Aspects of Induced Hypothermia following Cardiopulmonary Resuscitation

Cerebral and Cardiovascular Effects

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Abstract

Hypothermia treatment with cooling to a body temperature of 32-34°C has been shown to be an effective way of improving neurological outcome and survival in unconscious patients successfully resuscitated after cardiac arrest (CA). The method is used clinically but there are still many questions on the biological mechanisms and on how the treatment is best performed. This thesis focuses on cerebral and haemodynamic effects of hypothermia and rewarming.

A porcine model of CA was used. To shorten time to reach target temperature, induction of hypothermia, by means of infusion of 4°C cold fluid, was started already during ongoing cardiopulmonary resuscitation. The temperature was satisfactorily reduced without obvious haemodynamic disturbances.

Cerebral effects of hypothermia and rewarming were studied. Microdialysis monitoring showed signs of cerebral energy failure (increased lactate/pyruvate-ratio) and excitotoxicity (increased glutamate) immediately after CA. There was a risk of secondary energy failure that was reduced by hypothermia. Intracranial pressure (ICP) increased gradually after CA irrespectively of if hypothermia was used or not. There were no indications of increasing cerebral disturbances during rewarming.

Haemodynamic effects of hypothermia treatment and rewarming were examined in a study of patients successfully resuscitated after CA. Hypothermia was induced by means of cold intravenous infusion. No negative effects on the cardiovascular system were revealed. There were indications of decreased intravascular volume in spite of a positive fluid balance.

Cerebral microdialysis and ICP recording were performed in four patients. All patients had signs of energy failure and excitotoxicity following CA. ICP was only exceptionally above 20 mmHg. In contrast to the experimental study indications of increasing ischemia were seen during rewarming. Glycerol had a biphasic pattern, perhaps due to an overspill of metabolites from the general circulation. As most patients become extensively anti-coagulated following CA, intracranial monitoring is not suitable to be used in routine care.

Keywords: Cardiac Arrest, Cardiopulmonary Resuscitation, Hypothermia, Rewarming, Brain Ischaemia

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urn:nbn:se:uu:diva-9562 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-9562)
”Hemulen körde under hög jämmer ner sin nos i den våta sanden. Nu går det för långt! Sa han. Varför kan inte en stack- 
ars oskyldig botanist få levå sitt liv i frid och ro!
Livet är inte fridfullt, sa Snusmumriken förtjust.”

Tove Jansson ur ”Trollkarlen hatt”
List of papers

This thesis is based on the following papers which will be referred to in the text by their Roman numerals:

I    Induction of mild hypothermia with infusion of cold (4°C) fluid during ongoing CPR
      Nordmark J, Rubertsson S
      Resuscitation (2005), 66(3), 357-365

II   Cerebral energy failure following cardiac arrest - Hypothermia treatment reduces secondary lactate/pyruvate-ratio increase
      Nordmark J, Enblad P, Rubertsson S
      Resuscitation. Accepted for publication.

III  Intracerebral monitoring in comatose patients treated with hypothermia after cardiac arrest
      Nordmark J, Rubertsson S, Mörterberg E, Nilsson P, Enblad P
      Acta Anaesthesiologica Scandinavia. Accepted for publication.

IV   Decreased intravascular volume following cardiac arrest – Patient observations with echocardiography during hypothermia and rewarming
      Nordmark J, Johansson J, Sandberg D, Huzevka T, Covaciuc L, Mörterberg E, Rubertsson S
      Submitted.
Abbreviations

AMPA \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionate
CA Cardiac arrest
CBF Cerebral blood flow
CO Cardiac Output
CPR Cardiopulmonary resuscitation
CSF Cerebrospinal fluid
CVP Central venous pressure
ERK Extracellular signal-regulated kinases
Et-CO\(_2\) End-tidal carbon dioxide
ICP Intracranial pressure
L/P-ratio Lactate/pyruvate-ratio
LVEF Left ventricular ejection fraction
MAP Mean arterial pressure
MPT Mitochondrial permeability transition pore
NMDA N-methyl-D-aspartate
NO Nitric oxide
PCI Percutaneous Cardiac Intervention
ROS Radical oxygen species
ROSC Restoration of spontaneous circulation
SAPK Stress-activated protein kinases
ScvO\(_2\) Central venous oxygen saturation
SD Standard Deviation
SjvO\(_2\) Jugular bulb oxygen saturation
TTE Transthoracic echocardiography
VF Ventricular fibrillation
Introduction

Cardiac arrest (CA) results in a sudden cessation of blood flow generating an ischemic situation in the entire organism. The primary goal of cardiopulmonary resuscitation (CPR) is to promote blood flow and oxygen delivery to the heart and the brain. Even if circulation is restored, mortality and morbidity after CPR remains high (1-6).

In 1991 Cummins et al (7) defined the “Chain-of-survival” concept;

*Early access – Early CPR – Early Defibrillation – Early Advanced Cardiac Life Support*

However, even if spontaneous restoration of circulation (ROSC) is attained, the ischemic event has affected the entire body. The pathological state thus created has been defined as “post-resuscitation disease” by Nenkovsky (8). Recently the term “post-cardiac arrest syndrome” has been suggested as a more appropriate definition (9). The four key components of this syndrome are: (I) brain injury, (II) myocardial dysfunction, (III) systemic ischemia/reperfusion response and (IV) persistent precipitating pathology. The identification of this syndrome has led to the proposition of a fifth link to the “Chain-of survival”;

*Early Post-Resuscitation Care*

Survivors after CA and post-cardiac arrest syndrome present a wide variety of neurological deficiencies (3,4). Ischemic-anoxic encephalopathy is caused by three different phases of disturbed cerebral circulation during and following CA. Initially there is the period without circulation. Secondly, during resuscitation cerebral circulation is inadequate. Thirdly, after ROSC there is a period of haemodynamic instability combined with impaired cerebral autoregulation in addition with injury cascades, disrupted blood brain barrier and self-intoxication with extracerebral metabolites and blood dearrangements. To improve outcome after restoration of circulation it is of vital importance to shorten the time interval for the two first phases (i.e. to restore spontaneous circulation) and to halt secondary injuries after ROSC (i.e.post-resuscitation care). Several pharmacological and mechanical interventions have been used and evaluated for these purposes over the years.

During the 1950’s hypothermia treatment was used successfully after CA. Temperature range used was below 30 °C. Because of severe side effects, the method was abandoned. However, later on, animal studies showed beneficial results with considerable attenuation of negative side effects when mild (32-34°C) hypothermia was used. During the last years the method has
regained clinical popularity as a way of reducing mortality and neurological injuries after CA. The reason for using hypothermia has primary been to reduce cerebral injuries but the method might also help to protect other organs (such as the heart). This thesis focuses on hypothermia treatment and mainly on cerebral and haemodynamic effects of hypothermia and rewarming. Aspects of methods of inducing and maintaining hypothermia are also considered.
Background

Cerebral blood flow

The brain receives a blood flow of 50 ml/100 g/minute (15 % of cardiac output) during normal conditions. Cerebral autoregulation (through changes in cerebral vascular resistance) keeps cerebral blood flow relatively constant over a wide range of blood pressure changes.

The unique energy metabolism of the brain with limited intrinsic energy stores and dependence on aerobic glucose metabolism makes the brain vulnerable to reduced or ceased blood flow. Development of cerebral infarction is dependent on duration of ischemia as described by Heiss et al (10) and by intensity of ischemia. Different blood flow thresholds for ischemic changes in different animal species has been summarised by Hossmann (11) (Table 1).

Table 1. Cerebral flow thresholds for pathophysiological changes (Modified from Hossmann (11))

<table>
<thead>
<tr>
<th>Cerebral Blood Flow (ml/100ml/min)</th>
<th>Pathophysiological events</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>Protein synthesis and selective gene expression is inhibited</td>
</tr>
<tr>
<td>35</td>
<td>Increased lactate accumulation and glucose utilisation</td>
</tr>
<tr>
<td>26</td>
<td>Severe acidosis</td>
</tr>
<tr>
<td>23</td>
<td>Neurological dysfunction and suppression of EEG</td>
</tr>
<tr>
<td>5-18</td>
<td>Infarction, anoxic depolarisation and cessation of ion gradients</td>
</tr>
</tbody>
</table>
In humans, at a cerebral blood flow reduced to 20 ml/100 g/minute, cerebral functions cease and at a flow lesser than 10 - 15 ml/100 g/minute, cerebral tissue necrosis occurs (12,13).

Cerebral blood flow during CPR is insufficient. Using transit-time ultrasound flowmetry, Rubertsson et al have shown that open chest CPR generates better blood flow than closed chest CPR (14,15). Manual external chest compressions generate a flow of 10 - 40 % of normal (16). In an experimental study mechanical CPR (with LUCAS™ device (17)) comparably improved cerebral blood flow reaching about 65% of normal flow (18). The duration of the no-flow interval during CA influences the reflow during CPR. Prolonged no-flow leads to decreased reflow (19). This is probably due to increased blood viscosity, perivascular swelling and vasoconstriction.

After successful resuscitation following CA cerebral blood flow continues to be impaired. The no-reflow phenomenon is characterized by a lack of reperfusion immediately after ROSC (20). The severity of this phase is believed mainly to depend on the duration of the ischemic event (21). After the no-reflow period there is a brief hyperaemic (22-24) phase followed by a prolonged period of global and multifocal hypoperfusion (22,25). During the hypoperfusion period cerebral metabolic rate is decreased and global cerebral blood flow seems to be adequate for the metabolic demand (25). However there may still be regional circulatory deficits (26). In addition reperfusion accelerates injury processes (27,28) and cerebral auto-regulation is disturbed (29).

Cerebral biochemistry and cerebral cellular effects of ischemia

**Energy substrates**

Under physiologic conditions glucose can be metabolized anaerobically in astrocytes, producing lactate that is subsequently consumed aerobically by neurons (30). Cessation of blood flow interrupts the supply of glucose and oxygen to the brain. Tissue oxygen tension dramatically falls, causing arrest of mitochondrial respiration and ATP production by oxidative phosphorylation. Ischemia and increased metabolic demand activates glycolysis. Glucose and glycogen stores are limited and glucose levels are reduced (31-33) but residual glucose is anaerobically metabolized, both by astrocytes and by neurons to lactate. This results in acidosis and lactate accumulation accompanied with decreased levels of pyruvate (31,33,34). A decreased glucose level might be related to a decreased cerebral blood flow while an increase in lactate probably is more related to the cerebral metabolic rate of oxygen according to an experimental study by Frykholm et al (32). However, an
increased intracerebral lactate level alone is not necessarily the result of ischemia and hypoxemia but can be caused by a hypermetabolic condition (35,36). Increased lactate levels combined with a decrease in pyruvate levels are therefore considered to be a more specific marker of ischemia than lactate alone (34). In the absence of oxygen, lactate cannot be further metabolized leading to energy failure with altered redox state and NADH accumulation. Acidosis alters cell metabolism and mitochondrial membrane function (28,37,38).

**Glutamate**

Glutamate is the main fast neurotransmitter in the brain, acting on N-methyl-D-aspartate (NMDA)-; α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)-; and kainate - receptors. Presynaptic release of glutamate leads to receptor activation and opening of ion channels and thereby to cellular influx of sodium and calcium. Glutamate also acts on metabotropic (GluM)- receptors and thereby via G-protein coupled mechanisms modulates a variety of intracellular signal transduction events (39,40). The effects of glutamate are terminated via reuptake of glutamate into glial cells where it is converted to glutamine or lactate (38).

Ischemia leads to cellular depolarization with release of glutamate combined with inhibition of reuptake mechanisms (energy-dependent). The result of this is a large increase in extracellular glutamate concentration. Furthermore, there is a leakage of glutamate across disrupted cellular membranes and across the disrupted blood – brain barrier adding to the accumulation of extracellular glutamate (40). The increase of glutamate induces an uncontrolled activation of receptors and an intracellular influx of calcium. This, in turn, starts a number of destructive events and to stimulation of ATP-dependent processes, adding an extra metabolic demand. The glutamate-induced cascade of injuries is named excitotoxicity and was first described by Olney following observations of neuronal damage after application of glutamate to mouse brain slices (41) and of brain damage after oral intake of glutamate (42). Increase of extracellular glutamate levels have been observed in several studies of cerebral ischemia (43-45).

**Calcium**

Calcium concentration increases intracellular during primary ischemia as well as during reperfusion and secondary increase in mitochondrial calcium has been demonstrated several hours after the primary ischemic insult (46). Glutamate activation of AMPA- and NMDA–receptors causes an excessive influx of calcium. As calcium sequestration within the cell is dependent on ATP and/or oxygen there is a release of calcium from intracellular stores (mitochondria, endoplasmatic reticulum and calciosoms). In addition acidosis activates Ca$^{2+}$-permeable acid-sensing ion channels (ASIC) (37). All
these events add together to yield a dramatic increase of intracellular calcium (47).

The cytoplasmic calcium overload induces neuronal dysfunction and destruction and causes both immediate and secondary brain damage (48). Intracellular calcium overload can cause activation of various $\text{Ca}^{2+}$ dependent degradative enzymes which may contribute to cell death (49). Activation of endonucleases leads to DNA fragmentation. Proteases initiate cytoskeleton breakdown. Kinases, a family of signal enzymes, are activated and may contribute to ischemic neuronal damage (50). Kinases can be either extracellular signal-regulated kinases (ERKs) or stress-activated protein kinase (SAPK). Activation of ERKs are probably related to cell survival (51). In contrast, activation of SAPK is related to cellular stress and associated with apoptosis and degeneration (52,53). Phospholipases stimulate lysis of membrane phospholipids resulting in release of fatty acids e.g. arachidonic acid and glycerol. Cyclooxygenase and lipoxygenase metabolize arachidonic acid leading to a production of reactive oxygen species (ROS) (54). Calcium also activates other pathways of ROS-production via nitric oxide synthases (55) and xanthine oxidases (48).

In addition, intracellular increase of calcium causes intra-mitochondrial calcium concentration to increase. This, eventually, leads to activation of a mitochondrial permeability transition pore (MPT), resulting in additional efflux of calcium to the cytoplasm (28,38). The activation of MPT leads to swelling of the mitochondria due to osmosis and to cessation of ATP production.

**Radical oxygen species**

Oxygen free radicals are highly reactive molecules, considered to be important mediators of cerebral injury during reperfusion after cerebral ischemia (56). During normal conditions the electron flow in brain mitochondria produces a small amount of superoxide anion radicals ($\text{O}_2^{-}$) and hydrogen peroxide ($\text{H}_2\text{O}_2$) (57). They are then scavenged and detoxified in a balanced system. During reperfusion this system is disturbed as a result of overproduction of ROS, inactivation of detoxification mechanisms and consumption of antioxidants (55,58). The enhanced production of ROS is triggered by increase of intracellular calcium. ROS are directly involved in oxidative injury processes of lipids, proteins and nucleic acids. In addition evidence has been raised that ROS are also involved in redox signalling, targeting the mitochondria and DNA repair enzymes, thus leading to neuronal apoptosis (55).

**Inflammation**

The situation after cardiac arrest, has been described as a “sepsis-like syndrome” (59). After cerebral ischemia, especially during reperfusion, there is a marked release of pro-inflammatory mediators (such as tumour necrosis
factor-alpha, TNF-α and interleukin-1, IL-1) from astrocytes, microglia and endothelial cells (47,60). The increased levels stimulate accumulation of inflammatory cells. It is described, in humans, that there is an activation of leukocytes after cardiac arrest (61). The activated leukocytes may contribute to further cerebral damage by several mechanisms. Micro-vessels can be occluded, cytotoxic enzymes and cytokines are released and generation of ROS is stimulated (62).

Isoprostanes
The isoprostanes (or the eicosanoids) are a family of prostaglandin derivates synthesised from arachidonic acid (63). Increase of isoprostanes in plasma indicates oxidative injury. They are known biomarkers of oxidative stress (8-iso-PGF$_{2α}$) (64) and inflammatory response (15-keto-dihydro-PGF$_{2α}$) (65) and known to increase in plasma after cardiac arrest (66).

Cellular membrane breakdown
Cellular membrane phospholipids are degraded after cerebral ischemia as a result of glutamate-mediated activation of phospholipases and oxidative stress (67,68). This leads to accumulation of free fatty acids, lysospholipids and diacylglycerol (69). Diacylglycerol is further degraded into glycerol and arachidonic acid. Increased interstitial glycerol is a reliable signal of phospholipid degradation (70,71) and may also indicate cerebral ischemia (72).

The cellular membrane breakdown leads to increased release of calcium. ROS is produced by oxidation of arachidonic acid (54,73). These effects add together in promoting the circles leading to cellular death.

Cerebral cellular death
The mitochondria has a central role in cellular death after cerebral ischemia (74,75). After an ischemic insult the mitochondria will become both a source of and a target for increased intracellular calcium and the production of ROS leading to a double vicious circle. Ischemic necrotic death results from acute or delayed disturbed ionic homeostasis and osmotic cell lysis due to lack of mitochondrial ATP production. Damaged mitochondria release cytochrome C, an electron transport protein. This induces activation of caspase 3 leading to DNA fragmentation and apoptosis (55,74). Activation of kinases (SAPK) is another pathway to apoptosis (52,53). Cerebral ischemia may lead to either necrosis or apoptosis, depending on the severity of the ischemia. A more severe ischemia results in acute necrosis but a lesser degree of ischemia might lead to apoptosis as there is need for a certain degree of ATP-production for the apoptosis processes.
Cerebral oedema after cerebral ischemia

Intracranial Pressure (ICP)
Intracranial dynamics was first described in the late eighteenth century and early nineteenth century by Monro (76) and Kellie (77). Their observations gave birth to what is known as the Monro-Kellie doctrine stating that the intracranial space has a constant volume restricted by the rigid skull and that any expansivity has to be on expense of its original volumes (cerebral tissue, blood volume and cerebrospinal fluid – CSF). In case of intracranial expansivity there is initially no change of intracranial pressure as long as there is a possibility for compensation by reduction of CSF, but later in the course only a small increase of volume induces a marked increase in ICP.

Cerebral oedema after cardiac arrest
Several pathophysiological mechanisms tend to increase risk of intracranial oedema after CA. Lack of ATP leads to disturbed ionic homeostasis and cellular swelling, as discussed above. Blood-brain barrier alterations have been demonstrated after global ischemia (78,79). Impaired autoregulation and postischemic hyperperfusion may add to the risk of developing intracranial hypertension (29).

Limited knowledge exists about the occurrence of cerebral oedema after cardiac arrest and global ischemia. Cerebral oedema has been described in rats after cardiac arrest by means of wet-to-dry weight ratio and CSF pressure (80). In humans, cerebral oedema after cardiac arrest has been described using CT scan (81) and post-mortem histopathology (82). These studies suggest that high intracranial pressure is mainly a problem after cardiac arrest from non-cardiac origin. Increased ICP after cardiac arrest of different origins, has been demonstrated in humans, emerging after 24 hours in combination with hyperaemia (83).

Cerebral oxygen extraction
The cerebral venous blood drains into the internal jugular vein via the jugular bulb located at the skull base. By collecting and analysing blood gases from the jugular bulb, global cerebral oxygen extraction can be calculated. If haemoglobin concentration and arterial oxygen content are estimated to be relatively constant jugular bulb oxygen saturation (SjvO2) can be used as an estimation of cerebral oxygen extraction. Normal values of SjvO2 are considered to be in the range of 55 -75 %, although this range is still debated (84). A low SjvO2 indicates an ischemic situation with oxygen debt and is considered a bad prognostic sign (85). High levels, near-arterial, on the other hand, indicate low oxygen-extraction in the brain (86). Higher SjvO2 than oxygen-
content in mixed venous blood is related to poor neurological outcome as well as poor survival after cardiac arrest (87,88).

The myocardium after cardiac arrest

Post-ischemic left ventricular dysfunction was first described in 1975 (89) and in the 1980’s this phenomenon was described as “myocardial stunning”(90). This is defined as a reversible myocardial dysfunction which persists after myocardial reperfusion, in absence of an irreversible lesion, after restoration of normal or near-normal coronary artery flow. Initially this was described after infarction but myocardial dysfunction after resuscitation from cardiac arrest is a known to exist in animal experimental setting (91-93) as well as in humans (94-96) and this dysfunction is believed to be at least partly reversible (91,92,94,96). Cardiac output is reduced after CA, a decrease that is reversible. However, time to restoration of normal cardiac output is dependent on the duration of the cardiac arrest (92). In the experimental setting, swine ventricular function was shown to improve after 24 hours and to have returned to baseline values at 48 hours after CA (91). In humans, Laurent et al, have shown cardiac index values to be improved after 24 hours and almost uniformly returned to normal after 72 hours in a study on 73 patients (96). More sustained depression of ejection fraction with reversibility over the first month after CA has also been described (94).

The heart is one of the most energy demanding tissues of the body and ischemia during cardiac arrest damages the myocardial cells. Ischemic and reperfusion injuries to the myocardium bear much resemblance to cerebral traumatic mechanisms in the same situation.

During CPR coronary artery perfusion pressure is low with inadequate perfusion of the myocardium leading to cessation of oxidative phosphorylation causing decreased ATP concentration. Declining ATP concentration causes inhibition of cellular membrane ion pumps which increases intracellular calcium. Excessive intracellular influx of calcium mediates ischemic cardiac myocyte death (38,97). Another feature of ischemia contributing to cellular damage is the production of ROS (98).

No-reflow phenomenon is described following restoration of blood flow after myocardial ischemia (99). This phenomenon is probably associated with micro-vascular damage induced by ischemia together with capillary plugging of accumulating neutrophils.

After a period of myocardial ischemia, reperfusion may paradoxically cause increased cell mortality (100). Reperfusion is associated with a burst of ROS (98). Although a large portion of cell death occurs via necrosis there is evidence that apoptosis is also involved (101). Mitochondrial dysfunction probably plays a central role in both necrotic and apoptotic components of myocardial cell death (100,102). Mitochondrial proteins are susceptible to
ROS induced damage and the combined effects of ROS and increased intracellular calcium are believed to play a critical role in the transition from reversible to irreversible reperfusion injury. One of the critical events induced is the opening of the mitochondrial permeability transition pore (MPT) (102). The opening of the MPT interrupts oxidative phosphorylation and ATP production leading to disruption of metabolic and ionic homeostasis and activation of degradative proteins eventually resulting in necrotic cell death. The other consequence of MPT opening is cellular, osmotic swelling with rupture of the outer mitochondrial membrane, leading to release of proteins, including cytochrome c. These factors play an active role in apoptotic cell death (103).

Hypothermia treatment after cardiac arrest

Hypothermia is defined as a core temperature of \( \leq 35^\circ\text{C} \). It has traditionally been divided into five grades; mild (32-35°C), moderate (28-32°C), severe (20-28°C), profound (14-20°C) and deep (<14°C) (104). During the last years the clinical use of mild hypothermia as neuroprotection after cardiac arrest has increased worldwide.

Hypothermia as clinical treatment has ancient roots. It was recommended by Hippocrates to reduce haemorrhage in wounded patients (105) and was also used by the Romans and the ancient Egyptians (106,107). During the Napoleonic wars, in the early nineteenth century, General Baron Larrey, a surgeon in the French army observed that injured and hypothermic soldiers that were placed close to a fire survived to a lesser extent than those who remained hypothermic (108). In the 1930’s - 40’s several case reports on successful resuscitation of hypothermic drowning victims led to a growing interest for hypothermia as clinical treatment. During the 1950’s hypothermia was used in thoracic and neurosurgery for neuroprotection (109,110). Experimentally benefit was shown on dogs treated with moderate hypothermia during cerebral ischemia (111). In 1958 came the first scientific report on successful use of hypothermia after cardiac arrest in humans (112). During this period of years moderate to severe hypothermia was used (less than 30°C) and the therapy lost popularity because of unwanted side effects.

In the 1980’s and 1990’s the interest for hypothermia treatment as cerebral protection re-emerged. Experimental animal studies have shown that beneficial neurological effects could be obtained with mild hypothermia (32-35°C) with reduction of harmful, unwanted side-effects (113-119). This knowledge together with improved intensive care capabilities made it possible to perform clinical trials on patients that remained unconscious after successful resuscitation from cardiac arrest. Clinical studies performed in the 1990’s showed promising results of neurological outcome and survival when using mild hypothermia, compared to historical controls (120-123). The
success of these studies resulted in the initiation of two randomized controlled studies, published in 2002 (124,125). The first study, performed by Bernard and co-workers, included 77 patients with improved neurological outcome in the hypothermic group, but no difference in survival (124). The second study was carried out as a multi-centre study in Europe. It included 273 patients and showed both improved neurological outcome and reduced mortality in the hypothermic group (125). During the last years therapeutic hypothermia after successful resuscitation from cardiac arrest has become clinical practice (126) and is recommended by the European Resuscitation Council (127) as well as the American Heart Association (128).

Initially hypothermia treatment was merely used after cardiac arrest due to ventricular fibrillation (VF) (124,125). Animal experiments has proved usefulness of hypothermia after other types of cardiac arrest (e.g. asystolia and pulseless electrical activity – PEA) (129,130). It is now recommended to use hypothermia treatment following cardiac arrest due to VF/ventricular tachycardia and to consider it after asystolia or PEA (127,128,131,132).

Mechanisms of neuroprotection

Most evidence underlying the understanding of neuroprotective mechanisms of hypothermia comes from animal models. The beneficial effects are believed to comprise several factors (60). The riddle is not completely solved and there is still need for further research. It is also a problem that many of the experiments have been performed with hypothermia induced prior to the insult which is rarely the situation in clinical practice.

Cerebral metabolism

Traditionally, slowing of cerebral metabolism and thus reducing the brain’s oxygen and energy needs, has been the explanation to the beneficial effects of hypothermia (133). Cerebral metabolism is reduced by 5 - 7% for each degree Celsius reduction (114). At a body temperature of 20°C, oxygen demand is reduced to 18 % ± 2 of baseline at 37°C (134). Cooling during arrest has been shown to eliminate detrimental effects of prolonged cardiac arrest on metabolic rate (19). Using nuclear magnetic resonance spectroscopy, Chopp et al were able to show a more rapid return of adenylate intensities in hypothermic animals compared to normothermic (118) and the rate of metabolic recovery after reperfusion seems to be higher during hypothermic conditions (135). Hypothermia (34°C) has also been shown to attenuate ATP depletion during hypoxia (136).

However, although decrease in cerebral metabolism probably plays a role, other mechanisms may be of superior importance (114,137). This can be exemplified by the finding that cerebral metabolism decreased to the same level as induced by hypothermia, has been shown to be without protective mechanisms (138). In addition, protective effects of hypothermia has been found after the total store of cerebral ATP was emptied (139).
Excitotoxicity

Hypothermia is believed to protect against increased intracellular calcium levels as well as to decrease glutamate release (137). Microdialysis has been used to examine glutamate release during cerebral ischemia and reperfusion with the finding of decreased glutamate levels during hypothermia treatment in several animal experiments. The amount of decrease in glutamate release seems to be dependent on the depth of hypothermia but even during mild hypothermia there is a noticeable difference (45,140). Busto et al showed complete inhibition of glutamate release in rats whose brain temperature was maintained at 33°C and 30°C during cerebral ischemia(141). Hypothermia also reduces levels of glycine, a glutamate co-agonist (142). Reduced glutamate levels decreases excitotoxicity and reduces intracellular calcium influx and calcium mediated injury pathways. For example, hypothermia is described by Hicks et al to increase levels of neuroprotective kinases (ERKs) and at the same time to reduce SAPK, stress induced kinases leading to apoptosis and neuronal degeneration (143). There are indications though, that hypothermia has to be induced very early or before onset of ischemia to sufficiently depress glutamate levels (144).

Radical oxygen species

Levels of ROS, produced after cerebral ischemia, are dependent on brain temperature. Hyperthermia led to increased levels after global cerebral ischemia in rats in an experiment by Globus et al. However, in this study hypothermia (30°C) did not decrease levels when compared to normothermic conditions (58). On the other hand the same group was able to show protective effects of hypothermia with reduced ROS level after traumatic brain injury (44). It has recently been shown that antioxidative defences are preserved in children treated with hypothermia after traumatic brain injury(145). This indicates that hypothermia probably reduces ROS levels and thus allows endogenous, protective effects to perform their task with less restrain.

Inflammation

In humans hypothermia has been described to depress release of pro-inflammatory mediators (104,146). Leukocyte count is reduced (147). In addition hypothermia prevents reperfusion-related DNA injury, lipid peroxidation and leukotriene production (60,141,148). Taken together hypothermia depresses the inflammatory response to cerebral ischemia thus promoting neuroprotection.

Cellular membrane and intracellular acidosis

There are indications of hypothermia protecting against cellular membrane breakdown although experimental results are not entirely conclusive. Busto
et al. found no evidence of reduced fatty acid accumulation during hypothermia (141) showing no indications of attenuation of cellular membrane disintegration. On the other hand, Fisher et al. showed reduced cellular membrane permeability in brain microvascular cells, induced both by mild and severe hypothermia (149). Secondary events, such as development of intracellular acidosis, has also been shown to be reduced (118).

**Development of cerebral oedema**

Therapeutic hypothermia has been shown to reduce ICP and improve survival in patients suffering from traumatic brain injury (150-152). It should be noted that the studies in this field have been contradictory and there is no certain evidence that hypothermia treatment is beneficial after traumatic brain injury (153, 154). In specific circumstances it may anyhow be of use (155). The relation between brain oedema formation and neurological injuries after traumatic brain injury is well known. However oedema formation may be of importance following other causes of cerebral ischemia including hypoxia after cardiac arrest.

Hypothermia reduces mannitol induced blood brain barrier disruption (156) and also protects the blood brain barrier after global cerebral ischemia (78). Oedema formation is reduced in both white and grey cerebral matter after bilateral occlusion of the carotid artery (148) and after cardiac arrest (80).

**Cerebral blood flow and hypothermia**

The reduced metabolism during hypothermia causes cerebral blood flow to decrease via cerebral auto regulation. In experimental models of ischemia a strong relationship between CBF and brain temperature has been demonstrated (157). In humans several studies on brain temperature and CBF after traumatic brain injury has revealed the same relationship with lower global CBF during hypothermia treatment (157-159). In contrast to this, Metz et al. found no reduction in patients treated with mild hypothermia compared to values preceding cooling (160). Hypothermia seems to improve cerebral reflow during CPR and to reduce the detrimental effects of a prolonged no-flow period during CA (19).

**Hypothermia and protection of the myocardium**

Hypothermia as a mean for myocardial protection is clinically used in thoracic surgery. Cold cardioplegic solutions are used to protect the heart and are believed to reduce myocardial metabolism (161). In recent years mild hypothermia has also proven successful after myocardial infarction during percutaneous cardiac intervention (PCI) – the NICAMI study (162).

Evaluation of the effects of hypothermia on the ischemic heart has been made predominantly in experimental models. Hypothermia seems to reduce infarct size if induced during the ischemic event (163-165). If induced after
the ischemic event the effectiveness of the method is unclear. Although there are indications of the necessity for induction during ongoing ischemia (163), Hale et al has shown improved reflow and reduced no-reflow injury and necrosis when hypothermia was induced late during the ischemic event (166,167). These findings are supported by Miki et al describing reduced infarction in beating rabbit hearts even when hypothermia was induced after onset of ischemia (168). On the other hand protection was completely lost if onset of hypothermia therapy was delayed until after 15 minutes of reperfusion in a study by Shao et al on chick myocardocytes (169).

The protective mechanisms of hypothermia on the myocardium seem to be multi-factorial. In part it is believed to be caused by reduced metabolism and metabolic demand (161) and reduced ATP utilization (170). Anaerobic metabolites exacerbate myocardial injury during reperfusion. Hypothermia is believed also to reduce anaerobic ATP synthesis (171), although there are some uncertainty regarding the importance of this effect (172). Collagen network is better preserved after hypothermic myocardial hypoxia (171). Hypothermia during hypoxia influences the mitochondria, preserving mitochondrial gene expression (172). There are indications that hypothermia may inhibit development of MPT and reduce ROS expression (173). In addition apoptosis-linked protein expression is reduced together with promoted expression of proteins involved in cell survival (171).

**Methods of inducing and maintaining hypothermia**

There are several evidences that the most beneficial effects of from hypothermia after cardiac arrest are achieved if, the therapy is induced as soon as possible after the insult. In experimental animal studies positive neurological outcome has been shown when hypothermia was induced during or even before the ischemic insult (113,174-176). Improved neurological outcome in dogs treated with mild hypothermia was detected if hypothermia was induced immediately after reperfusion but not when induced with a delay of 15 minutes after reperfusion (115). Glutamate release after cardiac arrest has been shown to be reduced only if hypothermia treatment was induced no later than at the onset of resuscitation. This indicates that time factor is of great importance if hypothermia is to halt the excitotoxic brain damage (144). As described above, there are also indications of need for early induction regarding myocardial protection. However, there are experimental studies showing beneficial effects of hypothermia treatment even after later onset. This can be exemplified by Mori et al describing improved hippocampal neurohistology even after a 2 hour delay of hypothermia induction (177) and is further supported by findings of Hickey et al, showing reduced neuronal damage after delayed, spontaneous hypothermia (129).

When inducing hypothermia in animal studies several methods have been used, such as cardiopulmonary and femoral carotid bypass (115,139,177,178), cold aortic flush (175), peritoneal cooling (117,179),
nasal lavage (180), infusion of ice slurry (181), ice cold infusion (181) and external cooling (19,130). Many of these methods (excluding external cooling and ice cold infusion) are rather complicated and not suitable for clinical emergency use.

In clinical use of hypothermia after cardiac arrest, there is need for a simple and safe method that induces hypothermia rapidly. In order to shorten the time period spent until target temperature is reached it is also desired to be feasible in the prehospital setting. Clinical studies have been performed using external cooling with icepacks, cooling blankets (122-124,182,183), forced cooled air (125), a cooling helmet device (184) or intravascular cooling catheters (185,186). All these methods are suitable for both induction and maintenance of hypothermia. The problem with these devices however, is a slow onset of hypothermia and/or that they may be too complicated to use in the pre-hospital setting. Among the methods described intravascular cooling system seems to be the most reliable to maintain a stable temperature (187).

**Inducing hypothermia with ice-cold intravenous infusion**

In 2003 Bernard et al reported preliminary results of rapid intravenous infusion (30 minutes) with a large volume (30 ml/kg) of 4°C lactated Ringer’s solution, used to cool patients after cardiac arrest (188). The results were promising with a rapid initial reduction (1.7°C) of core temperature without any serious adverse haemodynamic effects. The method has several advantages as it is easy to induce, easy to handle for medical staff and inexpensive. It has also proven feasible in the prehospital setting (189). The method is now used in clinical practice. After induction of hypothermia with cold infusion, additional methods like ice packs, cooling suits, intravascular cooling catheters etc is needed for maintenance (190).

**Rewarming after hypothermia treatment**

Little is known about the effects of rewarming after hypothermia. In clinical practice rewarming rate is often set to 0.5 - 1.0 °C/hour. The reason for choosing this rate is without any specific scientific evidence. Rewarming is a potentially dangerous phase with increased cerebral blood flow, metabolism and oxygen consumption. After hypothermia treatment of traumatic brain injury brain temperature allowed to exceed 37 °C seems to be correlated with disturbed cerebro-vascular autoregulation (152). There are indications that harmful processes are inhibited by hypothermia only to result in a rebound phenomenon during restoration of normal body temperature. Detrimental effects of the cardiovascular system with inadequate cerebral blood flow and depleted brain energy stores are described with rewarming after prolonged (24 hours) hypothermia (191). In an experiment by Nakamura et al, using fast rewarming from deep hypothermia, CBF was not restored and
protective effects of hypothermia was counteracted (192). Slow rewarming has also been shown superior to fast in an experimental stroke study (193).

**Side effects of hypothermia treatment**

Hypothermia affects many physiological and intracellular processes. Some of these are directly related to the protective effect while others become unwanted side effects of the treatment.

**Coagulation**

Hypothermia causes disturbances in the coagulation system. Bleeding time increases due to the effect on platelet function (194,195) and on enzymes in the coagulation cascade (196-198). In spite of these abnormalities the risk of significant haemorrhage in mild hypothermia treated patients seems to be very low, even when used in patients with traumatic brain injury (199) and does, probably not, clinically affect patients after cardiac arrest.

**Infection**

Hypothermia is known to impair immune function. For example, cell-mediated immunity is impaired in human Antarctic population compared to individuals from temperate zones (200). As earlier described, the release of pro-inflammatory cytokines and leukocyte count is reduced (104,146,147) and, in addition, leukocyte migration and phagocytosis are suppressed (201).

There are several studies reporting higher risk of pneumonia in patients treated with hypothermia, especially when prolonged hypothermia (> 48 hours) has been used and there are also reports on a higher risk of wound infections (147,202). The increased risk of infections should be kept in mind when handling these patients.

**Fluid balance and electrolytes**

Hypothermia-induced polyuresis is caused by central pooling of the blood volume and by reduced re-absorption of Na⁺ in the distal tubuli. This may lead to the loss of significant amount of fluids with the risk of hypovolemia. Attention must therefore be kept on the patient’s fluid balance and diuresis.

Electrolyte balance disorders are another well known problem. Low levels of potassium, magnesium, calcium and phosphate can be seen (203) probably due to diuretic losses and intracellular shifts. Electrolyte disturbances may lead to cardiac arrhythmias and hypotensive episodes and electrolytes should be monitored closely.

**Blood glucose**

Insulin sensitivity and insulin secretion is reduced and is reflected by hyperglycaemia (204). The insulin level needed to maintain normal blood glucose during hypothermia is likely to increase (202). During rewarming it is most
important to closely monitor blood glucose as endogenous insulin production gradually will be restored.

Shivering
When hypothermia develops, physiological counteractive measurements include vasoconstriction of the skin to reduce heat loss and shivering. Shivering leads to an increase in oxygen consumption of about at least 40% (205) which can be fatal in a patient already with oxygen debt. Shivering can be blocked primarily by deepened sedation and analgesia followed by the use of muscle relaxants.

Pharmacokinetics
Only limited data describe the effects of hypothermia on drug distribution. Most enzyme-dependent processes in the body are temperature dependent and likely to slow down during hypothermia. Hepatic enzymes activity is also decreased leading to a reduced clearance of drugs relying on liver metabolism (206,207). Cardiac arrest causes down-regulation of drug metabolizing. This down-regulation is probably attenuated by hypothermia (208).
Aims

The aims of the studies described in this thesis were:

- To evaluate whether it is possible to induce hypothermia by means of ice cold infusion already during ongoing CPR in the experimental setting
- To examine haemodynamic effects of hypothermia treatment induced by cold infusion and rewarming in clinical practice
- To study the effects of mild hypothermia and rewarming after cardiac arrest on cerebral biochemical environment in the experimental as well as in the clinical setting
- To examine cerebral oxygen extraction during hypothermia treatment after cardiac arrest in the experimental setting
- To study ICP following cardiac arrest and during hypothermia treatment and rewarming in the experimental as well as in the clinical setting
Materials and methods

Experimental studies (study I and II)

Study design
Studies I and II were randomized, controlled, blinded, experimental studies. The experimental studies were conducted and reported in accordance with the Utstein-style guidelines for laboratory research (209). The care and handling of the animals involved were reviewed and approved by the Institutional Review Board for Animal Experimentation in Uppsala, Sweden.

Animals
Pigs of Swedish country breed, 11-15 weeks old, were included in the studies (Table 2).

Table 2. Numbers of groups and animals and their mean weight, kg (±SD)

<table>
<thead>
<tr>
<th>Paper No</th>
<th>n</th>
<th>Mean weight</th>
<th>No of groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>20</td>
<td>25.6 (±0.7)</td>
<td>2</td>
</tr>
<tr>
<td>II</td>
<td>16</td>
<td>23.7 (±2.1)</td>
<td>2</td>
</tr>
</tbody>
</table>

In study II one additional animal was examined for microdialysis recovery changes due to varied temperature.

Anaesthesia and fluid administration
Anaesthesia was induced with an intramuscular injection of tiletamine and zolazepam 6 mg/kg, xylazine 2 mg/kg and atropine 0.04 mg/kg. Morphine 1 mg/kg and ketamine 100 mg were given intravenously as a bolus injection after which anaesthesia was maintained by continuous intravenous infusion of 8 mg/kg/h of pentobarbital, 0.25 mg/kg/h of pancuronium bromide and 0.5 mg/kg/h of morphine. This combination of anaesthetics is documented to give reliable induction with maintenance of good cardiovascular function.
The use of anaesthetics is important for ethical reasons as well as to avoid stress that could interfere with the results (211). The drugs used for induction have short half life and the effects of these negligible by the time baseline values were collected. For maintaining anaesthesia pentobarbital and morphine were utilized. Pentobarbital is known to reduce both cerebral metabolism and thereby cerebral blood flow (134) and this probably, inevitably, has influenced the results.

Water losses were compensated for by a bolus infusion of 30 ml/kg of acetylated Ringers solution during 1 hour before the experiment and a continuous infusion of 2.5 % glucose at a rate of 10 ml/kg/h during the entire experiment. The amount of fluid replacement was based on earlier experiments by our research group (23,24).

**Surgical preparation**

The pigs were tracheotomised and mechanically ventilated (Servo Ventilator 900C, Siemens-Elema, Solna, Sweden) with a 70/30 mixture of N₂O/O₂ during preparation. Volume-controlled ventilation was used and minute ventilation adjusted to maintain the arterial pCO₂ within a range of 5.0-5.5 kPa (38-41 mm Hg) and a PEEP of 5 cm H₂O was applied. A pulmonary artery catheter (CritiCath Ohmeda®, 7 French) was inserted via the right external jugular vein for pressure monitoring. In addition three catheters were inserted; one into the right atrium (7 French) for drug administration and pressure monitoring; one into the aortic arch via a branch of the right external carotid artery (18 Gauge) for pressure monitoring and one into the femoral artery (18 Gauge) for blood sampling. Another catheter (3.8 French) was inserted into the left internal jugular vein and passed retrogradely as far as possible towards the jugular bulb for blood sampling. In order to ensure correct placement, position was confirmed by x-ray. The catheter was secured with a purse-string suture around the incision of the vein. Thus the vein itself was not ligated allowing continuous blood flow (212).

In study I, a Laser-Doppler flow probe (Periflux® Laser-Doppler flow meter PF2B, Perimed, Stockholm, Sweden) was placed directly over the surface of the right frontal cortex through a burr hole 1 cm anterior to the coronal suture and 1 cm lateral to the sagital suture for continuous measurement of cerebral cortical blood flow.

In study II, a two-channel skull bolt was used for insertion and fixation, in the right frontal lobe, of one combined intracranial pressure and temperature Camino probe (Integra Camino, Integra Neurosciences, Plainsboro; NJ; USA) and one microdialysis catheter (CMA/70, Microdialysis Bolt Catheter, 10 mm membrane length, CMA, Stockholm, Sweden).
**Cardiopulmonary resuscitation protocol**

*Induction of cardiac arrest*

After preparation the animals were ventilated with 30 % O₂ in air for 45 minutes. Ventricular fibrillation (VF) was induced with a brief application of an alternating current shock of 40-60 V. Cardiac arrest was defined as VF on the ECG and the loss of arterial pressure. Ventilation was stopped when CA was induced.

*Experimental Protocol*

Time schedules for the two experiments were identical regarding length of non-intervention CA interval, duration of closed chest compressions, time for interventions with intravenous infusions and vasoactive drug and time for defibrillation. (Fig 1)

![Figure 1](image)

*Figure 1*. Experimental protocols of study I and II. Time zero minutes is set at time for restoration of spontaneous circulation (ROSC). The animals suffered cardiac arrest (CA) for 8 minutes followed by 9 minutes of cardiopulmonary resuscitation (CPR). In study I the animals were randomized to the hypothermic group (H) and the control group (C) and in study II to the hypothermic (H) and the normothermic (N) group. In study II microdialysis baseline values (MD-BL) were collected from 30 minutes before start of the experiment. Following 1 minute of CPR, infusions (I) were started. In the hypothermic groups the infusions were cooled to 4°C and in the control group and the normothermic group infusions were at room temperature (24°C) and body temperature (38°C) respectively. Infusions were continued until 14 minutes after ROSC. After 3 minutes of CPR, all animals received a bolus dose of vasopressin (V). After CPR, the animals were defibrillated (D) and restored spontaneous circulation. In study II, 5 minutes after ROSC, collection of microdialysis samples was initiated and continued throughout the experiment. The hypothermic group was cooled for three hours and then rewarmed. The animals were observed for 180 minutes (study I-E) respectively 360 minutes (Study II).
After 8 minutes of cardiac arrest the pigs received mechanical closed-chest cardiopulmonary resuscitation (CPR) with a LUCAS™ device (17,18) to ensure standardized chest compressions. Ventilation was resumed with 100% O₂. In Paper I the animals were randomized into two groups with one group (hypothermic group) receiving an intravenous infusion of ice-cold (4°C) acetated Ringer’s solution 30 ml/kg at a rate of 1.33ml/kg/min starting after one minute of CPR and one group (control group) receiving the same amount of fluid but at room temperature. In paper II there were two groups receiving the same amount of fluid (isotonic saline) at 4° C (hypothermic group) or at body temperature (38°C) (normothermic group). After three minutes of CPR, the animals were given an intravenous bolus of vasopressin 0.4 U/kg. Vasopressin has shown to be effective in porcine models to improve vital organ flows, cardiac perfusion pressure and ability to resuscitation (213,214) and has proved to be useful during hypothermic conditions (215). CPR was continued for another 6 minutes following vasopressin administration. After nine minutes of CPR one external defibrillatory shock of 200J was administered. If ROSC was not achieved, CPR was discontinued. ROSC was defined as a pulsatile rhythm with a systolic aortic blood pressure of >60 mm Hg maintained for at least 10 minutes. In animals that received ROSC, FiO₂ was reset to 0.3 after 5 minutes of ROSC. In paper I no other interventions were performed during the remaining three hours of observation. In paper II the animals were observed for six hours. If mean arterial pressure was less than 60 mmHg or cerebral perfusion pressure was less than 40 mmHg the animals were given an infusion of dobutamine (Dobutrex®). In the hypothermic group, in addition to the cold fluid infused, the animals were cooled with ice-packs to a target temperature of 32-34 ºC and kept cold until 180 minutes after ROSC. After that they were rewarmed by the use of a heating mattress at the rate of 0.5-1 °C/ hour until the end of the experiment.

**Measurements**

Standard lead II ECG, systemic arterial blood pressure, right atrial pressure, pulmonary artery blood pressure and intracranial pressure (study II) were continuously monitored and recorded. Cardiac output, pulmonary wedge pressure and end-tidal CO₂ were measured and recorded intermittently. Blood gases and oxygen saturation were repeatedly measured in arterial and jugular venous blood. Core temperature was continuously measured in the pulmonary artery and recorded. Cerebral temperature was measured via the Camino device and recorded (study II).

**Cortical cerebral blood flow**

Cerebral cortical blood flow was continuously measured through a Laser-Doppler flow probe in study I. Laser-Doppler flowmetry is a method for continuous measurement of volume flow (216,217). The Doppler shift of a laser beam scattered by moving blood cells is analysed. Absolute perfusion
values cannot be determined with the equipment used. CBF was recorded every fifth second and presented as a fraction of the baseline flow level.

**Microdialysis**

Microdialysis is used to monitor the chemistry of the extracellular space in living tissue (218). The technique was first described in the 1960’s (219) and was later refined by Ungerstedt and colleagues (220, 221). When performing microdialysis a double lumen catheter with a permeable membrane on the tip is placed in the tissue. Perfusion fluid is pumped via an inlet tube to the distal end. Across the membrane molecules diffuse between the extracellular fluid and the perfusion fluid and an equilibration takes place. The dialysate is then collected via outlet tubing for later analysis. Several chemical markers have been validated for clinical use (222).

**Recovery**

The microdialysis technique does not allow a true sample of extracellular fluid. Instead the concentration of a solute in the dialysate is a fraction of the true interstitial fluid. The recovery is defined as the ratio between the concentration of a solute in the dialysate and its concentration outside the probe (223). In vivo recovery is dependent on several additional factors i.e. flow rate, time after start of perfusion, diffusion coefficient, membrane area, composition of perfusion fluid, substance concentration in tissue and – central for our study - temperature. All these factors are important to consider when comparing absolute values of interstitial solutes obtained by microdialysis, between different studies.

**Microdialysis sampling and chemical analysis**

In study II microdialysis baseline values were obtained 30 minutes before inducing of CA. Samples were then collected from 5 minutes after ROSC at 10 minutes interval until the end of the experiment. Artificial cerebrospinal fluid (containing 148 mmol/l Na⁺, 1.2 mmol/l Ca²⁺, 0.9 mmol/l Mg²⁺, 2.7 mmol/l K⁺ and 155 mmol/l Cl⁻) was delivered as perfusion medium by a microinjection pump (CMA/102 Microdialysis Pump, CMA Microdialysis AB, Solna, Sweden) at a rate of 2 μl/minute. Analysis of lactate, pyruvate and glutamate were done by an enzymatic fluorometric assay on a CMA/600 Analyser (CMA/Microdialysis). An increased Lactate/Pyruvate–ratio (L/P–ratio) was defined as a ratio above 30. This level was obtained by comparison with tentative normal values (222). A change in glutamate level was considered significant when there was a minimum increase of 20 % for a period of at least 20 minutes compared to baseline.

We performed an experiment with one anaesthetised animal (sham) that underwent the same cooling procedure as the animals in the hypothermic
group but without cardiac arrest to investigate the influence of temperature on microdialysis recovery.

**Intracranial pressure**

Intracranial pressure (ICP) monitoring was introduced into clinical neuro-surgical practice in the 1970s after the pioneering work by Nils Lundberg in the 1960s (224) and is today considered mandatory in comatose head-injured patients (225, 226).

In study II, ICP was continuously monitored from 30 minutes after ROSC (when the animal was stable in prone position) until the end of the experiment.

**Jugular bulb oxygen saturation/ cerebral oxygen extraction**

To obtain values reflecting global cerebral oxygen consumption blood gases were repeatedly measured in arterial and jugular blood (ABL 300, Radiometer, Copenhagen, Denmark) and oxygen saturation was analysed (OSM3, Radiometer, Copenhagen, Denmark).

All blood samples were obtained at baseline, after 1 and after 6 minutes of CPR, at ROSC and 10, 15, 30, 60, 120, 180 minutes following ROSC in study I and II and for further 240, 300 and 360 minutes in study II.

In study I, the cerebral oxygen extraction ratio (CExO₂) was calculated by using arterial and jugular venous oxygen saturation (SaO₂ and SjvO₂), haemoglobin concentration (Hb) and arterial and venous partial pressure of oxygen (PaO₂ and PjvO₂);

\[
\text{CExO}_2 = (\text{SaO}_2 \times \text{Hb} \times 1.31 + 0.003 \times \text{PaO}_2) \ - \ (\text{SjvO}_2 \times \text{Hb} \times 1.31 + 0.003 \times \text{PjvO}_2)
\]

The groups were compared after calculation.

If arterial oxygen content and haemoglobin concentration are considered stable, SjvO₂ can be used as estimation of cerebral oxygen extraction and in study II, SjvO₂ was compared between the groups.

**Isoprostanes**

In study II, unextracted plasma samples, derived from jugular bulb blood samples, were analysed for 8-iso-PGF₂α (an indicator of oxidative injury) and 15-keto-dihydro-PGF₂α (an indicator of inflammatory response) by a highly specific and validated radioimmunoassay at baseline, and 5, 15, 30, 60, 120, 180, 240, 300 and at 360 minutes after cardiac arrest. These data were not presented in the article based on study II.
Clinical studies (study III and IV)

Study design
Studies III and IV were clinical descriptive patient studies. The study was approved by the human ethics committee of Uppsala, Sweden. Consent for participation was always obtained from a legal next of kin and later by survivors when considered competent.

Patients
Thirty patients, admitted to our unit after cardiac arrest, were enrolled in study IV. Four of these patients were also enrolled in study III. Patient characteristics are presented in table 3.

Table 3. Characteristics of Patients (n=30). Values are median (with first and third quartile) or n (%).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age, years</td>
<td>63 (72-56)</td>
</tr>
<tr>
<td>Sex; Female</td>
<td>10 (33 %)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>81 (71-88)</td>
</tr>
<tr>
<td>Previous cardiovascular morbidity</td>
<td>16 (53 %)</td>
</tr>
<tr>
<td>Previous diabetic mellitus</td>
<td>6 (20 %)</td>
</tr>
<tr>
<td>Presenting rhythm; Ventricular fibrillation</td>
<td>15 (50 %)</td>
</tr>
<tr>
<td>Asystolia</td>
<td>10 (33 %)</td>
</tr>
<tr>
<td>Pulseless electrical activity</td>
<td>5 (17 %)</td>
</tr>
<tr>
<td>Genes to cardiac arrest: Cardiac</td>
<td>27 (90 %)</td>
</tr>
<tr>
<td>Asphyxia</td>
<td>2 (7 %)</td>
</tr>
<tr>
<td>Anaphylaxis</td>
<td>1 (3 %)</td>
</tr>
<tr>
<td>Place of arrest; Out-hospital</td>
<td>26 (87 %)</td>
</tr>
<tr>
<td>In-hospital</td>
<td>4 (13 %)</td>
</tr>
<tr>
<td>Time from cardiac arrest until ROSC (minutes)</td>
<td>20 (11-29)</td>
</tr>
<tr>
<td>Percutaneous Cardiac Intervention (PCI) during intensive care</td>
<td>9 (30 %)</td>
</tr>
</tbody>
</table>

Inclusion criteria
Inclusion criteria for the study were patients after CA with age ≥18 years, systolic pressure ≥ 80 mm Hg (with or without inotropic support) for more than five minutes after ROSC and unconscious with Reaction Level Scale (RLS) ≥ 3, Glasgow Coma Scale (GCS) ≤ 8.
Exclusion criteria
Patients were excluded with a primary coagulation disorder, terminal disease, known pregnancy, known acute intracranial haemorrhage or cardiac arrest secondary to massive bleeding and inclusion > six hours after CA.

In study III a disturbed coagulation due to primary coagulation disorder or medication was added to the exclusion criteria.

Intensive Care Protocol
Hypothermia treatment was started as soon as possible after ROSC with intravenous infusion of 4°C normal saline, 30 ml/kg at a rate of 100 ml/minute. The saline was infused into an intravenous line placed in the arm. Ice packs were placed in the groins, armpits and along the neck. Target temperature 32-34°C, was maintained until 26 hours after cardiac arrest. Thereafter the patients were slowly re-warmed (passively) to normothermia (defined as 36.5°C). During hypothermia treatment, patients were sedated with infusion of propofol 0.5 – 2.5 mg/kg/hour and fentanyl 0.5 - 2μg/kg/hour. In addition, during induction of hypothermia, if needed to avoid shivering, the patients received either intravenous boluses of rocuronium 0.6 mg/kg and or an infusion of 0.15 mg/kg/hour. At normothermia, the sedation was stopped to allow evaluation of neurological status and possible extubation.

During mechanical ventilation the aim was to maintain PaO₂ of ≥12 kPa and PaCO₂ between 5.0-5.5 kPa.

Target for blood pressure was mean arterial pressure of 65-100 mmHg with inotropic support if needed. Inotropics used were infusions of dobutamine as first line medication followed by nor-epinephrine or epinephrine if needed.

The aim for blood-glucose level was to keep the level between 5-8 mmol/l with insulin infusion if needed.

All patients received an arterial line in the radial artery and a central venous line in the internal jugular vein. Appropriate location of the central venous line tip was confirmed by X-ray.

Monitoring
Core temperature was recorded in the bladder hourly (Curity temperature KAD, Coviden/Tyco). Respiratory parameters were monitored according to general practice in the intensive care unit. Diuresis was measured hourly.

Haemodynamic information was obtained by measurement of arterial blood pressure and heart rate continuously with hourly records. Central venous pressure was recorded repetitively.

Fluid balance was calculated every 24 hours. Losses (diuresis, stools, gastric tube and possible drainages) were subtracted from given fluids (intravenous infusions, medications, enteral nutrition) to yield either a positive, with fluid overload, or a negative balance.
Repeated blood samples were analysed for blood-glucose, arterial blood-gases and electrolytes, central venous oxygen saturation and serum lactate.

**Intracranial Pressure-ICP**

In study III a two-channel skull bolt was inserted bedside and used for insertion and fixation of one combined intracranial pressure and temperature Camino probe (Integra Camino, Integra Neurosciences, Plainsboro; NJ; USA). ICP was continuously measured and recorded every 15 minutes.

**Microdialysis**

The inserted skull bolt was also used for insertion and fixation of one microdialysis catheter (CMA/70, Microdialysis Bolt Catheter, 10 mm membrane length, CMA, Stockholm, Sweden). The microdialysis catheter was perfused with artificial cerebrospinal fluid (containing 148 mmol/l Na⁺, 1.2 mmol/l Ca²⁺, 0.9 mmol/l Mg²⁺, 2.7 mmol/l K⁺ and 155 mmol/l Cl⁻) using a microinjection pump (CMA/106) at a rate of 0.3 μl/minute. Samples were collected hourly. The first two samples were not analysed. Interstitial lactate, pyruvate, glucose (metabolic markers), glutamate (marker of exitotoxicity) and glycerol (marker of membrane degeneration) were measured by an enzymatic fluorometric assay on a CMA/600 Analyser (CMA/Microdialysis). Lactate-pyruvate ratio (L/P-ratio) was calculated (indicator of ischemia).

Tentative normal microdialysis values used (222) are presented in Table 4.

Table 4. Tentative normal cerebral microdialysis values according to Hillered et al

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td>&lt; 4 mmol/l</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>&gt; 130 μmol/l</td>
</tr>
<tr>
<td>L/P-ratio</td>
<td>&lt; 25</td>
</tr>
<tr>
<td>Glucose</td>
<td>&gt; 1 mmol/l</td>
</tr>
<tr>
<td>Glutamate</td>
<td>&lt; 25 μmol/l</td>
</tr>
<tr>
<td>Glycerol</td>
<td>&lt; 100 μmol/l</td>
</tr>
</tbody>
</table>

One patient, in addition, received a microdialysis catheter in the abdominal subcutis (CMA/60, 30 mm membrane length, CMA, Stockholm, Sweden.). It was perfused with the same artificial fluid at the same rate as the cerebral catheter. Samples were collected hourly for comparison with the brain.

**Transthoracic echocardiography**

In study IV transthoracic echocardiography (TTE) was performed and recorded twice during hypothermia treatment (at 12 hours and 24 hours after cardiac arrest) and once after rewarming (at 48 hours). TTE was performed with Philips iE33 or 7500 systems using a small footprint harmonic imaging multifrequency probe.
The echocardiography protocol included parasternal long and short axis views, apical two and four chamber views and subxiphoid view. Five R-R-intervals of each were sampled and archived digitally. One physician with speciality training in echocardiography read all studies in random sequence blinded of patient identity, chronological order and clinical details. Assessment of filling was performed subjectively based on ventricular end-systolic area in available planes and degree of atrial end-diastolic area in the four chamber view. A scale was introduced according to which the estimations of intravascular volume were semi-quantified into five levels from -2 to +2, where -2 represented an obvious decreased intravascular volume, -1 a slightly decreased volume, 0 normal volume, +1 slight increased volume and + 2 obvious increased volume. Left ventricular ejection fraction (LVEF) was also assessed subjectively based on left ventricular dilatation, mitral ring motion and segmental systolic wall thickening. An effective range of 5-85% was used in order to allow for very low values in highly dilated hearts as well as for super-normal values in conditions with hypovolemia, hypertrophy, inotropic and or vasopressor drugs and stress. A new blinding of patient recordings was done and after that a second identical LVEF assessment was performed and the mean value of each pair was used.

Analysis and statistics

Data were presented as mean for variables considered normally distributed (study I and II) and otherwise as median (study III and IV). Range was presented as 0.95 confidence interval (study I and II), and first and third quartile (study IV). Significance was set at the < 0.05 level.

For normally distributed data t-test was used for testing differences between groups and paired t-test is used for differences between two time point recordings within a group. One-way ANOVA was used for differences within a group at repeated recording times. To compare differences between groups at repeated recording time points repeated measure ANOVA was used. Fisher’s exact test was used for analysis of categorical differences.

For values considered non-parametric, changes over time was analysed with the Friedman ANOVA test.
Results

Rate of survival

Experimental studies (I and II)
In study I, in one animal, in the hypothermic group ROSC was not achieved and one animal did not survive during the entire experiment. All animals in the control group survived during the entire experiment.

In study II all animals had ROSC and survived during the entire experiment.

Clinical studies (III and IV)
Thirty patients were enrolled in the study and of these 15 survived until hospital discharge. Of the initial 30 patients four were enrolled in study III and all of these survived until hospital discharge.

Temperature

Experimental studies (I and II)
In study I the animals were given infusions of 4°C or room temperature (22 °C) Ringer’s solution. Maximal decrease of temperature in the hypothermic group was 1.62 (±0.23) °C and 1.14 (±0.23) °C in the control group.

In study II the animals in the hypothermic group were given ice-cold saline and in the control group 38 °C saline. The animals in the hypothermic group were then further cooled with ice packs. In the hypothermic group the target temperature (<34°C) was reached 60 minutes after ROSC for core temperature and after 75 minutes for cerebral temperature (fig 2).
Figure 2. Core and cerebral temperature in hypothermic and normothermic groups (Paper II) from start of infusion (after 1 minute of cardiopulmonary resuscitation - CPR) until the end of the experiment. Values are expressed as mean ± 0.95 confidence interval. Time zero equals restoration of spontaneous circulation (ROSC). Infusion is started at minus eight minutes from ROSC.

Clinical studies (III-IV)
The patients (n=30) reached goal temperature (34°C) after a median time of 4.5 hours (3.0-8.5 hours) following cardiac arrest. The time from cardiac arrest until normothermia (36.5°C) was reached after rewarming was 36 hours (34-37 hours). Duration of time within target temperature (range 32-34°C) was 24 hours (21-28 hours).

Cerebral monitoring

Cerebral biochemical environment
Cerebral microdialysis – experimental study (II)
Lactate-pyruvate – ratio
All animals showed a peak in L/P-ratio associated with cardiac arrest, followed by a decline. L/P-ratio then remained normal in some cases but in 9/15 cases one or more secondary rises were seen. This pattern is exemplified in figure 3 and the incidence is described in table 5.
Figure 3. Example of microdialysis results in two animals (animal No 3 and No 14, paper II) after restoration of spontaneous circulation (ROSC). No 3 without secondary increase of lactate/pyruvate-ratio (L/P-ratio) after cardiac arrest and with no indication of secondary energy failure. No 14 with secondary increase of L/P-ratio after cardiac arrest and indication of secondary energy failure.

The duration of the secondary increase and the peak L/P-ratio value differed between individuals (table 5). A secondary increase in the L/P-ratio was found in 2/7 animals in the hypothermic group and in 7/8 animals in the normothermic group (table 5). This difference was statistically significant (p=0.04).

Pyruvate decreased secondary (compared to baseline) in most cases with secondary increased L/P-ratio, except in one pig in the normothermic group where pyruvate remained stable. Lactate was increased in all cases with increased L/P-ratio.
Table 5. Microdialysis (lactate/pyruvate results) and increased intracranial pressure (ICP) in study II. Cases with secondary increased Lactate/Pyruvate-ratio (L/P-ratio), duration of increase in minutes (mins) and peak value of the increase. Secondary increase defined as increases 60 minutes after cardiac arrest. Duration defined as the total duration of an L/P-ratio above 30. Cases with ICP above 20 mmHg marked with +.

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Secondary increase in L/P-ratio</th>
<th>Increase in ICP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothermic group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>+ (260 mins, 127)</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>+ (300 mins, 239)</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>excl</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Normothermic group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>+ (230 mins, 42)</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>+ (300 mins, 351)</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>+ (180 mins, 156)</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>+ (70 mins, 35)</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>+ (300 mins, 229)</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>+ (150 mins, 89)</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>+ (30 mins, 60)</td>
<td>+</td>
</tr>
</tbody>
</table>

**Glutamate**

All animals showed an initial increase in glutamate after insult. Two animals in each group (a total of four) showed a secondary rise in or remaining high levels of glutamate. In all cases with high glutamate there was an association with secondary increased L/P-ratio.

**Microdialysis during rewarming**

In the hypothermic group, no differences in L/P–ratio or glutamate over time were detected during the rewarming phase.

**Sham animal**

No specific effect of modulating temperature was observed.

**Cerebral microdialysis – clinical study (III)**

Four individuals underwent cerebral monitoring started between 6-10 hours after cardiac arrest.

All patients initially had increased levels of L/P-ratio, compared to tentative normal values (20). In three out of four patients normal levels were never reached during the entire observation period. Patient number 4 had a pe-
period of normal levels during hypothermia treatment. In patients number 1 and 4 there were a tendency of increased levels during rewarming. The pattern of glucose levels differed between the patients. High glutamate levels were recorded at the first observations but then fell to normal levels in all 4 individuals. Glycerol showed a biphasic pattern in all patients. These changes are exemplified by patient no 1 (fig 4).

**Figure 4.** Core and cerebral temperature, microdialysis parameters, intracranial pressure (ICP) and cerebral perfusion pressure (CPP) in patient 1 (paper III). I= Induction, M= Maintenance, R= Rewarming. Terms referring to hypothermia treatment. Recording of cerebral temperature was started after 8.5 hours and was then in the target temperature area (33.2º C). Target temperature according to core temperature
was reached 4.5 hours after cardiac arrest. Normothermia was reached after 37 hours according to core temperature (36.25 hours according to cerebral temperature). Microdialysis was started 10 hours after cardiac arrest and continued until extubation at 37 hours.

*The isoprostanes (study II)*

The eicosanoids 8-iso-PGF2α and 15-keto-dihydro-PGF2α increased after cardiac arrest with peak values 1.5-2 times baseline values 5 and 15-30 minutes after ROSC respectively. After this there was a gradual reduction and baseline values were reached about 4 hours after ROSC. No difference between the groups was detected.

*Intracranial pressure – ICP*

*Experimental study (II)*

Both groups showed increasing ICP from 30 minutes after ROSC until the end of the experiment (fig 5). In the hypothermic group the increase was 11.5 mmHg ±3.1 (p<0.001) and in the normothermic group 9.4 ±6.6 (p=0.01). No difference between the groups was detected. ICP increase above 20 mmHg (the level where intervention usually is considered in clinical neurointensive care) showed no relation to increased L/P-ratio (table 5). For the animals with increased ICP the duration of increase > 20 mmHg was 241 minutes ±88.

![Graph](image)

*Figure 5.* Intracranial pressure (ICP) in the hypothermic and the normothermic group (paper II) from 30 minutes after restoration of spontaneous circulation (ROSC) until the end of the experiment. Values are mean ± standard deviation.
Clinical study (III)

None of the 4 patients had a sustained period of increased ICP. Patient number 1 had a peak up to 30 mm Hg and remained above 20 mm Hg for about 30 minutes. Hourly recordings (to match microdialysis samples) of ICP and CPP in patient no 1 is presented in figure 4. In table 6 characteristics of ICP and CPP for the different patients are described.

Table 6. Mean (± standard deviation) and median (range 10th to 90th percentile) values of intracranial pressure (ICP) and cerebral perfusion pressure (CPP) in the different individuals (paper III). ICP and CPP in mmHg. Proportion of monitoring time (%) with measurements above or below the stated values (mmHg).

<table>
<thead>
<tr>
<th></th>
<th>ICP</th>
<th></th>
<th>CPP</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proportion of monitoring time</td>
<td>Proportion of monitoring time</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
<td>&gt;15</td>
<td>&gt;20</td>
</tr>
<tr>
<td>Pat 1</td>
<td>14 ±4</td>
<td>14 (11-18)</td>
<td>0</td>
<td>2.7</td>
</tr>
<tr>
<td>Pat 2</td>
<td>11 ±3</td>
<td>11 (8-15)</td>
<td>9.2</td>
<td>1.7</td>
</tr>
<tr>
<td>Pat 3</td>
<td>5 ±3</td>
<td>5 (1-9)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pat 4</td>
<td>15 ±3</td>
<td>16 (11-19)</td>
<td>43.4</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Cerebral oxygen extraction

Cerebral oxygen extraction ratio (study I)

In paper I, cerebral oxygen extraction ratio was calculated. Both groups (hypothermic and control) showed an increase in extraction ratio during CA followed by a decrease during CPR. After ROSC, there was an increase followed by a slow reduction down to values close to baseline values. No significant difference was detected.

The jugular bulb venous oxygen saturation - SjvO2 (study II)

In paper II, SjvO2 was used as an estimation of cerebral oxygen extraction. The SjvO2 was reduced during CPR followed by a minor peak 5 minutes after ROSC. After 30 minutes, the SjvO2 stabilised. The hypothermic group had comparably higher values (p<0.001), (fig 6).
Figure 6. Venous jugular bulb oxygen saturation from start of infusion (after 1 minute of cardiopulmonary resuscitation - CPR) until the end of the experiment (paper II). Time zero equals restoration of spontaneous circulation (ROSC). Reflects cerebral oxygen consumption. Low values indicate oxygen debt. Near arterial values indicate low oxygen extraction. BL = Baseline

Cerebral blood flow
Cerebral blood flow was measured in paper I. After ROSC there was hyperaemic phase followed by an adjustment to a level slightly higher than baseline. No difference between the groups was detected. (fig 7)
Haemodynamics

Experimental studies (I and II)
In neither study I nor study II, were there any differences in haemodynamic variables between the different groups (hypothermic – control; study I and hypothermic – normothermic study II).

During CPR the mean arterial pressure (MAP) initially reached values of about 30 % of baseline, increasing after the vasopressin bolus. After ROSC, there was a brief hypertensive phase followed by a hypotensive period and then an adjusting at a level lower than baseline values (study I). In study II mean arterial pressure was titrated with dobutamine infusion and there was no difference in terms of the amount of dobutamine/kg administered to the animals.

Mean right atrial pressure and mean pulmonary arterial pressure increased during CPR. After ROSC mean right atrial pressure (equivalent to central venous pressure - CVP), mean pulmonary arterial pressure and diastolic pulmonary arterial pressure (used as an equivalent to pulmonary arterial wedge pressure) stabilised on increased levels compared to baseline values.

Et-CO₂ (end-tidal carbon dioxide) was studied as an indirect marker of cardiac output (CO) during CPR. There was a decrease during CPR and a
further reduction was seen after administration of vasopressin. After ROSC, CO was followed via the conventional thermodilution method. CO initially was hyperdynamic, followed by a hypodynamic phase and a stabilisation on a decreased level compared to baseline, study I (fig 8).

Figure 8. Cardiac output (CO) before cardiac arrest and after restoration of spontaneous circulation (ROSC) (study I).

Clinical study (IV)

Mean arterial pressure, central venous pressure and heart rate

Values for MAP, CVP and heart rate are presented in table 7. MAP was in the range of 65-90 mmHg affected by the use of inotropics and vasopressors. No differences over time were found in either MAP or CVP.

Heart rate was stable at 70 beats/minute during hypothermia treatment, increased during rewarming and stabilised on 100 beats/minute at normothermia (p<0.001).
Table 7. Median values (with first and third quartile) of mean arterial pressure, central venous pressure, central venous oxygen saturation, serum lactate, heart rate, diuresis and used inotropics after restoration of spontaneous circulation following cardiac arrest in 30 patients

<table>
<thead>
<tr>
<th>Hours after cardiac arrest</th>
<th>6</th>
<th>12</th>
<th>24</th>
<th>36</th>
<th>48</th>
<th>60</th>
<th>72</th>
<th>108</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>72 (62-86)</td>
<td>73 (66-86)</td>
<td>67 (64-73)</td>
<td>72 (64-88)</td>
<td>80 (66-89)</td>
<td>80 (67-90)</td>
<td>85 (73-90)</td>
<td>76 (68-88)</td>
</tr>
<tr>
<td>Central venous pressure (mmHg)</td>
<td>10 (7-12)</td>
<td>11 (7-12)</td>
<td>10 (8-12)</td>
<td>11 (9-14)</td>
<td>12 (10-14)</td>
<td>11 (8-13)</td>
<td>10 (8-13)</td>
<td>10 (8-14)</td>
</tr>
<tr>
<td>Central venous oxygen saturation (%)</td>
<td>71 (65-76)</td>
<td>76 (70-81)</td>
<td>78 (72-81)</td>
<td>81 (74-82)</td>
<td>79 (77-83)</td>
<td>N/A</td>
<td>75 (71-80)</td>
<td>73 (71-77)</td>
</tr>
<tr>
<td>Serum Lactate (mmol/l)</td>
<td>2.4 (1.1-3.6)</td>
<td>2.1 (1.4-3.1)</td>
<td>2.1 (1.5-3.1)</td>
<td>1.8 (1.4-2.4)</td>
<td>1.7 (1.1-2.1)</td>
<td>N/A</td>
<td>1.1 (0.9-1.4)</td>
<td>1.1 (0.9-1.6)</td>
</tr>
<tr>
<td>Heart rate (beats per minute)</td>
<td>75 (60-88)</td>
<td>70 (60-85)</td>
<td>75 (56-89)</td>
<td>95 (85-120)</td>
<td>100 (90-110)</td>
<td>100 (92-110)</td>
<td>95 (90-100)</td>
<td>90 (80-95)</td>
</tr>
<tr>
<td>Diuresis (ml per hour)</td>
<td>110 (40-220)</td>
<td>95 (50-220)</td>
<td>35 (15-75)</td>
<td>62 (40-105)</td>
<td>135 (45-195)</td>
<td>90 (55-245)</td>
<td>70 (54-170)</td>
<td>78 (60-94)</td>
</tr>
<tr>
<td>Dobutamine (mg/kg/h)</td>
<td>N/A</td>
<td>0.1 (0-0.3)</td>
<td>0.20 (0.1-0.4)</td>
<td>N/A</td>
<td>0 (0-0.2)</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
</tr>
<tr>
<td>Nor-epinephrine (μg/kg/h)</td>
<td>N/A</td>
<td>0 (0-3)</td>
<td>2.1 (0-4.5)</td>
<td>N/A</td>
<td>2 (0.5-6)</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
</tr>
<tr>
<td>Number of patients observed</td>
<td>30</td>
<td>29</td>
<td>29</td>
<td>28</td>
<td>27</td>
<td>25</td>
<td>22</td>
<td>13</td>
</tr>
</tbody>
</table>

**Echocardiography**

A total of 24 patients underwent transthoracic echocardiography examinations. Sixteen patients fulfilled the entire echocardiography protocol. Of the remaining patients, 5 underwent examinations both during hypothermia (4 at 12 hours and one at 24 hours) and after rewarming. Three patients were only examined during the hypothermic phase.

In Figure 9 individual LVEF are plotted. Median LVEF did not change (p=0.38 over time (Figure 11). In figure 10 median LVEF are plotted together with the ScvO2 levels at the time for echocardiography.
Fig 9. Left Ventricular Ejection Fraction (LVEF) for each individual.

Fig 10. Left Ventricular Ejection Fraction (EF) and central venous oxygen saturation (ScvO₂) expressed as percentage at three different recording times after cardiac arrest. ScvO₂ values were recorded at the time of echocardiography for each individual. Values are expressed as median ± first and third quartile.
Results from the estimation of intravascular volume are presented in Figure 11. At the first registration, 44% of the patients were estimated to have a decreased intravascular volume (volume degree -1 and -2). At the second and third registration, this number had increased further to 74% and 62% respectively.

Fig 11. Percentage of cases with different cardiac volume filling degree at different observation times. Volume degree 2 = Obvious increased intravascular volume; Volume degree 1= Slight increased intravascular volume; Volume degree 0 = Normal intravascular volume; Volume degree -1= Slight decreased intravascular volume; Volume degree -2= Obvious decreased intravascular volume.

Central venous oxygen saturation, arterial oxygen saturation and lactate
Central venous oxygen saturation (ScvO$_2$) values are presented in table 7 and figure 10. Median values were above or equalling 70% throughout the entire observation period. There was no difference detected over time. In 37% (11/30) of the patients values below 60% were detected.

Arterial oxygen saturation was between 95-99% throughout the entire study period.

Lactate was increased with a value of 9 mmol/l during the first hour after cardiac arrest. Lactate levels then declined over time (p=0.009) and from 28 hours after cardiac arrest median levels were equal to or below 2 mmol/l (table 7).

Fluid balance and diuresis
The median calculated fluid balances are presented in table 8.
Table 8. Median fluid balance and median furosemid amount administered during day 1-5. Range is first and third quartile. + indicates positive fluid balance, - negative fluid balance

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid balance</td>
<td>+4056 (+3272 to +5108)</td>
<td>+2476 (+1431 to +3087)</td>
<td>-998 (-1898 to +526)</td>
<td>-900 (-1552 to +10)</td>
<td>-1030 (-1700 to +185)</td>
</tr>
<tr>
<td>Furomsemid</td>
<td>0 (0-0)</td>
<td>10 (0-15)</td>
<td>15 (0-26)</td>
<td>0 (0-10)</td>
<td>0 (0-10)</td>
</tr>
<tr>
<td>administration</td>
<td>(mg/ 24 hours)</td>
<td>(mg/ 24 hours)</td>
<td>(mg/ 24 hours)</td>
<td>(mg/ 24 hours)</td>
<td>(mg/ 24 hours)</td>
</tr>
</tbody>
</table>

Diuresis is presented in table 7. High values were detected during the first few hours after cardiac arrest.

Administration of diuretic medication (furosemid) is presented in table 8.

**Inotropic and vasopressor support**

All patients needed inotropic and/or vasopressor support. Dobutamine was given to all patients except one, who was directly put on epinephrine-infusion. The amount of dobutamine given was 9.7 mg/kg (4.8 - 19.0) during a time period of 37.5 (26.5-51.0) hours (table 7). The median amount administered was doubled (0.1 mg/kg/hour to 0.2 mg/kg/hour) between the first and the second registration but at the third registration the median was 0.

Fourteen patients received nor-epinephrine in addition to dobutamine. Amount infused was 0.14 (0.06 - 0.29) mg/kg during a time period of 28 (22 - 40) hours (table 7).

Five patients received epinephrine-infusion. The median amount epinephrine given was 0.14 (0.10-0.25) mg/kg during 26 (21-32) hours.
Discussion

This work is focused on hypothermia treatment after successful resuscitation following cardiac arrest and questions of why, when, where and how.

- **Why?** – The reason for using hypothermia in the post resuscitation period is obviously the improved survival and neurological outcome. The question of why it works, however, is not that clear. Even though there are theories on the mechanisms of cerebral protection by hypothermia the question is far from solved. This problem is investigated and discussed in study II and III. In addition, there are indications that hypothermia treatment may also be used as myocardial protection, mentioned in study IV.

- **When?** - Most studies indicate that the earlier hypothermia is induced the better is the effect of the method. This is discussed in study I and, partly, in study II.

- **Where?** – If earlier induction is beneficial, hypothermia should be induced even prehospital. This is, partly, discussed in study I.

- **How?** - There are many questions related to hypothermia treatment. For example, how to best perform induction and maintenance of hypothermia and how to rewarm the patient. Methods of induction and limitations of the methods are dealt with in study I and IV. Problems with rewarming are discussed in study II, III and IV.

Induction of hypothermia with 4°C solution

**Induction with cold infusion during ongoing CPR**

Core cooling by central venous infusion of 4°C solution has been shown by Rajek et al to be an effective method of reducing core temperature in healthy volunteers (227). One experimental study has suggested improved haemodynamics and tissue perfusion with intravenous volume loading during CPR (228). There are also indications of improved neurological outcome using hypothermia and haemodilution to promote cerebral blood flow and reduce no-reflow (117).

Using an ice-cold intravenous volume bolus to induce hypothermia is an easy and inexpensive method. As earlier described, there are evidence suggesting that the earlier hypothermia is induced, the better are the effects of
the method (115,119,144). This also seems to apply to the protective effects of hypothermia on the myocardium (169). In study I, we wanted to examine the possibility of starting infusion already during ongoing CPR to achieve a quicker induction of hypothermia. The temperature decrease was 1.6 °C which correlates well to Bernard’s clinical study (188). This should be compared to 0.3-0.9 °C reductions with external cooling as observed in different studies, both experimental and clinical (122,124,184,215). By inducing hypothermia during ongoing CPR, the time lag from the injury to the reached target temperature evidentially is reduced. The time gained can be even more increased if hypothermia is induced in the pre-hospital setting. The method has recently been described in the clinical setting in two studies with promising results. In the first, a reduction of nasal temperature of 2.5 °C was achieved although a reduced infusion volume (14 ml/kg) was used (229). The other study reported that target temperature was reached within 16 minutes after ROSC using 2 litres of 4°C saline (230). The other method suitable for pre-hospital use, external cooling, has not proven equally effective in decreasing body temperature.

Haemodynamic considerations with hypothermia treatment and induction by ice-cold infusion

The main concern with infusion to induce hypothermia is the fear of side effects of volume loading on the cardiovascular system. The possibility that induction of hypothermia with a large amount of cold intravenous infusion could be harmful for the heart that has been traumatised by ischemia after cardiac arrest (231) has been a concern in the clinical practice. The negative effects might be even greater if the myocardium is affected with volume loading during ongoing CPR. Our results from the experimental studies (I and II), however, demonstrated no evidences of a negative effect of volume loading during CPR. It seems as if induction of hypothermia with cold intravenous infusion can be conducted during CPR without any major disadvantages.

However, our studies have been performed on young healthy pigs and a history of previous heart disease might have changed these results. Inducing hypothermia with cold infusion has been performed in the clinical setting without major haemodynamic side effects (188,189). Bernard et al has even reported indications of an improved general circulation (188). Inducing hypothermia with cold infusion during ongoing CPR was successfully performed in one, in-hospital, case report (232) and in two out-of hospital studies (229,230). In the former of these studies, performed by Kämäräinen et al, one patient out of five survived until hospital discharge. In the later study, performed by Bruel et al, 20 patients out of 33 survived until discharge from the intensive care unit. None of the surviving patients (except one who had a pulmonary oedema) presented any major adverse haemodynamic effects, indicating that it is possible to induce hypothermia during ongoing CPR also
in the clinical situation. However, all of the mentioned studies (188,189,229,230) were primary designed to focus on mortality outcome and on the feasibility of the method and not on haemodynamic effects of the method. There is one study in humans focusing on the cardiac effects of inducing therapeutic hypothermia by cold infusion (233). In this, no effect on the heart was found one hour after completion of fluid infusion when examined by determining LVEF with TTE.

In our study (IV) no evidence of changes in LVEF after cold infusion and hypothermia treatment was found, neither over an extended time (a total of 48 hours) after cardiac arrest, nor when comparing hypothermic to normothermic conditions. ScvO$_2$ did not change over time either. In addition both ScvO$_2$ and serum lactate values were normal or near to normal at the time of echocardiography exams indicating a sufficient global circulation. However, it must be kept in mind that ScvO$_2$ is difficult to evaluate during hypothermic conditions. With a reduced metabolism global oxygen extraction is reduced, which could lead to increased levels of ScvO$_2$ compared to normothermic values. In addition, hypothermia causes a leftward shift of the oxyhemoglobin dissociation curve leading to increased saturation values at a lower oxygen tension. Taken together, this makes interpretation of ScvO$_2$ complicated (234).

Another problem with evaluating differences over time in the clinical study (IV) was the fact that varying amounts of inotropic and vasopressor support were used. This, most likely, has affected the LVEF and compromised our results. There was an increased need for inotropic and vasotropic support during hypothermia treatment. This could be caused by decreased intravascular volume or by hypothermia in itself. Most probably it was also a result of post-resuscitation myocardial dysfunction (91,94). However, it could also be a result of an over-use due to inability to assess the cardiovascular system during hypothermic conditions.

**Decreased intravascular volume following cardiac arrest**

As discussed above there are several indications that volume loading in the doses we have used during or after CPR does not cause any major haemodynamic disadvantages. Furthermore, Jameson et al showed results suggesting that volume loading improves haemodynamics (228). Even though the mentioned study was performed during normothermic conditions and with 4 ml/kg of hypertonic saline-dextran, making it difficult to compare with the method used in the clinic at present, it indicates that fluid loading during resuscitation might be beneficial. Earlier studies have shown the need for a positive fluid balance in order to keep a central venous pressure or right ventricular pressure of 8-12 mmHg and 8-13 mmHg respectively (96,235). This is in line with our findings in study IV. About half of the patients were estimated to have a decreased intravascular volume at the first observation and this amount increased further in spite of a positive fluid balance. The volume
estimation was made by subjective evaluation of TTE-recordings and a non-validated range was used. This could limit the interpretation of the results. However, no patients displayed any obvious increased intravascular volume at any observation time; although all patients had a calculated positive fluid balance on day 1 and 2 after resuscitation. This is of interest as the patients, during the time of recordings, were calculated to have a positive fluid balance of 1.4-5.1 litres.

The reason behind why many of the patients had a decreased intravascular volume, in spite of volume loading to induce hypothermia, is unclear. After successful resuscitation, a “sepsis-like” syndrome, with high levels of circulating cytokines and the presence of plasma endotoxin has been described (59), which might lead to capillary leakage. It has also been shown that during surface cooling to 28 °C, a shift of plasma from circulation to interstitial space occurs (236). This observation, on the other hand, is contradicted by findings of Jurkovich et al (237), who found decreased posts ischemic capillary permeability in hypothermia treated animals. Another possible explanation for the decreased intravascular volume could be the phenomenon of cold diuresis. However, the high diuresis during the first hours after cardiac arrest was probably related to the fluid loading rather than to temperature decrease. Diuresis subsequently normalised, and was even low during hypothermia treatment. The fact that no obvious cold diuresis emerged could strengthen the hypothesis that there was a relative hypovolemia due to post-resuscitative capillary leakage.

**Cerebral blood flow**

Most studies indicate that hypothermia treatment reduces cerebral blood flow at least in experimental settings and clinically after traumatic brain injury. In our study (1) we found no difference in CBF between the two groups. This could be explained by the fact that both groups reduced their body temperature.

**Neurochemical changes following cardiac arrest and hypothermia treatment**

**Lactate/Pyruvate-ratio**

In the experimental microdialysis study (study II), 60% of the animals developed a secondary increase in L/P-ratio, indicating secondary energy failure. The incidence of secondary energy failure was lower in the hypothermic group. However, in the clinical study (study III) all patients had increased L/P-ratio levels although the first samples were taken 6 hours after the insult.
In three of the four patients completely normalised L/P-ratio was never reached.

The results suggest that after successful resuscitation following cardiac arrest, the individual may develop a secondary ischemia (presenting as an increased L/P-ratio). To our knowledge, our results from study II are the first indicating that hypothermia treatment diminishes secondary energy failure following resuscitation after cardiac arrest. In contrast to our results, Natale et al showed that dogs that were cooled before cerebral ischemia showed no difference in lactate accumulation compared to normal temperature controls (180). This could perhaps be explained by different methods used. In Natale’s study dogs were cooled prior to cardiac arrest (duration 10 minutes) and cooling was stopped after 30 minutes following CA. Lactate was then analysed in cerebral biopsies.

The mechanisms behind secondary energy failure after CA are unclear. So is the question why some pigs were more prone to develop it. The fact that there was no correlation between increased L/P-ratio and increased ICP, indicates that there was probably no relation to brain swelling, although this is a blunt way of estimating intracerebral oedema. Disturbed haemodynamics cannot explain the energy failure as individuals with secondary increased L/P-ratio did not have a more compromised general circulation compared to those without. One could speculate that the secondary ischemia is caused by disturbed auto-regulation or reperfusion injury (27,29). Another explanation could be delayed, post-ischemic mitochondrial dysfunction and cellular death (28). Hypothermia is traditionally believed to reduce oxygen need through diminished metabolism (133). This may be one explanation for the reduced incidence of secondary energy failure in the hypothermic group. The increased SjvO₂ in this group supports this assumption. However, reduced metabolism is not the only explanation for the neuroprotective effect of hypothermia (138,139). Protection from intracellular increased Ca²⁺ (137) and excitotoxicity(140), cellular membrane disintegration (149) and reduced production of radical oxygen species (44) could be additive factors behind the reduction of secondary energy failure in the hypothermic group.

In our clinical material (study III), all patients had increased levels of L/P-ratio after more than 6 hours following CA indicating secondary energy failure. In three out of four patients normal levels were never reached indicating energy perturbation, even though all patients were neurologically intact after the insult. This result differs from the experimental study (study II) where 40 % of the animals only had a primary increase related in time to the cardiac arrest. The fact that all patients presented with secondary increased L/P-ratio could be caused by longer CA and, perhaps, more insufficient chest compressions during CPR. On the other hand the patient material is small and without any control group and conclusions are difficult to make. In accordance with study II, one could anticipate that without hypothermia treatment, ischemia would have been worse and increase of L/P-ratio even
higher. All patients were neurologically intact; indicating that the insult either was halted by hypothermia treatment or was rather small. Prior to our study (III) there was only one case report on microdialysis during spontaneous CA (238). This was a patient with a middle cerebral artery infarction who underwent CA during ongoing microdialysis monitoring. L/P-ratio was elevated directly after CA. L/P-ratio has also been monitored by Mendelowitsch et al in three patients having neurosurgery performed during hypothermia and induced circulatory arrest (239). Lactate was increased in one patient, mildly and transiently increased in one and without changes in the third. However these results are difficult to compare with ours as the hypothermia was profound (15 °C) and as the patients were on extracorporeal circulation and thus there were no global circulatory disturbances.

**Glutamate**
An increase in glutamate after cerebral ischemia indicates excitotoxicity and brain injury (40). Reduction of excitotoxicity is believed to be one of the protective effects of therapeutic hypothermia. However, in the experimental study (study II), we were not able to show any difference in glutamate levels between the hypothermic and the normothermic group. This is in contrast to earlier studies that show a reduction of cerebral glutamate in hypothermia treated animals (44,45,140,141). The above mentioned studies differ from ours in time of inducing hypothermia as the animals, in all these studies, were cooled before the insult. More in line with our results, is a study by Takata et al (144), showing that glutamate levels are reduced only if hypothermia is attained before cardiac arrest or immediately upon resuscitation but not if hypothermia is induced five minutes or more after CPR. In the patient material (study III) high levels of glutamate were recorded in the first observation but then fell to normal values in all 4 individuals. Probably we caught the end of the glutamate peak. This too could be explained by excitotoxicity being an early event in the process of cerebral injury after cardiac arrest. Taken together this indicates that hypothermia should be induced as soon as possible after the insult if excitotoxic injury is to be avoided.

**Glycerol**
Intracerebral glycerol was only examined in study III. Increase of cerebral glycerol originates from cellular membrane degeneration during ischemia (71,72). There is also a possibility of glycerol formation from glucose (72). However, this is probably not relevant in the ischemic, energy depleted situation after CA. Another source of glycerol is lipolysis. No triglycerids are present in the brain but when using microdialysis after cardiac arrest certain considerations must be kept in mind. The blood-brain barrier may be injured by the ischemia (78,81) and with a total body ischemic situation there is a possibility of overspill of metabolites from the general circulation...
to cerebral microdialysis samples. This was further investigated by performing simultaneous subcutaneous microdialysis in the fourth patient. Glycerol was higher subcutaneously than in the brain during the entire observation period and increased even further during rewarming and normothermic phase. This increase was followed by an increase in the brain. Cerebral glycerol showed a biphasic pattern in all patients that consequently may be explained by an overspill from the general circulation.

**Rewarming**

The phase of rewarming after hypothermia treatment is potentially dangerous (191,192). In the experimental study (II), there were no obvious indications of further ischemia, expressed by an increased L/P-ratio or excitotoxicity, expressed by increased glutamate levels during rewarming. It is earlier described that fast rewarming is more harmful than slow (214) and the results might have been different with more rapid rewarming. On the other hand, there was an increase in L/P-ratio during rewarming in two of the patients (study III). Maybe, this increase was influenced by a greater primary ischemic insult, but conclusions are hard to make, especially as all patients were neurologically intact. Glycerol increased in all patients during rewarming. However, this may not be a sign of further ischemic membrane degeneration, but could be caused by overspill from the general circulation (as described above).

**Recovery and temperature changes**

A methodological problem to consider was the possibility that temperature changes influenced *in vivo* recovery. Temperature is known to change diffusion coefficients and alter the relative recovery (218). This could have affected our glutamate values but not the L/P-ratio (as the effect of recovery on lactate and pyruvate concentrations should be identical). However, the effect of temperature changes on recovery is fairly small in the temperature range employed in this study (218). This is further supported by the fact that the microdialysis results from the sham animal (study II) showed no effect of temperature changes.

**Isoprostanes**

Hypothermia is believed to suppress oxidative injury (44) and inflammatory reactions (141,146,147). The isoprostanes are known biomarkers of oxidative stress (8-iso-PGF$_{2\alpha}$) and inflammatory response (15-keto-dihydro-PGF$_{2\alpha}$). With this in mind one might have anticipated a reduced isoprostane release in the hypothermic group, but this was not the case (study II). This finding may be due to the fact that we used mild hypothermia, where changes probably are more discrete. We had a fairly large inter-individual variation, which seems to be characteristic of these biomarkers (240) thus making group-depending differences harder to detect in a small sample.
Intracranial pressure and hypothermia treatment

Theoretically, several mechanisms could lead to an increase in ICP after CA. Hypothermia is known to reduce ICP after traumatic brain injury (150) and in an experimental study by Xiao et al, brain oedema formation was reduced when mild hypothermia was induced before CA (80). In spite of this we were not able to show any lowering effect of hypothermia treatment on the ICP, study II. It is notable that ICP was above 20 mm Hg in 8 out of 16 animals at the end of the experiment, the level above which intervention is usually considered in neurointensive care.

None of the four patients (study III) had a sustained period of increased ICP. One patient had a peak up to 30 mmHg that was easily treated with increased sedation. In one study by Iida et al, no rise in ICP was observed during the first 24 hours after CA but developed in half of the patients thereafter (83) a phenomenon not reproduced in study III. It is reasonable to assume that in our patient population, hypothermia treatment might have influenced the occurrence of increased ICP. However, there are also previous studies (81,82) suggesting that high intracranial pressure is mainly a problem after cardiac arrest from non-cardiac origin. In these studies high ICP was diagnosed by means of either post-mortem histopathology or signs on CT-scan and not by intra-parenchymatous pressure measuring complicating comparisons. The patients in study III all had cardiogenic CA and this could also be an explanation to why ICP was kept low.

Several possible reasons for the increase in ICP in the experimental study II may be considered. After ROSC there is a recognised period of hyperaemia (24) that could influence ICP due to an increased cerebral blood volume. This commences soon after the insult and at 30 minutes following ROSC, cerebral blood flow values have generally returned to normal or subnormal levels. Therefore the results in study II should not have been influenced as ICP was not analysed until 30 minutes post-ROSC. Another explanation could be that intravenous volume loading together with a deranged blood-brain barrier (78,79) may lead to an intracerebral vasogenic oedema. On the other hand, in earlier studies showing increased ICP after CA no volume bolus was given. A third mechanism for the increase in ICP may be that the global ischemia during cardiac arrest was severe enough to cause irreversible brain injury and cytotoxic brain oedema. Irrespective of the mechanisms explaining the rise in ICP, there was no correlation between the occurrence of increased ICP and secondary ischemia (defined by a prolonged or secondary high L/P-ratio). The reason for why the pigs in our experiment seemed more prone to develop an increased ICP compared to our patient material as well as to earlier patient studies may be caused by a narrower intracranial space in young pigs, making them more sensitive to develop a high ICP as a result of brain swelling.
Cerebral oxygen extraction and hypothermia treatment

Hypothermia reduces cerebral metabolism and, thus, cerebral oxygen consumption (133,134). SjvO₂ can be used as an indirect measure of cerebral oxygen extraction. Abnormally high levels of SjvO₂ after cardiac arrest are known to be related to poor survival in humans (88) during normothermic conditions. In study II the hypothermic group presented increased SjvO₂ values compared to the normothermic group. The levels approached arterial oxygen content. However, as the incidence of secondary energy failure (measured by increased microdialysis L/P-ratio) was comparably lower in the hypothermic group the decreased cerebral oxygen consumption probably was a result of reduced metabolism rather than a sign of cerebral injury. These findings point out the problem of interpreting SjvO₂ values during hypothermic conditions.

Neurointensive care monitoring after cardiac arrest

After successful resuscitation following cardiac arrest, the brain has been exposed to global ischemia causing both primary and secondary insults. If modern neurointensive care principles were to be introduced following CA, there may be a possibility to avoid secondary injuries and further improve outcome. To be able to reduce secondary brain injury after CA it is mandatory to understand cerebral dynamics i.e. ICP-changes, cerebral blood flow and cerebral metabolism. In order to accomplish this, neurointensive care monitoring with ICP-measuring and cerebral microdialysis could be used. In the experimental study II, these parameters were examined. Monitoring used was chosen to be feasible in the clinical setting. In study III, four patients were clinically monitored with ICP and microdialysis during hypothermia treatment and rewarming.

The use of intracerebral catheters possibly adds extra risk of complications, especially when used during hypothermic conditions. Hypothermia is known to increase the risk of infections (147,202). None of the patients examined suffered any cerebral infection during ICU stay but this risk must be kept in mind if cerebral monitoring is performed during hypothermic conditions.

Another side effect of hypothermia is influence on the coagulation (195,198). This is of great importance as the insertion of catheters may lead to unwanted intracerebral bleedings. This was the reason for excluding all anticoagulated patients in study III. After CA most patients, in whom an ischemic cardiac disease is suspected, are investigated with coronary angiography. If a stenosis is found they are treated with percutaneous coronary intervention. This requires extensive anticoagulation therapy making intracerebral monitoring impossible. This is the reason for the small number of
patients suitable for intracerebral monitoring. In addition, this also makes routine intracerebral monitoring after CA impossible. However, in selected patients it may add extra information and a possibility to adjust the therapy, for example regarding the best time and rate for rewarming. It may also give us possibility to obtain more information about intracerebral dynamics after CA.

**Future perspective**

In order to promote optimization of hypothermia treatment for cerebral and myocardial protection further studies on the underlying mechanisms should be performed. The reduced incidence of secondary energy failure during hypothermia treatment demonstrated in this work is one example of findings needing further investigation. However, as hypothermia treatment today is an established part of post-resuscitation care it is hard to ethically justify patient studies with a normothermic control group. Questions on why hypothermia works must therefore be answered by future experimental research.

In the clinic, therapeutic hypothermia is most often accomplished according to strict protocols. Perhaps a more individualized care, for example, different therapeutic time periods and rates of rewarming should prove superior. To be able to accomplish this there is need for the development of non-invasive cerebral monitoring when using hypothermia treatment. Analysis of biochemical markers can be used for prognostic prediction (241). Bedside EEG may prove useful (242). Anyhow, these methods need further evaluation.

Despite the scientific evidence on beneficial effects of therapeutic hypothermia world-wide implementation rate is low (243). This stresses the need for further studies on clinical feasibility of induction and maintenance methods both for prehospital and in-hospital use. There is also need for further implementation studies in order to promote adherence to recommendations.
Conclusions

Inducing therapeutic hypothermia with intravenous infusion of cold (4 °C) solution is a method that can be started already during ongoing CPR. The effect on the reduction of body core temperature is fast and fairly good and without any major haemodynamic disadvantages. **Study I**

Inducing hypothermia after resuscitation following CA, using cold intravenous infusion is a safe method without serious haemodynamic side effects. This method may even prove beneficial as many patients, in spite of a positive fluid balance, had a decreased intravascular volume, during hypothermia treatment and rewarming. **Study IV**

In humans, there are changes indicating cerebral ischemia and excitotoxicity after the cardiac arrest. There is a risk of secondary energy failure following successful resuscitation after cardiac arrest, expressed by a secondary increase in L/P-ratio in the experimental setting. The experimental study indicates that hypothermia treatment reduces the occurrence of secondary energy failure. The hypothermia treatment might have attenuated the L/P-ratio increase in the patients and contributed to their good neurological outcome. **Study II and III**

There were no indications that rewarming by 0.5 - 1.0 °C/ hour could induce cerebral ischemia, secondary energy failure or excitotoxicity in the experimental setting. However, in humans, signs of ischemia were found during the rewarming phase and conclusions in this matter are hard to make. **Study II and III**

All patients showed a biphasic pattern in cerebral glycerol. This could, at least partly, be due to an overspill from the general circulation. **Study III**

Hypothermia treatment increases jugular bulb venous oxygen content, indicating reduced cerebral oxygen extraction. This is probably due to decreased cerebral metabolism. **Study II**

ICP increases after CA, in the experimental setup, irrespective of whether or not hypothermia treatment is used. In humans, ICP are only exceptionally
above 20 mmHg, perhaps due to hypothermia treatment. Rewarming does not seem to affect ICP. **Study II and III**

Cerebral monitoring of ICP and microdialysis after cardiac arrest might prove to give additional knowledge in how to optimise the treatment. However, due to existing treatment regimes, where cerebral catheterising is incompatible with anticoagulation treatment, the method will probably be of limited routine use. **Study III**
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