific marker of cerebral ischemia in order to detect the threshold levels of BtipO2 and CPP for cerebral ischemia (see previously).

Statistical methods

To assess the critical threshold, we assumed a hyperbolic relationship between the L/P ratio and x = BtipO2 or CPP. The relation is characterized by the equation: L/P ratio = a/x ** b. With log transformation, this equation becomes the following linear model: log(L/P ratio) = a – b ** log(x). We applied regression analysis to estimate the regression coefficients, the squared correlation and the value of x (with 95 % confidence interval) that corresponded to a L/P ratio threshold = 30.
Ethics

The study was approved by The Uppsala Institutional Review Board for Animal Experimentation. The pigs were handled according to the guidelines of the Swedish National Board for Laboratory Animals and the European Convention of Animal Care. Qualified individuals in the experimental laboratory at the Uppsala University Hospital cared for the animals.

TBI in patients (Paper IV)

Twenty three patients (21 men and 2 women) with severe TBI (including cerebral contusions, diffuse axonal injury, extra cerebral hematomas) were studied. Mean age was 46 years (range 16-82). All patients were admitted to the neurointensive care unit (NICU) at the Uppsala University Hospital between year 2008 and 2012. Patients with Glasgow Coma Scale of ≤ 8 (not obeying commands or worse) at the NICU were included. CT scans were performed in all patients. All patients received continuous propofol infusion 1-4 mg/kg/h (Propofol-Lipuro®, B.Braun Melsungen AG, Melsungen, Germany) as sedation and morphine as analgesia, 1-3 mg intermittently (Morfin Meda®, Meda, Sollentuna, Sweden). In all patients advanced multiparameter neuromonitoring was applied for measurements of ICP, CPP, $B_{tip}O_2$, and MD for cerebral metabolism. The $B_{tip}O_2$ and MD probes were inserted into the brain as soon as possible after the injury or if a patient deteriorated during the stay in NICU (see below). The treatment was based on ICP and CPP guided protocols (ICP < 20mmHg; CPP > 60mmHg) including mild hyperventilation (PaCO$_2$ 30-35 mmHg) and head elevation to 30°. Normoventilation was applied as soon as possible. Mass lesions were removed when indicated. High ICP was controlled with hyperventilation, cerebrospinal fluid drainage, barbiturate coma treatment (Pentothal Natrium, Abbott Laboratories, IL, USA) and decompressive craniectomy in an escalated manner.

Advanced patient neuromonitoring

Brain tissue oximetry

A multiparameter Neurovent-PTO® (NV) probe (Raumedic, Munchberg, Germany) for continuous measurements of ICP, $B_{tip}O_2$ and brain
temperature was inserted via a burr hole usually in the right frontal lobe. The probes were placed in the left hemisphere in cases when hemicraniectomy or evacuation of mass lesion was indicated on that side.

Microdialysis

A microdialysis catheter (71 High Cut-Off Brain Microdialysis Catheter, M Dialysis AB, (formerly CMA Microdialysis) Solna, Sweden) was placed through a separate burr hole in close proximity to the NV probe. The MD catheter was connected to a microinjection pump (106 MD Pump, M Dialysis AB) and perfused with Perfusion Fluid CNS (M Dialysis AB) with a flow rate of 0.3 µL/min. The MD samples were collected in one-hour intervals and analyzed for Lactate, Pyruvate, Glucose, Glutamate and Glycerol with enzymatic techniques using a bedside analyzer (CMA 600, CMA Microdialysis, Solna, Sweden). The analyzers were automatically calibrated when started as well as every sixth hour using standard calibration solutions from the manufacturer. Imprecision values for between assay coefficient of variation was <10% for all analytes. In all patients the first six hours of the monitoring time was excluded due to the time needed for the measurement stabilization of the BipO₂ probe. The time periods with barbiturate induced coma were excluded from this study. Bold line on Y-axis (Figure 21, 22, 23) shows the tentative normal MD values based on Reinstrup et al. and Schulz et al. No correction for relative recovery (extraction efficiency) was made.

Classification of type of injury and probe placement

The type of injury was classified as focal (extra cerebral haematomas or contusions) or as diffuse axonal injury (DAI) based on dominant CT findings. The patient group with focal lesions was subdivided into two groups based on probe placement: ipsilateral (injured hemisphere) and contralateral (non-injured hemisphere).

Data collection

ICP, CPP, MD, BipO₂ and physiological data (heart rate, arterial blood pressure, peripheral oxygen saturation, arterial oxygen saturation) were acquired and processed using the Odin software for multi-modality monitoring in the NICU, developed at Uppsala University and Edinburgh University. The trend data were stored in a minute by minute time interval. Artifacts, which mainly occurred during the probe
recalibration or nursing interventions, were manually removed from the datasets. For the correlative MD data analysis, the continuous data of $B_{\text{tip}}pO_2$ and CPP were averaged for 1-hour intervals to match the microdialysis sampling periods.

Statistical analysis

Statistical analyses and graphical views were done using Statistica 10.0 for Windows (StatSoft Inc., Tulsa, OK, USA). All data were evaluated for normal distribution and did not meet the assumptions for parametric analysis. Therefore a non-parametric analysis was performed using Kruskal-Wallis analysis of variance (ANOVA) on the full set of evaluated $B_{\text{tip}}pO_2$ levels, and if this was significant, Mann-Whitney U test was used to determine which pairs of $B_{\text{tip}}pO_2$ levels were significantly different. Results were considered significant if $p<0.05$. The data are presented as mean values ± standard deviation (SD).

Ethics

This study was approved by the local ethics committee for human research. Informed consent to participate in the study was obtained for all patients from the nearest kin.
Results

Brain tissue oxygen sensors *in-vitro* (Paper I)

When testing brain tissue oxygen sensors *in-vitro* (Paper I) we found no technical failures during the calibration of any of the probes.

Accuracy of pO₂ sensors

The pO₂ accuracy measurements for the sensors using 5 different oxygen concentrations are shown in Figure 7 and Table 2.

*Table 2.* Calculated and measured pO₂ values using the NV and LX oxygen sensors

<table>
<thead>
<tr>
<th>Gas</th>
<th>pO₂ calculated (mmHg)</th>
<th>pO₂ measured (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NV</td>
<td>LX</td>
</tr>
<tr>
<td>1</td>
<td>4.85 ± 0.05</td>
<td>5.62 ± 0.14</td>
</tr>
<tr>
<td>2</td>
<td>10.0 ± 0.08</td>
<td>10.82 ± 0.50</td>
</tr>
<tr>
<td>3</td>
<td>19.78 ± 0.10</td>
<td>20.74 ± 0.76</td>
</tr>
<tr>
<td>4</td>
<td>29.76 ± 0.24</td>
<td>31.16 ± 0.62</td>
</tr>
<tr>
<td>5</td>
<td>39.72 ± 0.29</td>
<td>41.32 ± 0.77</td>
</tr>
</tbody>
</table>

In low (~5 mmHg) oxygen gas concentration (the exact O₂ was calculated to 4.85 mmHg, depending on the barometric pressure), the mean readings for NV probes were 5.62 ± 0.14 mmHg which was 0.76 ± 0.14 mmHg higher than the calculated O₂ value. LX probes showed a more accurate mean value of 5.12 ± 0.71 mmHg, i.e. 0.26 ± 0.71 mmHg higher than the reference value. When comparing the probe that gave the lowest oxygen value with the probe that gave the highest oxygen value, the spread was 8% of the true oxygen partial pressure for NV, whereas for LX the spread between the probes was 35% at this oxygen concentration. In ~10 mmHg oxygen concentration (10.02 mmHg calculated), the readings were for NV 10.82 ± 0.50 mmHg and for LX 10.20 ± 1.04 mmHg. When measured in ~20 mmHg oxygen (with a
calculated value of 19.78 mm Hg), the NV showed 20.74 ± 0.76 mmHg and the LX probes gave 19.36 ± 0.99 mmHg. In higher oxygen concentrations (with a calculated oxygen value of 29.76 mmHg), NV showed 31.16±0.62 mmHg and LX 29.92±1.33 mmHg. With oxygen concentration of ~40 mmHg (39.72 mmHg calculated), the NV mean readings were 41.32±0.77 mmHg and for LX 39.82±1.26 mmHg. Thus, in all gases the mean deviation from the calculated $pO_2$ was very small, for NV 0.76-1.6 mmHg and for LX -0.46 - 0.26 mmHg. For all gas concentrations, the small difference between NV readings and the calculated value were significant (p<0.05). For LX sensors, there were no significant difference between the calculated value and the recorded value. However, this was mainly an effect of the larger variation and spread in LX sensors, making the standard deviation larger.

Figure 7. The accuracy test of Neurovent and Licox sensors.

Response time

When changing from low (10 mmHg) to high (40 mmHg) oxygen concentration, the NV sensors reacted fast in 4 of 5 probes, within 36-55 seconds. In one NV probe it took 95 seconds for the 90% response time. For all 5 probes the mean was 56±22 s (Figure 8A). The LX probes reacted
somewhat slower in the same test, ranging from 45 to 103 seconds (mean 78±21 s) (Figure 8A).

Figure 8B reflects the 90% response time when switching sensors from high oxygen concentration (40 mmHg) to low (10 mm Hg). This test took longer time for both types of sensors. NV sensors needed 131±42 seconds and LX sensors 215±63 seconds, this difference was significant (p<0.05).

![Figure 8. The response time for LX and NV sensors. Time (seconds) needed to reach 90% of expected (calculated) $pO_2$ value when switching probes from (A) low (10 mmHg) to high (40 mmHg) oxygen concentration and in from (B) high (40 mmHg) to low (10 mmHg) oxygen concentration. NV was significantly faster than LX in the response from high to low oxygen concentration. Data of LX and NV are shown as Mean ± standard deviation (SD) and Mean ± 1.96*SD. * Statistically significant difference between LX and NV response time (p<0.05).](image)

Long-term drift

For long term measurements (240 hours), probes were inserted in the equilibrated tonometer containing gas with 10 mmHg oxygen concentration and readings were taken every 24 hours. The $pO_2$ in the equilibration gas was calculated depending on the barometric pressure for each day (mean...
value ± SD = 10.02 ± 0.11 mmHg, in absolute values ranging between 9.8 mmHg – 10.2 mmHg).

Individual NV sensors read $pO_2$ between 9.6 to 11.8 mmHg (mean value ± SD = 10.83±0.59 mmHg), as shown in Figure 9. Individual LX sensors read between 10.0 to 13.0 mmHg $pO_2$ (mean value ± SD = 11.09±0.74 mmHg). There was a significant difference between the calculated value and the NV probe measured values ($p=0.0003$). However, there was no trend towards a time dependent increase- or decrease in estimations. Instead, we observed only minimal fluctuations over time. For LX probes there were no statistical difference ($p = 0.36$) between calculated and measured oxygen values, due to larger variations between the probes.

![Figure 9. Long-term drift test for LX and NV sensors. The accuracy of the sensors in pO2 (mmHg) measurement was evaluated continuously for 10 days.](image)

**Response to temperature changes**

To detect the sensor response to temperature changes the tonometer was equilibrated with two different oxygen concentrations (10 and 20 mmHg) and the temperature was raised from 37.0 to 38.5 and to 40.0 ± 0.2 °C. With the oxygen concentration of 10mmHg, the $pO_2$ value for NV probes were slightly higher at all temperatures (ranging between 10.82 mmHg to 10.94 mmHg) compared to the calculated value (9.92-10.02 mmHg) (Figure 10). For LX sensors the mean value of 10.0±0.63 mmHg was equal to the calculated value. At higher temperatures (38.5-40.0°C), the standard deviations increased for LX probes up to 1.20 mmHg.
Figure 10. The LX and NV sensors accuracy in different temperatures. The temperature in the buffer solution was raised from 37.0 to 38.5 and to 40.0 ± 0.2 °C. The tonometer was equilibrated with two different oxygen concentrations. Response to temperature changes with 10 mmHg oxygen concentration. Data of LX and NV sensors are shown in Mean ± Standard deviation.

With 20 mmHg oxygen concentration, the pO2 measured was different for both NV (p<0.05) and LX (N.S.) (Figure 11). In all measurements, the LX probes read lower mean values (18.9 to 19.14 mmHg) and the NV probes read higher mean values (20.74 – 20.78 mmHg), compared to the calculated value (19.7±0.1 mmHg). As temperature became higher, LX sensors standard deviation increased from 0.4 to 1.55 mmHg, while the NV probes standard deviation decreased from 0.76 mmHg to 0.44 mmHg.

Figure 11. The LX and NV sensors accuracy in different temperatures. The temperature in the buffer solution was raised from 37.0 to 38.5 and to 40.0 ± 0.2 °C. The tonometer was equilibrated with two different oxygen concentrations. Response to temperature changes with 10 mmHg oxygen concentration. Data of LX and NV sensors are shown in Mean ± Standard deviation.
Standardized experimental brain death model (Paper II)

A standardized large animal model was created to enable studies of intracranial dynamics and evaluation of cerebral monitoring devices. To mimic the clinical situation, a gradual and prolonged increase of ICP was applied by inflation of an epidural balloon catheter, resulting in a volume-dependant elevation of ICP and decrease of intracranial compliance (IC) (Figure 12). All animals showed CPP ≤ 0 after 7-10 ml infusion (7ml = 2 pigs; 8ml = 3; 9ml = 0; 10ml = 1) in the epidural balloon. The results are presented in detail below where average values for all parameters are shown. Measured individual values are presented in an illustrative case.

![Figure 12. The volume-pressure relation observed during 1 ml stepwise increase of intracranial volume and the influence on IC.](image)

**Intracranial pressure and intracranial compliance**

The stepwise inflation of the balloon gave a gradual volume-dependent elevation of ICP (Figure 12). In all cases, when the intracranial volume was increased between 0 and 5 ml, there was a gradual exponential increase of ICP starting from baseline values of 13.2 ± 4.8 mmHg to ICP of 60.9 ± 11.9 mmHg. After increasing the volume with additional 2 ml of saline (totally 6 to 7 ml) the ICP did not increase, instead a slight decline was observed (Figure 12). Subsequently, at a total of 8 ml inflation ICP severely increased...
again, reaching $89.8 \pm 9.7$ mmHg at the total inflated intracranial volume of 10 ml. Intracranial compliance was gradually decreasing, starting from $0.137 \pm 0.069$ ml/mmHg and ending at the $0.007 \pm 0.001$ ml/mmHg at the end of the experiment (Figure 12). The decline of IC reflects a decreasing ability to compensate for added intracranial volume.

*Cerebral Perfusion Pressure*
Baseline CPP was $72.3 \pm 15.0$ mmHg. As shown in Figure 13, CPP declined after every additional volume inflation. After 7 ml of inflation the calculated CPP was below 0 mmHg. When 10 ml had been inflated the calculated CPP was $-28.6 \pm 26.2$ mmHg.

![Figure 13. CPP changes observed during 1 ml stepwise increase of intracranial volume.](image)

*Changes in hemodynamics*
Throughout the first 5ml of intracranial volume inflation mean arterial blood pressure (MAP) remained stable and close to the baseline levels of $85.0 \pm 10.4$ mmHg (Figure 14). Also the heart rate (HR) was maintained close to baseline values $98.5 \pm 15.4$ beats per min throughout the first half of the experiment (0 to 5 ml inflation) (Figure 14). The MAP decreased and the HR increased significantly when the intracranial volume was increased to between 5 and 6 ml. At the end of the experiment MAP was $59.0 \pm 7.5$ and the HR was $114 \pm 12.7$ beats per min.
Figure 14. Changes in MAP and HR observed during 1 ml stepwise increase of intracranial volume. All values are presented as mean±SD

**Brain tissue oxygenation**

No technical problem was detected in any of the NV sensors. As shown in Figure 15, baseline $B_{ti}pO_2$ levels were $26.0 \pm 9.5$ mmHg. $B_{ti}pO_2$ started to decrease after 2 ml inflated volume and at the volume of 7 ml $B_{ti}pO_2$ showed critical ischemic levels of $6.9 \pm 13.0$ mmHg.

Figure 15. Brain tissue oxygenation changes observed during 1-ml stepwise increase of intracranial volume.
After the inflation of 9 ml in the epidural balloon, $B_{\text{ip}}O_2$ rapidly displayed 1.1 ± 0.24 mmHg and remained at the same level to the end of observation period.

**Confirmation of total brain infarction by injection of microspheres**

After reaching the physiological brain death criteria used in this study (negative CPP for 60 min), we analyzed the possible presence of intracranial microspheres in five cases. A negligible amount of microspheres were found in the cerebrum 34.66 ± 9.947 per gram tissue and brain stem 60.41 ± 36.28 per gram tissue, confirming the absence of cerebral blood flow. The values should be compared to kidney tissue where 17979±5086 microspheres per gram tissue were found (Figure 16).

![Figure 16. The amount of microspheres found in kidney (control tissue), brain and brainstem.](image)

**Brain tissue oxygenation and cerebral perfusion pressure threshold levels of ischemia in an animal model (Paper III)**

One of the 6 pigs was excluded because of abnormal baseline microdialysis levels. The results refer to the remaining 5 pigs. The results are firstly presented as mean values of the 5 pigs at every volume inflated (Figure 17 and 18) and then for ischemic threshold estimations as scatter plots with L/P ratio data related to $B_{\text{ip}}O_2$ and CPP, respectively (Figure 19 and 20). Figure 17 and Table 3 shows the effect of the added intracranial volumes on ICP. Table 4 shows the L/P ratio values in relation to the Glucose, Glutamate and Glycerol values.
Figure 17. The volume-pressure relation observed during 1 ml stepwise increase of intracranial volume. All values are presented as mean ± SD.

Cerebral perfusion pressure and lactate/pyruvate ratio

Baseline mean CPP was 73±17 mmHg (Table 3, Figure 18). There were no obvious changes of the L/P ratio until the CPP decreased to a level of below 40 mmHg (Table 3). At this CPP level there appeared to be a very slight increase of the L/P ratio (21.5±8.7) (Table 3). When CPP continued to decrease the L/P ratio increased further (Figure 18). When CPP approached zero mmHg we observed very high L/P ratios (271.9±325.6, range 69.8 - 850.0) (Table 3). A correlation study between CPP and L/P ratios showed a CPP ischemia threshold level (L/P ratio > 30) of around 30 mmHg (mean 28 mmHg, 95% confidence interval =25.0-34.8 mmHg) (Figure 20).
As previously mentioned the CPP started to decrease immediately after the first addition of intracranial volume (Figure 18). In the beginning of this process $B_{tip}$O$_2$ remained unaffected. Later, at the volume of 4 ml, when CPP reached around 40 (40±20) mmHg, $B_{tip}$O$_2$ decreased to around 15 (15.1±9.6) mmHg. Thereafter, when CPP dropped to around 27 (27±16) mmHg, we observed a marked decline of $B_{tip}$O$_2$ to a mean value of 8.1±5.8 mmHg (Table 3). In some cases (n=2) the $B_{tip}$O$_2$ approached 0 mmHg at this CPP level. In all cases the CPP and $B_{tip}$O$_2$ were close to zero or negative values (CPP) at the end of the experiment.
**Brain tissue oxygenation and lactate/pyruvate ratio**

At the beginning of the experiment, before any intracranial volume was added, the mean baseline $B_{tp}O_2$ was $22.9 \pm 6.2$ mmHg and the baseline L/P ratio was $17.7 \pm 6.1$ (Table 3). Both variables remained stable in the beginning of the experiment, whereas starting at 4 ml of volume inflation a clear $B_{tp}O_2$ decrease was observed (Figure 18), simultaneously with a minor L/P ratio increase (Table 3). When $B_{tp}O_2$ dropped below 10 mmHg (mean 8.6 mmHg, 95% confidence interval=7.4-10.4 mmHg) the L/P ratio increased to clearly abnormal values (>30) (Figure 19).

Table 3. $B_{tp}O_2$, CPP and L/P ratio values during every step of added intracranial volume.

<table>
<thead>
<tr>
<th>Volume (ml)</th>
<th>Time (min)</th>
<th>$B_{tp}O_2$ (mmHg)</th>
<th>CPP (mmHg)</th>
<th>L/P ratio</th>
<th>ICP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_0$</td>
<td>-20 - 0</td>
<td>$22.9 \pm 6.2$</td>
<td>$73 \pm 17$</td>
<td>$17.7 \pm 6.1$</td>
<td>$13 \pm 5$</td>
</tr>
<tr>
<td>$V_1$</td>
<td>0 - 20</td>
<td>$24.0 \pm 7.3$</td>
<td>$71 \pm 16$</td>
<td>$16.1 \pm 6.0$</td>
<td>$17 \pm 6$</td>
</tr>
<tr>
<td>$V_2$</td>
<td>20 - 40</td>
<td>$24.0 \pm 10.0$</td>
<td>$64 \pm 16$</td>
<td>$18.7 \pm 5.5$</td>
<td>$25 \pm 8$</td>
</tr>
<tr>
<td>$V_3$</td>
<td>40 - 60</td>
<td>$22.2 \pm 10.6$</td>
<td>$53 \pm 18$</td>
<td>$18.8 \pm 7.2$</td>
<td>$35 \pm 6$</td>
</tr>
<tr>
<td>$V_4$</td>
<td>60 - 80</td>
<td>$15.1 \pm 9.6$</td>
<td>$40 \pm 20$</td>
<td>$21.5 \pm 8.7$</td>
<td>$48 \pm 7$</td>
</tr>
<tr>
<td>$V_5$</td>
<td>80 - 100</td>
<td>$8.1 \pm 5.8$</td>
<td>$27 \pm 16$</td>
<td>$44.3 \pm 25.8$</td>
<td>$64 \pm 9$</td>
</tr>
<tr>
<td>$V_6$</td>
<td>100 - 120</td>
<td>$4.1 \pm 3.9$</td>
<td>$15 \pm 13$</td>
<td>$65.6 \pm 38.2$</td>
<td>$68 \pm 14$</td>
</tr>
<tr>
<td>$V_7$</td>
<td>120 - 140</td>
<td>$1.7 \pm 0.8$</td>
<td>$2 \pm 11$</td>
<td>$271.9 \pm 325.6$</td>
<td>$64 \pm 9$</td>
</tr>
</tbody>
</table>

Table 4. The microdialysate Lactate, Pyruvate and L/P ratio values in relation to the Glucose, Glutamate and Glycerol (intracranial volume 1mL/20 min). Data presented as mean ± SD.

<table>
<thead>
<tr>
<th>Volume (ml)</th>
<th>Lactate (mmol/L)</th>
<th>Pyruvate (µmol/L)</th>
<th>L/P ratio</th>
<th>Glucose (mmol/L)</th>
<th>Glutamate (µmol/L)</th>
<th>Glycerol (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_0$</td>
<td>$0.9 \pm 0.3$</td>
<td>$53.8 \pm 15.5$</td>
<td>$17.7 \pm 6.1$</td>
<td>$1.1 \pm 0.5$</td>
<td>$4.5 \pm 1.3$</td>
<td>$27.4 \pm 5.2$</td>
</tr>
<tr>
<td>$V_1$</td>
<td>$0.9 \pm 0.2$</td>
<td>$50.6 \pm 18.7$</td>
<td>$18.1 \pm 6.0$</td>
<td>$1.1 \pm 0.4$</td>
<td>$4.3 \pm 1.4$</td>
<td>$25.0 \pm 3.9$</td>
</tr>
<tr>
<td>$V_2$</td>
<td>$0.9 \pm 0.3$</td>
<td>$47.8 \pm 12.0$</td>
<td>$18.7 \pm 5.5$</td>
<td>$1.1 \pm 0.4$</td>
<td>$4.7 \pm 1.8$</td>
<td>$26.8 \pm 5.5$</td>
</tr>
<tr>
<td>$V_3$</td>
<td>$1.1 \pm 0.4$</td>
<td>$62.0 \pm 25.8$</td>
<td>$18.8 \pm 7.2$</td>
<td>$1.2 \pm 0.4$</td>
<td>$4.6 \pm 1.7$</td>
<td>$20.0 \pm 6.8$</td>
</tr>
<tr>
<td>$V_4$</td>
<td>$1.5 \pm 0.6$</td>
<td>$73.4 \pm 34.7$</td>
<td>$21.5 \pm 8.7$</td>
<td>$1.2 \pm 0.7$</td>
<td>$5.7 \pm 1.2$</td>
<td>$33.8 \pm 18.5$</td>
</tr>
<tr>
<td>$V_5$</td>
<td>$2.1 \pm 0.4$</td>
<td>$64.0 \pm 36.6$</td>
<td>$44.3 \pm 25.8$</td>
<td>$0.8 \pm 0.3$</td>
<td>$5.8 \pm 2.8$</td>
<td>$27.0 \pm 4.4$</td>
</tr>
<tr>
<td>$V_6$</td>
<td>$2.7 \pm 0.9$</td>
<td>$58.2 \pm 41.5$</td>
<td>$65.6 \pm 38.2$</td>
<td>$0.4 \pm 0.2$</td>
<td>$24.3 \pm 20.0$</td>
<td>$40.6 \pm 14.0$</td>
</tr>
<tr>
<td>$V_7$</td>
<td>$3.0 \pm 1.0$</td>
<td>$22.8 \pm 20.7$</td>
<td>$271.9 \pm 325.6$</td>
<td>$0.2 \pm 0.1$</td>
<td>$53.4 \pm 30.9$</td>
<td>$63.0 \pm 19.6$</td>
</tr>
</tbody>
</table>
Figure 19. A hyperbolic relationship between the L/P ratio and B\textsubscript{a}pO\textsubscript{2}. The mean value of B\textsubscript{a}pO\textsubscript{2} when L/P reached 30 is shown with 95 % confidence interval. Different symbols reflect individual pigs.

Figure 20. A hyperbolic relationship between the L/P ratio and CPP. The mean value of CPP when L/P reached 30 is shown with 95 % confidence interval. Different symbols reflect individual pigs.
The main findings in the present study were a sequence of changes in CPP, \( B_{ip}\text{pO}_2 \) and MD levels during a standardized elevation of intracranial volume in the pig (Table 5). The mean baseline values were \( B_{ip}\text{pO}_2 \) of 22.9±6.2 mmHg, CPP of 72.9±16.9 mmHg and L/P ratio of 17.7±6.1 (Table 3). The ICP elevation caused profound changes in CPP and \( B_{ip}\text{pO}_2 \). When mean CPP moved from 50 to 40 mmHg, there was a drop in mean \( B_{ip}\text{pO}_2 \) from 20 to 15 mmHg, but the mean L/P ratio remained normal with minimal changes. When mean CPP fell from 40 to 27 mmHg, the mean \( B_{ip}\text{pO}_2 \) decreased from 15 to 8 mmHg and the mean L/P ratio increased from 21 to 44 (Table 5).

Table 5. A summary table of critical threshold level patterns. A CPP decline to 40 mmHg caused a slight initial \( B_{ip}\text{pO}_2 \) decrease but the L/P ratio showed only minimal increase, indicating reduced haemodynamic reserve/oligemia. A further CPP decline to 27 resulted in a \( B_{ip}\text{pO}_2 \) decrease to 8 mmHg and an increase in L/P ratio to clearly abnormal levels (44), indicating impaired cerebral metabolism/ischemia. \( B_{ip}\text{pO}_2 \), CPP and ICP presented as mean values in mmHg.

<table>
<thead>
<tr>
<th>CPP</th>
<th>( B_{ip}\text{pO}_2 )</th>
<th>L/P ratio</th>
<th>Brain status</th>
</tr>
</thead>
<tbody>
<tr>
<td>53 → 40</td>
<td>22 → 15</td>
<td>18 → 22</td>
<td>↓ Haemodynamic reserve/oligemia</td>
</tr>
<tr>
<td>40 → 27</td>
<td>15 → 8</td>
<td>22 → 44</td>
<td>Penumbra/Severe ischemia</td>
</tr>
</tbody>
</table>

Brain tissue oxygenation and metabolism in TBI patients (Paper IV)

The \( B_{ip}\text{pO}_2 \) and MD probes were inserted 35±23 hours (mean±SD) after the injury. The mean duration of the \( B_{ip}\text{pO}_2 \) and MD monitoring was 199 hours (range 13 – 496 h). The results are presented divided in focal (with ipsilateral or contralateral measurements) and diffuse injury (Table 6).

Focal brain injury and probe placement on the contralateral side

**ICP, CPP and \( B_{ip}\text{pO}_2 \)** - During monitoring ICP mean values ranged between 12-17 mmHg and CPP was in the range 70-90 mmHg at \( B_{ip}\text{pO}_2 \) levels of ≥10 mmHg. When \( B_{ip}\text{pO}_2 \) decreased below 10 mmHg ICP was significantly higher (21.0 ± 3.2 mmHg) and CPP was significantly lower (61.4 ± 6.1mmHg) (p<0.01) (Table 7 and Figure 21 A-B).

**MD-glutamate and \( B_{ip}\text{pO}_2 \)** - In this group of patients, MD-glutamate was around 3 to 9 µmol/L at \( B_{ip}\text{pO}_2 \) levels higher than 5mmHg (Table 7 and
However, when $B_{tipO2}$ was below 5 mmHg we observed a significant ($p<0.05$) increase of MD-glutamate ($15.8 \pm 6.7 \mu$mol/L).

Table 6. TBI patients investigated: Type of injury, NV probe and MD catheter location. *Ipsi* – probe placement frontal in injured hemisphere; *Contra* - probe placement frontal in non-injured hemisphere; *Right* – probe placement in right frontal hemisphere.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Extra cerebral haematoma</th>
<th>Cerebral contusions</th>
<th>Diffuse axonal injury</th>
<th>Probe location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>X</td>
<td></td>
<td></td>
<td>Contra</td>
</tr>
<tr>
<td>2</td>
<td>X</td>
<td></td>
<td></td>
<td>Contra</td>
</tr>
<tr>
<td>3</td>
<td>X</td>
<td></td>
<td></td>
<td>Contra</td>
</tr>
<tr>
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<td>X</td>
<td></td>
<td></td>
<td>Contra</td>
</tr>
<tr>
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<td>X</td>
<td></td>
<td></td>
<td>Contra</td>
</tr>
<tr>
<td>6</td>
<td>X</td>
<td>X</td>
<td></td>
<td>Contra</td>
</tr>
<tr>
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<td>X</td>
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<td>Ipsi</td>
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<td>Right side</td>
</tr>
<tr>
<td>23</td>
<td></td>
<td>X</td>
<td></td>
<td>Right side</td>
</tr>
</tbody>
</table>

*MD-glycerol and $B_{tipO2}$* - Similarly, in all patients in this group MD-glycerol was 44-66 $\mu$mol/l when $B_{tipO2}$ was $>10$ mmHg. $B_{tipO2}$ 5-10 mmHg was accompanied with a slight increase of MD-glycerol to $97.5 \pm 134.9$ $\mu$mol/L (Table 7 and Figure 21D). A significant increase of MD-glycerol to $291.3 \pm 144.5$ ($p<0.01$) was observed at very low oxygen levels ($<5$ mmHg).

*MD-L/P ratio and $B_{tipO2}$* - The MD-L/P ratio remained stable and within the normal levels (mean range 16-19) when $B_{tipO2}$ was between $>5$ mmHg. When the MD-L/P ratio significantly increased to $24.4 \pm 3.0$ ($p<0.01$) we observed a $B_{tipO2}$ decrease below 5 mmHg (Table 7 and Figure 21E).

*MD-glucose and $B_{tipO2}$* – Table 7 and Figure 21F shows the mean MD-glucose concentrations at different $B_{tipO2}$ levels. We did not observe any correlations of MD-glucose and $B_{tipO2}$.
Table 7. $B_{ti}pO_2$ and MD-dialysate concentrations at different ICP and CPP levels. NV probe and MD catheter placement in focal (Ipsi- or Contra lateral side) or diffuse axonal injury (right frontal hemisphere). All values are shown as Mean ± SD.

### Contralateral (n=6)

<table>
<thead>
<tr>
<th>$B_{ti}pO_2$</th>
<th>ICP</th>
<th>CPP</th>
<th>Glutamate</th>
<th>Glycerol</th>
<th>L/P ratio</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5</td>
<td>18.9 ± 6.0</td>
<td>61.9 ± 8.4</td>
<td>15.8 ± 6.7</td>
<td>291.3 ± 144.5</td>
<td>24.4 ± 3.0</td>
<td>2.4 ± 0.7</td>
</tr>
<tr>
<td>5-&lt;10</td>
<td>21.0 ± 3.2</td>
<td>61.4 ± 6.1</td>
<td>6.8 ± 5.5</td>
<td>97.5 ± 134.9</td>
<td>18.0 ± 2.8</td>
<td>2.4 ± 0.7</td>
</tr>
<tr>
<td>10-&lt;20</td>
<td>15.3 ± 6.5</td>
<td>74.0 ± 20.6</td>
<td>9.2 ± 10.7</td>
<td>44.6 ± 20.5</td>
<td>16.8 ± 2.7</td>
<td>1.9 ± 0.7</td>
</tr>
<tr>
<td>20-&lt;30</td>
<td>12.0 ± 5.4</td>
<td>89.0 ± 20.0</td>
<td>8.2 ± 7.7</td>
<td>41.0 ± 15.5</td>
<td>17.6 ± 4.6</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>30-&lt;40</td>
<td>14.7 ± 6.5</td>
<td>76.2 ± 16.5</td>
<td>5.7 ± 6.0</td>
<td>63.9 ± 25.8</td>
<td>19.2 ± 5.3</td>
<td>2.0 ± 0.4</td>
</tr>
<tr>
<td>≥40</td>
<td>17.1 ± 7.2</td>
<td>71.0 ± 11.7</td>
<td>3.3 ± 1.5</td>
<td>66.8 ± 22.3</td>
<td>16.5 ± 3.3</td>
<td>2.4 ± 0.4</td>
</tr>
</tbody>
</table>

### Ipsilateral (n=12)

<table>
<thead>
<tr>
<th>$B_{ti}pO_2$</th>
<th>ICP</th>
<th>CPP</th>
<th>Glutamate</th>
<th>Glycerol</th>
<th>L/P ratio</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5</td>
<td>14.9 ± 7.8</td>
<td>65.5 ± 12.0</td>
<td>14.8 ± 11.4</td>
<td>80.4 ± 35.8</td>
<td>22.8 ± 4.3</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>5-&lt;10</td>
<td>17.1 ± 6.8</td>
<td>68.8 ± 11.4</td>
<td>9.3 ± 8.3</td>
<td>75.7 ± 45.8</td>
<td>23.2 ± 2.8</td>
<td>1.5 ± 0.8</td>
</tr>
<tr>
<td>10-&lt;20</td>
<td>15.6 ± 6.3</td>
<td>78.2 ± 13.5</td>
<td>6.7 ± 7.1</td>
<td>87.4 ± 58.3</td>
<td>24.1 ± 2.9</td>
<td>1.6 ± 0.7</td>
</tr>
<tr>
<td>20-&lt;30</td>
<td>14.9 ± 5.6</td>
<td>82.1 ± 13.8</td>
<td>8.2 ± 6.8</td>
<td>92.1 ± 67.3</td>
<td>24.4 ± 4.0</td>
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<td>30-&lt;40</td>
<td>15.8 ± 6.2</td>
<td>79.0 ± 14.4</td>
<td>5.5 ± 5.1</td>
<td>100.1 ± 53.1</td>
<td>25.0 ± 4.5</td>
<td>1.7 ± 0.8</td>
</tr>
<tr>
<td>≥40</td>
<td>12.5 ± 6.1</td>
<td>80.6 ± 13.3</td>
<td>6.7 ± 5.5</td>
<td>74.2 ± 55.7</td>
<td>22.5 ± 5.5</td>
<td>2.1 ± 0.4</td>
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</table>

### Diffuse injury (n=5)

<table>
<thead>
<tr>
<th>$B_{ti}pO_2$</th>
<th>ICP</th>
<th>CPP</th>
<th>Glutamate</th>
<th>Glycerol</th>
<th>L/P ratio</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5</td>
<td>6.6 ± 4.7</td>
<td>82.6 ± 13.3</td>
<td>78.2 ± 73.9</td>
<td>65.8 ± 32.2</td>
<td>24.5 ± 11.2</td>
<td>2.3 ± 1.0</td>
</tr>
<tr>
<td>5-&lt;10</td>
<td>11.8 ± 5.9</td>
<td>75.6 ± 11.6</td>
<td>20.0 ± 27.4</td>
<td>44.1 ± 21.7</td>
<td>18.1 ± 5.4</td>
<td>2.2 ± 0.7</td>
</tr>
<tr>
<td>10-&lt;20</td>
<td>11.8 ± 5.1</td>
<td>74.0 ± 10.1</td>
<td>17.0 ± 21.7</td>
<td>62.9 ± 35.6</td>
<td>26.5 ± 8.1</td>
<td>1.8 ± 0.6</td>
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<td>20-&lt;30</td>
<td>12.5 ± 4.3</td>
<td>74.0 ± 10.6</td>
<td>19.2 ± 19.6</td>
<td>54.3 ± 30.3</td>
<td>26.7 ± 10.0</td>
<td>1.9 ± 0.8</td>
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<tr>
<td>30-&lt;40</td>
<td>13.0 ± 4.2</td>
<td>77.4 ± 25.4</td>
<td>28.7 ± 25.4</td>
<td>62.2 ± 44.5</td>
<td>23.8 ± 6.2</td>
<td>2.2 ± 1.0</td>
</tr>
<tr>
<td>≥40</td>
<td>15.0 ± 4.6</td>
<td>72.8 ± 12.4</td>
<td>45.1 ± 21.8</td>
<td>67.1 ± 29.0</td>
<td>26.9 ± 7.4</td>
<td>2.5 ± 1.0</td>
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</table>
Figure 21. Focal injury and contralateral probe location

ICP (A), CPP (B), Glutamate (C), Glycerol (D), L/P ratio (E) and Glucose (F) at different BtipO2 levels in TBI patients with focal injury and probe (MD and NV) placement on the contralateral side from the injury (non-injured hemisphere). Bold line on Y-axis shows tentative normal MD values based on Reinstrup et al. (2000) and Schulz et al. (2000). All values are expressed as Mean ± SD.
ICP, CPP and $B_{tip}O_2$ – Table 7 and Figure 22A show the ICP corresponding to different $B_{tip}O_2$ levels in patients with a focal injury and probe placement in the ipsilateral side. The lowest mean ICP (12.5±6.1mmHg) was seen at the highest $B_{tip}O_2$ levels (≥40mmHg), but the differences were not statistically significant. Mean ICP was within the normal levels, between 12 and 17 mmHg for all levels of $B_{tip}O_2$.

The mean CPP values with $B_{tip}O_2$ levels below 10mmHg were significantly (p<0.05) lower than mean CPP at $B_{tip}O_2 > 10$ mmHg (Table 7 and Figure 22B). When the $B_{tip}O_2$ levels were between 5-10 mmHg, the mean CPP was 68.8±11.4 mmHg. CPP decreased even more (65.5±12.0 mmHg) at the $B_{tip}O_2$ level below 5mmHg. At $B_{tip}O_2$ levels above 10mmHg the CPP levels were stable and somewhat high (mean range 78-82 mmHg).

$MD$-glutamate and $B_{tip}O_2$ – The mean $MD$-glutamate concentration ranged between 5-8 µmol/L at $B_{tip}O_2$ above 10mmHg. A slight but not significant increase in $MD$-glutamate concentration was observed at levels of $B_{tip}O_2 < 10$ mmHg (Table 7 and Figure 22C). A drop of $B_{tip}O_2$ below 5 mmHg resulted in a significant increase (p<0.05) of $MD$-glutamate concentration (14.8±11.4 µmol/L).

The $MD$-glycerol and $MD$L/P ratio – The mean range of $MD$-glycerol was higher in this group ranging between 75.7±45.8 and 100.1±53.1 at the different levels of $B_{tip}O_2$. No correlations with the $B_{tip}O_2$ levels were observed. The mean range of the $MD$L/P ratio were between 22.5±5.5 and 25.0±4.5, thus somewhat higher than in the previous group (Table 7 and Figure 22 D-E).

$MD$-glucose - Mean $MD$-glucose levels were 2.1±0.4mmol/L at the highest $B_{tip}O_2$ pressure and had a stepwise decreasing trend to 1.5±0.5mmol/L at $B_{tip}O_2$ levels 0-5 mm Hg (Table 7 and Figure 22F). This difference did not reach statistical significance.
Figure 22. Focal injury and ipsilateral probe location
ICP (A), CPP (B), Glutamate (C), Glycerol (D), L/P ratio (E) and Glucose (F) at different BtcpO2 levels in TBI patients with focal injury and probe (MD and NV) placement on the ipsilateral side from the injury (injured hemisphere). Bold line on Y-axis shows tentative normal MD values based on Reinstrup et al. (2000) and Schulz et al. (2000). All values are expressed as Mean ± SD.
Diffuse axonal injury

*ICP, CPP and $B_{tp}O_2$* - In patients with diffuse axonal injury, mean ICP ranged from 11 to 15mmHg when $B_{tp}O_2$ levels were >5mmHg (Table 7 and Figure 23A). At the level of $B_{tp}O_2$ below 5mmHg the mean ICP was even lower (6.6±4.7mmHg) but not statistically significant. At all $B_{tp}O_2$ levels CPP remained within normal levels ranging from 72 to 82 mmHg (Table 7 and Figure 23B). No correlation was found between CPP and $B_{tp}O_2$ levels.

*MD-glutamate and $B_{tp}O_2$* – MD-glutamate was abnormally high in all patients within this group (Figure 23C). It was significantly higher (78.2±73.9 µmol/L; p<0.05) when $B_{tp}O_2$ level was less than 5mmHg (compared to $B_{tp}O_2$ ≥5 mmHg) (Table 7 and Figure 23C). At $B_{tp}O_2$ levels of ≥5mmHg the mean MD-glutamate concentrations had no significant correlation with $B_{tp}O_2$ and varied between 16.9 to 45.1 µmol/L.

*MD-glycerol and $B_{tp}O_2$* - Mean MD-glycerol concentration ranged between 44.1 to 67.1µmol/L and had no significant correlation between different $B_{tp}O_2$ levels (Table 7 and Figure 23D).

*MD-L/P ratio and $B_{tp}O_2$* – Table 7 and Figure 23E presents the MD-L/P ratio correlation to different $B_{tp}O_2$ levels. The mean MD-L/P ratio values ranged between 18.1 and 26.9 and had no significant correlation with $B_{tp}O_2$.

*MD-glucose and $B_{tp}O_2$* – Table 7 and Figure 23F shows the MD-glucose concentrations in correlation to different $B_{tp}O_2$ levels. MD-glucose remained normal at all $B_{tp}O_2$ levels and varied between 1.8 and 2.5mmol. Differences in MD-glucose levels did not reach statistical significance.

Overall results of TBI patients

In summary, very low $B_{tp}O_2$ levels < 5 mmHg were associated with different response patterns for biomarkers of energy metabolism (MD-glucose, MD-L/P ratio) and cellular distress (MD-glutamate, MD-glycerol) depending on the clinical condition and MD catheter location.
Figure 23. **Diffuse injury and probe location on the right side**

ICP (A), CPP (B), Glutamate (C), Glycerol (D), L/P ratio (E) and Glucose (F) at different BtipO2 levels. TBI patients with diffuse axonal injury and probe (MD and NV) placements in the right frontal hemisphere. Bold line on Y-axis shows tentative normal MD values based on Reinstrup et al. (2000) and Schulz et al. (2000). All values are expressed as Mean ± SD.
Discussion

The performance of the brain tissue oxygen probes *in-vitro* (Paper I)

There are some clinical and experimental studies regarding the reliability of local brain tissue $pO_2$ sensors showing accurate, safe and stable measurements.\(^{35,44,67,72,86,167,180,181}\) However, there are only a few in vitro studies for electrochemical LX sensors\(^{35,67}\) and no studies for fiberoptic NV sensors. In clinical practice it is crucial to accurately differentiate ischemic (5-15 mmHg) from normal (20-50 mmHg) $pO_2$ values\(^{156,168,180}\) and therefore there is a need for in vitro validation studies of the monitoring devices.

To determine $pO_2$ system accuracy, sensitivity drift, response time and response to temperature changes, we used the same experimental setup for both sensors (LX and NV), similar to previous studies.\(^{35,67}\) To avoid technical errors: high precision $O_2$ gases were used, temperature was controlled and the $pO_2$ value in the tonometer solution was calculated depending on atmospheric pressure.

**Accuracy**

Our tests confirmed that both LX and NV oxygen probes were highly accurate in measuring the oxygen partial pressure at different clinically relevant oxygen concentrations. In all gas concentrations, the LX sensors mean value were the closest (0.1-0.42 mmHg) to the reference value, though with relatively high standard deviations (0.71-1.33 mmHg). On the other hand, NV probes read slightly higher mean values (0.76-1.6 mmHg), not significantly different from LX, but with higher precision, i.e. less standard deviations (0.14-0.77 mmHg). We conclude that both sensors, even though based on different technologies, read partial oxygen pressure ranging from low to high concentration satisfactorily good and both systems are clinically eligible. The results from the LX sensors are comparable with recent data from Hoelper et al.\(^{67}\) To our knowledge, there is no previous independent report using NV sensors.

When the measured values were compared with the calculated oxygen values in low oxygen concentrations (5 and 10 mmHg), our data for the
electrochemical LX probes showed a difference between 2.1-5.3% which should be compared with previous data from the study by Dings et al.\textsuperscript{35} where the difference was between -4.5% and 9.0%. For the NV probes in our study the difference from calculated values ranged between 4.0% to 10.2%. Neurotrend\textsuperscript{8}(NT), another fiberoptic oxygen sensor system, showed differences between 4.8% – 25.87% in the study by Dings et al.\textsuperscript{35} However, the experimental setup was quite different between ours and their study. For example, Dings et al. evaluated the sensors in a $pO_2$ of 0 and 42.7 mmHg but without using a buffer solution and not correcting $pO_2$ for local barometric pressure.\textsuperscript{35}

**Response time**

The $pO_2$ sensor response time is an important clinical factor. We analysed the response time to reach the $pO_2$ level from low (10 mmHg) to high (40 mmHg) oxygen concentration. The experimental setup was similar to the study reported by Hoelpner et al.\textsuperscript{67}, but different oxygen concentrations were used (7.13 mmHg to 57.03 mmHg) in their study. In the low to high oxygen response test, the LX sensors (in our study) required 78.2±21 seconds compared to 129±27 seconds in the study by Hoelpner et al.\textsuperscript{67} The fiberoptic sensors response time were practically the same for NV in our study (56.2±22 seconds) and NT used in the other study (55±19 seconds).\textsuperscript{67} When going from high to low oxygen concentration in our study, both LX and NV sensors needed longer response time, LX sensors 215±63 seconds and NV 131±42 seconds. In Hoelpler et al.\textsuperscript{67}, LX needed 174±26 seconds and NT 98±39 seconds.

The results from the two studies can not be compared directly due to different oxygen concentrations used, but the results are relatively consistent and illustrate the magnitude of the response time needed for the different systems, that the response time is shorter for NV and NT (fiberoptic systems) compared to LX (electrochemical system) and that the response time is longer from high to low oxygen concentration for all systems. It is obvious that the response time is good enough for the clinical situation for all systems.

**Long-term drift**

Of major clinical importance is the question weather there is a drift in sensor accuracy over time. In clinical practice, an observation period for $B_0pO_2$ of 7-10 days is to be expected. In our test of long-term drift we measured the accuracy for 10 days under stable conditions. We found that both LX and NV catheters performed well during this period, with a mean $pO_2$ difference from the calculated value of less than 1 mmHg, which is supported by the
results from Hoelper et al.\textsuperscript{67} who did a 5 day long-term drift test in $\sim$20 mm Hg oxygen concentration. Although, the in vitro long term drift tests showed stable levels, the stability after long term use in patient needs to be assessed.

Response to temperature changes
In clinical studies using the LX system, Stocchietti et al.\textsuperscript{168} observed brain tissue $pO_2$ changes related to changes in brain temperature. We therefore wanted to study the influence of temperature in the range between 37.0 and 40.0°C in two different oxygen levels. The results showed that there was no temperature effect on NV probes. With the LX system an increased standard deviation was observed with increasing temperature. In the clinical situation, this must be considered when the brain tissue oxygen value approaches the assumed critical threshold level.

Animal brain death model (Paper II)
In Paper II the objective was to create a reproducible standardized and clinically relevant large animal BD model. To mimic the clinical situation a more gradual and prolonged increase of intracranial volume and ICP were applied compared to previous models and the experiments were not finished until CPP had been below zero mmHg for at least 1 h, i.e. that the organs are exposed to influences both before and after complete cessation of cerebral blood flow. It was not found feasible to extend the experiment further for logistic reasons even if that would have mimicked the clinical situation even more. At this stage of developing and validating the basic model the animals were not exposed to e.g. hypotensive events and/or infections but this may be added in future studies in order to imitate the clinical setting further. The pig was selected due to its brain structure similarities to human brain and the size of the brain which allows the use of same neuromonitoring equipment as utilized in neurointensive care.

Porcine BD models have been widely used in transplant surgery studies. Ryan et al., Lyons et al. and Mclean et al. used sudden inflation of subdural balloon for BD induction.\textsuperscript{93,105,147} BD was confirmed with high ICP, low cerebral blood flow (CBF) or lack of electroencephalogram (EEG) activity.\textsuperscript{93,105,147} In order to establish a more clinically relevant model, Barklin et al. performed a study with a lengthening of the induction phase to 60 min with gradual epidural balloon inflation up to 15 ml.\textsuperscript{8} Furthermore, they suggested that prolonged BD process might give the necessary preconditions to trigger a systemic inflammatory response that might contribute to organ dysfunction.\textsuperscript{8} Compared to the clinical situation we
considered that 60 minutes was still a relatively short time. We therefore wanted to prolong the BD induction phase up to 200 minutes by stepwise increase of intracranial volume, followed by a 30 minutes observation period. We found it desirable to capture the pathophysiological process more in detail using neuromonitoring devices measuring ICP, CPP and BtipO₂, and to confirm BD by microsphere injections.

Analysis of the monitoring results showed a classical intracranial pressure-volume relationship (Figure 12). Furthermore, IC decreased gradually when ICP increased (Figure 12), which reflects a decreasing ability to compensate for added intracranial volume. At the end of the experiment IC was close to 0 ml/mmHg, indicating completely exhausted compensatory mechanisms. Looking at the pressure-volume curve in detail, we found that from 5 to 7 ml added volume, the ICP did not increase as much as earlier. This finding could be explained by that after 5 ml added volume the previously stable MAP started to decrease (Figure 14) which probably resulted in a decrease of cerebral blood volume and thereby an increased ability to compensate for added volume. This suggestion was supported by the results of the IC measurements.

The described so-called ‘Cushing response’ (CR) with arterial blood pressure increase, bradycardia and respiratory irregularities was demonstrated in another experimental BD study. However, in our study in most cases we observed the opposite, i.e. a decrease of the mean arterial blood pressure and tachycardia. The reason for this difference is unclear, but it is well known from the clinical situation that a distinct CR never is observed in many patients developing BD. The CR is usually followed by hypotension and tachycardia which probably was what we observed.

Regarding the CPP, we saw a gradual decrease by added epidural volume (Figure 13). Initially there were only small changes in BtipO₂ until 3-4 ml was injected (Figure 15). At this time the ICP was between 30 and 45 mmHg, resulting in a CPP of around 40 mmHg. From this point on there was a linear decrease in BtipO₂. Thus, CPP around 40 mmHg seems to be a threshold for the brain tissue oxygenation in the pig. From 7 ml of added volume there was zero perfusion pressure and but the BtipO₂ remained between 5 and 10 mmHg. Our previous study showed that there is a longer response time of BtipO₂ required to assess low oxygen levels when declining from higher oxygen tension values. At the end of the experiment (9-10 ml of volume injection) BtipO₂ reached almost 0 mmHg in all cases (1.1±0.23 mmHg ± SD) suggesting total absence of oxygen in the brain tissue, i.e. BD, even if the final BtipO₂ was not exactly 0 mmHg. Our previous study, using standardized oxygen conditions, showed that the NV sensors read slightly higher values (0.76-1.6 mmHg) than the actual values, suggesting that the near zero values could be interpreted as 0 mmHg.

For definite confirmation of BD, samples from the brain tissue were taken to prove the absence of micro-spheres in the cerebral vessels, which where
injected into arterial blood stream after BD was suggested by negative CPP and near zero BtipO2. This technique has been previously used for studies in organ capillary blood flow. The results confirms BD showing a negligible number of micro-spheres in the intracranial vessels compared to extensive amount found in the perfused extra cranial control tissues. We believe that the current model may be used for explorative studies of brain injury mechanisms and for evaluating new neuromonitoring devices.

**Threshold levels of cerebral ischemia in an animal model (Paper III)**

Recent studies report that BtipO2 could be a reasonable substitute for measurements of regional cerebral blood flow and BtipO2 guided treatment protocols may improve TBI patient outcome. Some investigators report a correlation between patient outcome and the duration of BtipO2 below different thresholds in severe TBI. However, these studies cannot be directly compared due to the wide spread of threshold levels used. Of particular interest is to detect and determine the exact critical BtipO2 threshold level to be able to avoid secondary brain injury using BtipO2 guided management protocols in TBI patients. The aim of the present study was to identify threshold levels of cerebral ischemia in a standardized situation of elevated ICP in the pig. The brain interstitial microdialysis (L/P ratio >30) is regarded to be a specific and sensitive marker of cerebral ischemia. Therefore, a correlation analysis of cerebral microdialysate L/P ratio and BtipO2 was used to define a critical cerebral oxygenation threshold level of cerebral ischemia.

The main findings in the present study were a sequence of changes in CPP, BtipO2 and MD levels during the elevation of intracranial volume in the pig resulting in ICP changes comparable to those seen in TBI patients. The ICP elevation caused a profound change in CPP and BtipO2. When mean CPP moved from 50 to 40 mmHg, there was a drop in mean BtipO2 from 20 to 15 mmHg, but the mean L/P ratio remained normal with minimal changes. When mean CPP fell from 40 to 27 mmHg, the mean BtipO2 decreased from 15 to 8 mmHg and the mean L/P ratio increased from 21 to 44 (Table 3 and 5). These observations were supported by correlation studies revealing a critical ischemia level for BtipO2 of around 10 mm Hg and for CPP around 30 mmHg (Figures 19 and 20).

Similar results have been reported using polarographic BtipO2 monitoring technique (Clark-type electrode) where BtipO2 levels below 10 mmHg indicated critical brain ischemia. When BtipO2 was correlated to global cerebral oxygenation i.e. jugular bulb oxygenation, BtipO2 values below 8-10 mmHg was considered critical. BtipO2 <10 mmHg was also found to be critical according to L/P ratio levels. This finding was
supported by Hlatky et al. who observed an increase of the L/P ratio when 
$B_ipO_2$ was below 10 mmHg.

Regarding the correlation between CPP and the L/P ratio, reports from 
human studies where MD probes were inserted in the pericontusional brain 
tissue demonstrated an increase of the L/P ratio when CPP decreased below 
50 mmHg. However, in another study an increase of the L/P ratio (>40) 
was observed despite CPP values >60 mmHg when MD probes were placed 
in pericontusional brain tissue. A recent report where MD probes were 
inserted into brain cortex of the pig suggested a threshold level of CPP at 40 
mmHg based on significant increase of L/P at this level. These different 
results show that MD and $B_ipO_2$ levels can vary significantly depending on 
the placement of the probe in peri- or contusional areas, and that the local 
values do not necessarily reflect the global brain situation. Thus, 
interpretation of probe data needs to take into account the placement of the 
probe. In our present study, we used an injury model affecting the entire 
brain. The overall results from paper III are depicted in table 5.

Clinical study (Paper IV)

In paper IV the measurements of $B_ipO_2$ and cerebral metabolism was used in 
patients with focal and diffuse TBI to evaluate these monitoring techniques 
and to get an impression of its clinical value. We found that there were 
different response patterns of $B_ipO_2$ and cerebral metabolism depending on 
the injury type and probe localization which will be discussed below.

Brain tissue oxygenation and intracranial dynamics

In patients with focal injury and probe placement in the ipsilateral 
hemisphere, periods with $B_ipO_2 < 10$ mmHg occurred at significantly lower 
CPP but there was no relation to ICP (Figure 22 and Table 7). In patients 
with diffuse injury periods with $B_ipO_2 < 5$ mmHg tended to be associated 
with lower ICP but there was no relation to CPP (Figure 23 and Table 7). 
Thus, the type of injury and probe placement appear to be factors influencing 
the relation between $B_ipO_2$ and ICP and CPP, respectively.

Brain tissue oxygenation and cerebral metabolism

Lactate to pyruvate ratio

The L/P ratio is a balance between lactate and pyruvate reflecting the state of 
cerebral oxidative metabolism and is known as a sensitive marker of cerebral
ischemia. MD-LP ratio was recently reported to be an independent positive predictor of poor outcome in a large cohort of TBI patients. Normal MD-LP ratio values have been reported previously as approximately 15-20. Prior studies have used different L/P ratio threshold levels of cerebral ischemia ranging from 25 to 40. Results from a study of focal TBI revealed that the L/P ratio values are higher in the tissue “at-risk” (ipsilateral side) compared to “normal” tissue (contralateral side). Similarly, in the current study L/P ratio seemed to be higher at all BtipO2 levels in TBI patients with focal injury and when probes were placed in the ipsilateral side and also in DAI patients. However, a decrease of BtipO2 to very low levels (<5mmHg) resulted in a significant increase of L/P ratio only in patients with focal brain injury and when probes were placed in contralateral side but not in the ipsilateral side (Figure 21E and 22E). Thus, a somewhat provocative assumption appeared that in the case of injured tissue in which the L/P ratio is already elevated, very low BtipO2 levels do not lead to even higher L/P ratios. In relatively uninjured tissue (contralateral side) with a normal L/P ratio, however, very low BtipO2 levels do lead to increases in the L/P ratio. Further studies with increased number of patients are needed to support this hypothesis.

**Glutamate**

MD-glutamate basal concentration in humans ranges between 5 to 15 µmol/L. A clinical BtipO2 TBI study identified MD-glutamate as the most sensitive and early marker of cerebral ischemia. In that study a significant increase of MD-glutamate was observed at BtipO2 level below 10mmHg. A positive correlation has been demonstrated in TBI patients between high levels of MD-glutamate and increased ICP and poor outcome. Increased MD-glutamate levels have also been reported in TBI patients mostly with CPP below 70 mmHg. In that study the authors also found patients with high MD-glutamate and CPP above 70 mmHg. In addition, they did not specify the location of the probe and the type of the injury which could explain potentially different pathophysiological processes. The effect of microdialysis catheter location has also been studied in TBI patients with focal injury, and higher MD-glutamate concentrations were found in the most injured brain hemisphere.

In the present study in patients with focal TBI, we observed that MD-glutamate increased significantly irrespective of the placement of the probe when BtipO2 decreased to extremely low (<5 mmHg) levels. DAI patients had higher MD-glutamate levels than patients with a focal injury, but even in this group a decrease of BtipO2 to <5 mmHg was associated with significantly higher glutamate levels (Figure 23C), illustrating the energy dependence of the astrocytic glutamate-glutamine cycle capacity to clear interstitial glutamate. The overall high MD-glutamate concentration
observed in diffuse injury could be explained by massive neuronal cell damage occurring in DAI and most likely originating from intracellular stores that leak into the extra-cellular space as the neuronal membrane loses its structural integrity.79

Glycerol

Glycerol is one of the end products in cell membrane phospholipid degradation. It can be used as a marker of phospholipid degradation in cerebral ischemia.63 Experimental and clinical studies have shown significant increases of MD-glycerol during cerebral ischemia.46,63,101 Normal cerebral MD-glycerol levels have been reported previously from patients during wakefulness, anesthesia and neurosurgical procedures.140 Clausen et al. reported increased MD-glycerol levels when BtipO2 decreased below 10 mmHg.27 We have recently shown in a pre-clinical study that the interstitial MD-glycerol concentration increases when CPP or BtipO2 decrease.137 In the present study under similar conditions (focal TBI on the contralateral side), periods with low CPP and BtipO2 significantly correlated with increased MD-glycerol levels. However, we did not see any correlation of BtipO2 and MD-glycerol levels in the more injured hemisphere (ipsi) or in DAI patients. These results are similar to those for L/P ratio in that relatively uninjured tissue seemed to be more sensitive to decreased BtipO2 than injured tissue. It is unclear which factors are responsible for the heterogeneity of the MD-glycerol levels between hemispheres in focal TBI and between focal TBI and DAI patients. Based on recent validation data implicating MD-glycerol as a biomarker of oxidative stress we submit that this may be an important additional factor to consider.26

Clinical aspects

In a clinical situation BtipO2 may be influenced by parameters such as cerebral metabolism, cerebral blood flow, oxygen diffusion, sedation, hyperventilation, low inspired oxygen, ICP- and CPP changes, age, trauma severity, and other traumatic changes in the cellular environment. It is obvious that the interpretation of BtipO2 needs to be carefully considered, preferably together with other parameters such as MD and cerebral blood flow assessments. The thresholds may vary in different patients. However, in all three patient groups analyzed very low BtipO2 levels < 5 mmHg were accompanied by increases in either the MD-L/P ratio, MD-glutamate or MD-glycerol, indicating an increased vulnerability of the brain at this level of oxygen despite fairly normal levels of ICP and CPP. Thus, BtipO2 monitoring adds valuable information about the brain vulnerability not disclosed by routine ICP and CPP surveillance. However, very low BtipO2 levels < 5 mmHg were associated with different response patterns for
biomarkers of energy metabolism (MD-glucose, MD-L/P ratio) and cellular distress (MD-glutamate, MD-glycerol) depending on the clinical condition, NV probe and MD catheter location suggesting that $BipO_2$ monitoring is a complement to MD monitoring rather than an alternative.
Conclusions

- High accuracy, reasonable response time, low response to temperature and long-term stability of brain tissue oxygen sensors in different oxygen tension levels were shown *in-vitro*. (Paper I)

- A standardized brain death model was developed in pigs simulating the clinical development of brain death in humans with a classical pressure-volume response and systemic cardiovascular reactions. Brain death was convincingly confirmed. We believe that this model is very useful in studies of ICP related brain injury mechanisms. (Paper II)

- Brain tissue oxygenation below 10 mmHg and cerebral perfusion pressure below 30 mmHg was associated with an increase of the cerebral microdialysate L/P ratio >30, thus $B_{tipO2} < 10$ mmHg seems to be a threshold level for ischemia in the pig. (Paper III)

- There are different patterns of changes in $B_{tipO2}$ and cerebral microdialysis biomarkers in focal and diffuse TBI patients. The placement of the probe in focal injury did also influence the results. However, despite fairly normal levels of ICP and CPP in all patient groups, increased cerebral MD levels of glutamate, glycerol or the lactate/pyruvate ratio were observed at $B_{tipO2} < 5$ mmHg, indicating increased vulnerability of the brain at this critical level of tissue oxygenation in TBI patients. (Paper IV)
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