Intranasal Cooling for Cerebral Hypothermia Treatment

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Abstract

The controlled lowering of core body temperature to 32°C to 34°C is defined as therapeutic hypothermia (TH). Therapeutic hypothermia has been shown to improve neurological outcome and survival in unconscious patients successfully resuscitated after cardiac arrest. Brain temperature is important for cerebral protection therefore methods for primarily cooling the brain have also been explored.

This thesis focuses on the likelihood that intranasal cooling can induce, maintain and control cerebral hypothermia. The method uses bilaterally introduced intranasal balloons circulated with cold saline.

Selective brain cooling induced with this method was effectively accomplished in pigs with normal circulation while no major disturbances in systemic circulation or physiological variables were recorded. The temperature gradients between brain and body could be maintained for at least six hours.

Intranasal balloon catheters were used for therapeutic hypothermia initiation and maintenance during and after successful resuscitation in pigs. Temperature reduction was also obtained by combined intranasal cooling and intravenous ice-cold fluids with possible additional benefits in terms of physiologic stability after cardiac arrest. Rewarming was possible via the intranasal balloons.

In these studies brain temperature was recorded invasively by temperature probes inserted in the brain. The fast changes in pig’s brain temperature could also be tracked by a non-invasive method. High-spatial resolution magnetic resonance spectroscopic imaging (MRSI) without internal reference showed a good association with direct invasive temperature monitoring. In addition the mapping of temperature changes during brain cooling was also possible.

In awake and unsedated volunteers subjected to intranasal cooling brain temperature changes were followed by two MR techniques. Brain cooling was shown by the previously calibrated high-spatial resolution MRSI and by the phase-mapping method. Intranasal cooling reduced body temperature slightly. The volunteers remained alert during cooling, the physiological parameters stable, and no shivering was reported.

Keywords: Selective brain cooling, cerebral hypothermia, therapeutic hypothermia, cardiac arrest, stroke, traumatic brain injury, brain temperature, magnetic resonance spectroscopy, trigeminal reflex

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List of Papers

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


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<td>Cho</td>
<td>Choline</td>
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<tr>
<td>CPR</td>
<td>Cardiopulmonary resuscitation</td>
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<td>Cr</td>
<td>Creatinine</td>
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<td>ECG</td>
<td>Electrocardiogram</td>
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<td>ENT</td>
<td>Ear, nose and throat</td>
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<td>ICP</td>
<td>Intracranial pressure</td>
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<td>MMSE</td>
<td>Mini mental state examination</td>
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<td>MR</td>
<td>Magnetic resonance</td>
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<td>MRSI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>NAA</td>
<td>N-acetylaspartate</td>
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<tr>
<td>PEEP</td>
<td>Positive End-Expiratory Pressure</td>
</tr>
<tr>
<td>pCO2</td>
<td>Carbon dioxide pressure</td>
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<td>ROSC</td>
<td>Return of spontaneous circulation</td>
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<td>SI</td>
<td>Spectroscopic imaging</td>
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<td>SVS</td>
<td>Singel voxel spectroscopy</td>
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<td>SBC</td>
<td>Selective brain cooling</td>
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<td>TH</td>
<td>Therapeutic hypothermia</td>
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<td>VOI</td>
<td>Volume of interest</td>
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Therapeutic hypothermia (TH) is defined as the controlled lowering of the core body temperature to between 32°C and 34°C [1]. It is thought that this temperature range represents the optimal balance between a positive clinical effect and the occurrence of complications. In two randomized trials therapeutic hypothermia improved survival and neurological outcomes after successful resuscitation for sudden cardiac arrest [2, 3]. Subsequently, the International Liaison Committee on Resuscitation published consensus recommendations for the use of TH after sudden cardiac arrest resuscitation [4, 5]. Data also supported the use of TH in neonates with suspected hypoxic-ischemic encephalopathy [6, 7].

Brain injury in the setting of global or focal cerebral ischemia is the result of a complex chain of events at cellular and molecular level set in motion by ischemia and eventually continued by the restoration of blood flow during the recirculation phase. These processes continue for hours or days after the initial insult and could resume with new ischemic episodes. In this dynamic time-sensitive context hypothermia has a protective effect via various pathways by downregulating the cellular metabolism [8].

The induction and maintenance of hypothermia require an interruption of the body’s normal thermoregulation mechanisms as well as active heat exchange. Therapeutic hypothermia can be initiated by methods that cool the whole body or by methods that primarily target the brain [9-12].

The initiation of cerebral hypothermia by cooling via the upper airways is an appealing alternative [13] that has been tested clinically [14-16]. Recently, trans-nasal evaporative cooling was successfully applied in humans after cardiac arrest [17]. Evaluating the effect of selective cooling methods on the human brain, other than in neurosurgical care, is challenging because direct measurements of the brain temperature cannot be made without the need for surgery. Furthermore, invasive temperature measurements do not inform on the intra-cerebral distribution of temperature changes induced by these methods.

A new intranasal cooling method for induction of cerebral hypothermia where cold saline was circulated within intranasal balloons was studied in this thesis.

This thesis focused on the likelihood that intranasal cooling via balloons circulated with cold saline could preferentially initiate and maintain cerebral hypothermia while having minimal effects on the rest of the body.
Background

General considerations on therapeutic hypothermia

Therapeutic hypothermia (TH) has an established or at least a promising place in the treatment of a variety of pathological states [18-25]. At a time when a radical insult threatens the delicate balance of a complex open non-equilibrium system such as a cell or an organism [26], hypothermia induces a much needed thermodynamic calm, allowing time to recover. It has the ability of acting at different levels by ultimately preserving cellular metabolism. Hypothermia modulates ischemia-induced alterations in cellular metabolism and protects against reperfusion injuries, late inflammation and cell death processes [27-33]. Cerebral metabolism is reduced by 5-7% for each degree reduction in temperature [34] while coupling with the cerebral blood flow is preserved [35]. Hypothermia may decrease intracranial pressure (ICP) and cerebral oedema after stroke, liver failure, traumatic brain injury, and global brain ischaemia [18,20,36,37], and while evidence for influencing outcomes in the treatment of head injury has been discussed [21], it may have a role in the prevention of fever in neuro-intensive care [24]. There is no support for TH as standard therapy in acute myocardial infarction [22] but if induced in early phase it may reduce the infarct size [38] and it also reduces the no-flow injury and necrosis when induced later on in the process [39,40].

The currently available methods and devices for initiating and maintaining TH are diverse and of variable efficacy [9, 41]. Whole-body hypothermia can be induced by surface cooling, core-cooling or by a combination of both methods.

Surface cooling with either air or fluids can be accomplished easily with equipment already available in an intensive care or with commercially marketed devices. The various means require a large surface of the patient’s skin to be exposed or covered in order to be effective. The variety of surface cooling devices in use ranges from being very simple to more complex and more labour intensive [9,10]. If not used in conjunction with anaesthesia or sedation, these methods activate counter-regulatory mechanisms such as vasoconstriction and shivering in order to reduce heat loss [42]. These responses pose the risk of accelerated oxygen consumption and might impede further reductions in temperature.
Core-cooling methods, with the important exception of ice-cold fluids infusion, are invasive, labour intensive and expensive [9, 43]. Intravascular cooling catheters are effective in the initiation of TH and reliable in its maintenance and then rewarming, but they require expertise and are prone to catheter related complications [44]. Extracorporeal circulation is highly effective but this requires specialized centres [45]. The infusion of ice-cold (4°C) fluids is very rapid, effective and easily accomplished even outside the hospital [46]. However, this method requires a large volume of fluids and temperature control is poor. During the maintenance phase it needs to be combined with other methods because of its short-term effect [10,47]. The intravenous administration of cold fluid decreases the core body temperature by a combination of heat loss through the skin and systemic effects of the cold fluid per se while isolating the core from the peripheral thermal compartment [48]. The risks that are frequently associated with systemic hypothermia include infections and cardiovascular and pulmonary complications [49,50].

Brain cooling mechanisms

Brain temperature depends on the balance between metabolic heat production and heat dissipation. The brain is one of the most metabolically active organs in the body and it accounts for 20% of the resting total body oxygen consumption. At rest, it has a metabolic rate of ~35 μl O2 g⁻¹ min⁻¹ [51] corresponding to a cerebral heat production of ~0.6 J g⁻¹ min⁻¹ that elevates brain temperature by a calculated 0.3-0.4°C above the temperature of arterial blood [52].

The main physiological mechanisms responsible for cerebral heat loss are: (i) cooling by arterial blood supply from the body, which is cooled by venous blood from the skin; (ii) cooling by heat loss through the skull via the venous sinuses, diploic and emissary veins; (iii) cooling by heat loss from the upper airways [53]. Their contribution to the actual cooling varies and it is argued that heat exchange with the environment can only lead to temperature changes near the brain’s surface [54,55]. The main regulators of brain temperature are thus cerebral blood flow and the temperature of incoming arterial blood [54,55]. The rate of heat removal from the brain tissue can then be estimated by the product of regional cerebral blood flow and the temperature difference between brain tissue and arterial blood [52].

The term selective brain cooling (SBC) refers to the lowering of brain temperature, either locally or as a whole, below the temperature of arterial blood [56]. Hyperthermia triggers the occurrence of this mechanism, which is seen as a defense of the brain against high temperatures [57, 58]. Selective brain cooling was demonstrated in species both with and without carotid rete [57-59]. Carotid rete represents a vast vascular network at the base of the
brain arising from branches of the carotids before entering the polygon of Willis. This intricate network comes into close contact with the cavernous sinus which receives cool blood from the nasal mucosa and face. In species with carotid rete cold flow through the angularis oculi vein is being responsible for up to 80% of selective brain cooling [60]. The adrenoreceptors of the angularis oculi vein are of the α-type and are relaxed, whereas the facial vein with β-type receptors is normally contracted in resting states. Cold blood flows through the angularis oculi vein to the carotid rete if the vein sphincter is relaxed. This creates an efficient heat exchanger capable of lowering the temperature of the whole brain by 2°C or more [61]. However, high sympathetic activity constricts the angularis oculi sphincter and diverts the cold blood away from the cavernous sinus, via the facial vein, to the body. It was observed that while SBC was active during moderate hyperthermia and at rest it was inhibited during periods of stress and intense exercise. This arrangement allows fine tuning of the temperature of both the brain and the body. It provides the necessary stimulus to the hypothalamus to balance heat loss mechanisms with the heat stress. By allowing the temperature of the brain to rise, heat loss mechanisms such as panting and sweating are activated to their full capacity [62].

In non-carotid rete non-human species the cavernous sinus was shown to provide the necessary heat exchanger and to make regional cooling of the near-by brain possible [59,63].

In humans, selective brain cooling during hyperthermia is a matter hotly under debate [13,61,64]. While the anatomy may provide the necessary substrate for efficient heat exchange, the likelihood of this really happening is disputed [61,64,65]. The cold venous blood flows via the angularis oculi vein to the brain during hyperthermia, as in other species [66]. However, it was argued that the face and the surface of nasal mucosa are too small in relation to the mass of the brain and the carotid rete, where heat exchange might occur, is absent. These limitations add to the short contact time and make an efficient heat exchange in the cavernous sinus dependent on a temperature difference between vein and artery in the order of 120°C [61]. Another possible way that the incoming arterial blood to the brain cools is during its course in the neck. The arterial blood could theoretically lower its temperature in the neck by approximately 1.1°C [67]. However, it was shown that due to the short transit time in the neck the actual decrease in the arterial blood temperature was less than 0.1°C [65]. The biological necessity of separate regulatory mechanisms for brain and body temperatures that allow the brain to cool while the rest of the body boils was also discussed [64]. It was also speculated that while selective brain cooling may not be demonstrated in acute heat stress situations it may, however, serve to a long term biological adaptation leading to adaptive craniofacial geographical diversity [68].
Whether or not selective brain cooling exists as a physiologic mechanism in humans the heat exchange routes described can be used to induce preferential cerebral hypothermia. Surface cooling methods make use of heat exchange through the skull [69]. At room temperature and at rest heat loss through the skin of the head is between 15-20W and may increase to over 100W during exercise [70]. This is the simplest method of inducing selective cerebral hypothermia. It is used in the management of neonatal encephalopathy [11] and, in adults, the cooling helmet and neckband proved to be practical, relatively safe, easy to use and they might have improved the neurological outcome [12,71,72]. The main concern with surface cooling is the uneven distribution of the cooling [55,73]. Core-cooling by endovascular cooling is feasible and very effective in animal experiments but it remains mostly experimental in humans [74-76]. The same is true for epidural cooling, at least for the time being [77].

The upper airways could also be used for heat exchange [69]. The airway surface is cooled by a direct loss of heat to the air and by evaporation of water. This loss of heat under normal conditions varies between 8 and 15 W, i.e. 10% of the total heat loss of the body [78]. A low inspired air temperature produces greater convective cooling of the mucosa, whereas low humidity causes greater evaporative cooling [79]. In each condition, the increase in ventilation facilitates mucosal losses [79]. A continuous and cold nasal airflow in patients was found to reduce brain temperatures between 0.15-0.8°C [14-16,80]. Recently, trans-nasal evaporative cooling was successfully tested in patients after cardiac arrest [17]. Methods like flushing the nasopharynx of rats with cold saline [81] or the pharynx with cold water through thin Tygon tubing [82] were demonstrated to be both effective and neuroprotective in animals.

Brain temperature monitoring

Knowledge of brain temperature is important for assessments of the efficacy of cooling methods. Since a direct measurement of brain temperature cannot be made in healthy people or in patients who do not require invasive cerebral monitoring as a part of their routine care, research has focused on noninvasive techniques of measuring brain temperature.

The temperature of the brain is usually 0.3-0.4°C above the core temperature [52]. This means that, for practical reasons, temperatures reflecting the core temperature can be used to indicate brain temperature. Under normothermic steady-state conditions, these alternative sites might reflect the true cerebral temperature [83-85].

Tympanic temperature as a surrogate for brain temperature and proof of selective brain cooling has been much discussed [13,64]. Under conditions of good external isolation, the tympanic temperature was found to follow the
variations in brain temperature, as measured invasively [86]. However, the
temperature changes were relatively low and close to the accuracy of the
probes which makes those measurements uncertain. The main criticism
against tympanic temperature as an indicator for brain temperature is its
anatomy. The blood supply of the tympanic membrane comes via branches
of the external carotid artery that transports cold blood from the face to the
auditory canal [64]. This would imply that tympanic temperature reflects the
face rather than brain’s temperature. The use of tympanic temperature as a
proof for selective brain cooling was strongly disputed in a re-
analysis of
data coming from studies that argued in favour of selective brain cooling
[87]. However, the author did not argue against tympanic temperature as a
non-invasive measurement of deep-body temperature, provided that it was
properly measured [87]. The difference between tympanic and core tempera-
ture in normothermia varies between ±0.08°C using a near-infrared ear ther-
mometer [84] and ± 0.5°C using Genius tympanic thermometers[85]. How-
ever, when hypothermia is rapidly induced and reversed, temperature meas-
urements made at standard monitoring sites - nasopharynx, oesophagus,
pulmonary artery, tympanic membrane, urinary bladder, rectum, axilla, foot,
in this order - may not reflect the cerebral temperature [88].

Magnetic resonance techniques, in the context of studying the effect of
brain cooling, could provide an interesting non-invasive alternative to the
usual methods of temperature measurement [89]. Their advantage is the abil-
ity to measure absolute temperature as well as temperature changes and to
compute temperature maps with good spatial and temporal resolutions. The
application of magnetic resonance (MR) to the non-invasive thermometry of
biological tissues is based on the effects of temperature on proton $^1$H density,
relaxation times T1 and T2, diffusion coefficient and chemical shift [90].

Among these parameters, chemical shift of the water hydrogen $^1$H was
recognized as the most reliable and practical indicator of temperature [91].
The resonance frequency of a proton at a certain temperature is determined
by the strength of the external magnetic field and by the shielding effect of
the electron cloud. When temperature rises, the motion of water molecules
intensifies, distorting, extending and/or breaking hydrogen bonds of the
molecules; thus, an electron cloud at higher temperatures is freer from the
restraint of the electrical bonding force than at lower temperatures. The at-
tenuation of hydrogen bonding induces more current in the electron cloud,
strengthening the shielding effect. As a result, the resonance frequency of the
water proton becomes lower. This frequency change with temperature can be
determined from the shifts of water spectral line [91]. Three methods, phase-
mapping [92], single-voxel spectroscopy (SVS) [93, 94] and spectroscopic
imaging (SI) [91] have been used for the measurement of temperature using
the proton chemical shift.

In phase-mapping techniques, phase differences between successive MR
images are used to monitor relative temperatures [90]. The advantages of
phase-mapping methods are their simplicity and high temporal as well as spatial resolutions. Their weakness is the computing of temperature using the phase difference. Subtraction makes phase-mapping techniques sensitive to interscan motions.

Spectroscopic techniques, such as single voxel $^1$H spectroscopy (SVS) and conventional magnetic resonance spectroscopy imaging (MRSI), distinguish water resonance from other resonances. They have the potential to reduce the effects of interscan motions and temperature-dependent magnetic susceptibility changes by using a temperature-insensitive internal reference spectral line. The choice of the reference compound depends on the application. Lipids have been used for liver and muscles while N-acetylaspartate (NAA) is most suitable for the brain. Other substance such as choline (Cho) and creatinine (Cr) could also be used for the same purpose. Spectroscopic techniques are capable of estimating both absolute and relative temperatures. Their disadvantage is a poor spatial resolution and long acquisition time (minutes) to obtain an acceptable signal-to-noise ratio of water-suppressed spectra. The absolute brain temperature is measured using single voxel $^1$H spectroscopy (SVS)[89,93,94]. In SVS the signal is received of a volume limited to a single volume of interest (voxel). A voxel is a volume element representing a regularly-sampled space. Magnetic resonance spectroscopic imaging (MRSI) has the capability of measuring spectra from multiple adjacent voxels covering a large volume [91]. It provides wide coverage of the brain and allows selective evaluation of different parts of the target volume (temperature mapping).

The nose

The nose is a unique anatomic structure with a complex physiology. It is responsible for the functions of olfaction, sensation, immunology, mucociliary clearance, air conditioning and airflow dynamics.

The bony roof of the anterior part of the nasal cavity consists of the nasal bones and the ascending process of the maxillary bones [95,96]. The covering is completed by the upper and lower lateral cartilages. The anterior part of the nasal cavity opens anteriorly into the nostril while the nose communicates posteriorly with the rhinopharynx. The nasal cavity is divided into three parts: the nasal vestibule, the olfactory region and the respiratory region. The nasal vestibule is the largest part of the upper airway and has the lowest resistance to air inflow. The upper airway is otherwise responsible for up to 70% of the total airway resistance needed to allow the lungs to expand optimally. The olfactory region consists of the upper part of the nasal cavity and the superior turbinates. The remainder of the nasal cavity constitutes the respiratory region. The total surface area of both nasal cavities reaches about 150 cm$^2$ and the total volume is about 15 ml. The nasal septum divides the
nasal cavity and separates the two nasal airways. The lateral nasal wall supports the three turbinates that divide this lateral wall into three meatuses (inferior, middle, superior) [95, 96].

The paranasal sinuses are air-containing cavities in the facial bones that are connected to the nasal cavity [95]. The maxillary sinus supports the orbit at its superior part and its posterior part is also the anterior wall of the pterygopalatine fossa containing the maxillary artery, the sphenopalatine ganglion and branches of the trigeminal nerve and the autonomic system. The frontal sinus posterior wall is the anterior wall of the cranial fossa and its floor covers the orbit. The ethmoid sinuses, localized in the medial part of the orbit and the inferior part of the skull base, contains six to ten cells. The sphenoid sinus is placed at the junction of the anterior and middle cerebral fossae. It is closely related to the optic nerve and the carotid artery, which lie in its lateral wall [95].

The arterial supply of the nose is very rich [97]. Anteriorly, the blood supply of nasal cavity comes from the anterior ethmoidal artery. The sphenopalatine artery supplies the posterior and a branch of the greater palatine artery the inferior part of nasal cavity. The direction of arterial blood flow in the nose runs anteriorly against inspiration and may help in warming incoming air. The veins accompany the arteries. The venous system is complexly interconnected and communication exists between the orbit, the nasal cavity, the sinuses, the skin of the nose and the cavernous sinus. Various veins drain into large venous sinusoids which make up the venous erectile tissue of the mucosa. They form the major component of the mucosa called turbinates. These vascular cushions line the lateral nasal wall and act as a heat exchanger in the upper airway [97].

Blood flow through the nasal blood vessels is controlled by the autonomic innervation of the nasal mucosa [98]. Stimulation of the sympathetic nerves to the nose causes a reduction in nasal blood flow and a pronounced decongestion of nasal venous erectile tissue via α 1- and α 2-adrenoceptors [99,100]. The parasympathetic fibres to the nose relay in the sphenopalatine ganglion before distribution to the nasal glands and blood vessels. Their stimulation initiates increased mucous secretion and vasodilatation [95].

The function of sensation originates from free nerve endings scattered throughout the nasal mucosa and is mainly mediated by branches of the trigeminal nerve, but also the glossopharyngeal nerve, which all input to the spinal trigeminal nucleus, the thalamus and the somatosensory cortex [96,101]. It is different from olfaction but both have a protective role and can initiate a protective sneezing reflex, tears or nasal secretions. Branches from the anterior ethmoidal nerve and infraorbital nerve provide sensation to the nasal skin, nasal tip, the anterior part of nasal cavity and the lateral walls. The nasopalatine nerve, a branch from maxillary nerve, provides the major sensory supply to the posterior two thirds of the nasal septal mucosa, maxillary gingival and anterior palatal mucosa [96,101]. Trigeminal sensory in-
nervation of the nose is involved in the sensation of pain and mucus production due to the release of substance P from sensory nerve endings [102].

The nasal epithelium covering the inside of the nasal cavity is a physical barrier that entraps and clears foreign material through coordinated events of mucus secretion and ciliary activity [97]. A squamous and transitional epithelium is found in the first third of the nasal cavity. A pseudostratified columnar epithelium (respiratory epithelium) composed of four major types of cells: ciliated cells, nonciliated cells, goblet cells and basal cells, is found in the posterior two-thirds of the nasal cavity.

Approximately 10 000 litres of air per day pass through the nose at different temperatures and humidity levels [95]. The air conditioning function is assured by the nasal epithelium. This complex activity depends on the quantity of the sero-mucous glands and goblet cells, the beating quality of the cilia and the microvilli, the ability to change the nasal internal diameter, the efficacy of the vascular network in the lamina propria, the contribution of watery secretion and the surface contact between inspired air and mucosa. About 20–40 ml of mucus is secreted from the nose each day [103]. Nasal mucus provides a continuous blanket lining the nasal cavity onto which particles in the turbulent inhaled air-stream can impact and stick. The blanket of mucus can be moved by the coordinated waves of the cilia from the front of the nose to the nasopharynx, where they can be swallowed or expectorated. Ciliary action stops after 8-10 min at 50% relative humidity of inspired air and after 3-5 min at 30% relative humidity [97]. Ciliary beating frequency shows consistent readings in the temperature range of 32 to 40°C. Between 19 and 32°C it increases in a linear fashion, and above 40°C it declines. Ciliary activity ceases at between 7 and 12°C [104].
Aims

Paper I
- To investigate whether or not cold saline circulating within intranasal balloons could induce and maintain selective brain cooling in pigs with a normal circulation.

Paper II
- To study whether or not cold saline circulating within intranasal balloons is effective in inducing hypothermia in pigs during and after cardiac arrest.

- To compare an intranasal cooling method alone and associated with intravenous ice-cold fluids in terms of cooling efficiency and physiologic stability after induced cardiac arrest in pigs.

- To assess the possibility and the effects of rewarming via intranasal balloons after cooling by these two different methods.

Paper III
- To evaluate the possibility of monitoring and mapping brain temperature changes using a non-invasive MR technique during intranasal cooling in pigs and to verify the accuracy of this non-invasive method of measurement with invasive brain temperature measurements.

Paper IV
- To study the possibility of inducing and maintaining brain temperature reductions in healthy and unsedated volunteers subjected to intranasal cooling using the non-invasive MR technique calibrated in study III. Moreover, the acceptance, tolerance and adverse effects of the method were investigated.
Material and methods

Experimental studies (studies I, II and III)

Study designs

Studies I and III were descriptive and study II was a randomized, controlled experimental study. The design of the studies and the care and handling of the animals were reviewed and approved by the Institutional Review Board for Animal Experimentation in Uppsala, Sweden. The animals were handled according to the guidelines of the Swedish National Board for Laboratory Animals and the European Convention on Animal Care.

Animal model

A total of 38 pigs of Swedish country bred were used in all studies:
Paper I: 12 pigs with mean weight of 30 ± 4 kg,
Paper II: 20 pigs with a mean weight of 26.7 ± 1.9 kg
Paper III: 6 pigs with mean weight of 27.6 ± 4.2 kg.

Anaesthesia and fluids

Premedication consisted of an intramuscular injection of a mixture of 6 mg/kg tiletamine and zolazepam, 2 mg/kg xylazine and 0.04 mg/kg atropine. A peripheral ear vein was then cannulated for the induction and maintenance of anaesthesia and for fluid administration. The induction of anaesthesia was performed with 1 mg/kg morphine and 100 mg ketamine given as a bolus injection. 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.

Fluid losses were compensated for by a bolus infusion of 30 ml/kg Ringer’s acetate solution 1 h before the experiment and a continuous infusion of 2.5% glucose at a rate of 10 ml/kg/hr during the experiment. The fluids were at room temperature (24 ± 2°C).
Mechanical ventilation
The pigs were tracheotomised and mechanically ventilated (Servo-i Ventilator, Maquet, Solna, Sweden) with volume controlled, FiO2 0.30 and positive end-expiratory pressure (PEEP) of 5 cm H₂O. End-tidal CO₂ was monitored continuously (Lidingö, Marquette Sweden). Minute-ventilation was adjusted to maintain an arterial p₅₅CO₂ of 5.0–5.5 kPa.

Materials and Monitoring

Study I, II, III – common to all studies
For intranasal cooling we used a thin-walled balloon catheter with inlet- and outlet ports (William Cook Europe, Bjaeverskov, Denmark) specially designed to fit a pig’s nasal cavity. The balloon catheters were introduced bilaterally into the nasal cavity of the pig and their position in nasopharynx confirmed by X-ray imaging. The catheters were connected to a cold saline circuit comprising a pump and a modified cardioplegia set including a heat-exchanger Myotherm Set 4/B(Medtronic, Kerkrade, The Netherlands), which was connected to a heater-cooler unit (Stöckert, Munich, Germany)(Fig.1). The flow and temperature of the saline were measured on-line.

Brain temperature was measured invasively by two needle thermistors (Genesee Biomedical Inc, Denver, CO, USA) (study I and II). In study III brain temperature was measured by two MR-compatible fiber-optic probes of a fluoroptic thermometer (study III) (Biomedical LAB KIT, Luxtron Corp., Santa Clara, CA, U.S.A.). The probes were placed through bilaterally positioned burr holes 0.25 cm anterior to the coronal suture and 1 cm lateral to the sagital suture at a depth of ~14 mm.

A pulmonary artery catheter (CritiCath™, BD, UT, USA) and a central venous catheter (BD Careflow™, BD Critical Care Systems, Singapore) were surgically inserted via the right external jugular vein for drug administration and pressure monitoring. A 4F arterial catheter (BD Careflow™, BD Critical Care Systems, Singapore) was surgically introduced into the aortic arch via a branch of the right external carotid artery.

Standard ECG, systemic arterial blood, and right atrial and pulmonary artery blood pressures were continuously monitored and recorded (Marquette, Solar 8000, Hellige Systems, Freiburg, Germany).

Blood gases (ABL 300, Radiometer, Copenhagen, Denmark) and oxygen saturation (OSM3, Radiometer, Copenhagen, Denmark) were measured hourly in arterial blood.

Study I - specifics
Body temperature was measured using a right atrial, oesophageal and a rectal temperature probe (Biopac Systems Inc, Santa Barbara, CA, USA). All
thermistors used were calibrated against the control using test probes-fixed resistors according to YSI 400 (Yellow Spring Standard) standard.

*Study II - specifics*

In addition to brain temperature thermistors an intracranial pressure transducer (ICP) (Camino, Neuro Care Group, USA) was introduced posterior to the right brain thermistor.

Cardiac output and mixed venous saturation were measured according to the protocol during the experiment.
Fig. 1. Cooling circuit with intranasal balloons circulated with cold saline from heat exchanger via a saline bag. The height of the bag relative to the intranasal balloons gives the pressure inside the balloons which was about 20 cm H$_2$O. Warmer saline from the intranasal balloons is circulated back to the heater-cooler unit by the roller pump.

**Study III - specifics**

Basal monitoring consisting of an arterial line and a venous line was used. No conventional temperature probes were inserted to monitor body temperature because of incompatibility with the magnetic field. All MR measure-
ments were performed on a 1.5 T Philips Intera system (Philips Medical Systems, Best, the Netherlands).

Relative changes of the brain temperature during cooling were obtained by determining the water spectral line positions. A high-spatial resolution MRSI technique was used for this purpose. A water and vegetable oil phantom was used to check the feasibility and accuracy of the proposed MRSI technique. *In vivo* calibration experiments were done in a ~ 0.1 cm$^3$ voxel (4.68 x 4.68 x 5 mm) placed around the implanted temperature probes (Fig.2). The reference temperature of the brain was measured by fiber-optic sensors just before and immediately after MRSI. These temperatures were used for calibration of the MRSI results.

![Fiber-optic probes](image)

*Fig.2.* Axial slice of the pig’s head. Volume of interest - VOIs (6 x 6 voxels, 0.1 cm$^3$) were placed at the ends of fiber-optic temperature sensors in the right (V1) and left (V2) hemispheres.

The absolute brain temperature was non-invasively measured using single voxel $^1$H spectroscopy (SVS). The voxel where the measurement was taken had a size of 7.1 cm$^3$ (1.8 x 1.8 x 2.2 cm) and was placed around the implanted temperature probes.

The temperature map was obtained by subtracting the mean baseline magnetic field distribution from the magnetic field distribution which was obtained by averaging the last five magnetic fields measured at the end of the cooling. Magnetic field distributions in the measured slice were computed from the positions of the highest spectral line in each voxel.
Experimental Protocols

**Study I**

Intranasal cooling was started after baseline temperatures and physiological values were obtained. Saline cooled to 8-10°C was circulated through the intranasal balloons for a period of six hours. A heating mattress (OperaTherm202, KanMed AB, Bromma, Sweden) and a blanket were used for body heating. Temperature, haemodynamics and metabolic variables were recorded as well.

**Study II**

After the baseline values were obtained, cardiac arrest was induced with a brief application of an alternating current shock of 40-60 V. After 8 min of cardiac arrest, the pigs received 1 min closed-chest cardiopulmonary resuscitation (CPR) with LUCAS™ (Jolife AB, Sweden). Ventilation was resumed with 100% O₂. Cooling was initiated 1 min after the start of CPR and after randomisation. One group received an intravenous infusion of 30 ml/kg ice-cold (4 °C) saline solution via the peripheral vein at a rate of 1.33 ml/kg/min combined with intranasal cooling with 8°C saline solution circulating through the balloon catheters (N+S-group). In the other group hypothermia was induced by intranasal cooling alone (N-group). No external cooling was applied. After 3 min of CPR, both groups received an intravenous bolus of 0.4U/kg vasopressin. Chest compressions were then continued for another 6 min. After 9 min of CPR, one external defibrillatory shock of 200 J was administered. If this was unsuccessful, the energy level was raised to 360 J, and if needed, a bolus dose of 45 μg/kg adrenaline was given. Defibrillatory shocks were applied for a maximum of 5 min. If restoration of spontaneous circulation (ROSC) was not achieved during this time, CPR was discontinued (Fig.3).

![Fig.3. Experimental protocol study II](image-url)
Restoration of spontaneous circulation (ROSC) was defined as a pulsatile rhythm with a systolic aortic blood pressure of > 60 mmHg, maintained for at least 10 min. The fraction of inspired oxygen (FiO₂) was returned to 30% after 5 min of ROSC. If the arterial pH was less than 7.20 5 min after ROSC, acidosis was corrected with 1 mmol/kg of a tri-buffer mixture (Tribonate®, Pharmacia & Upjohn, Sweden). Minute ventilation was adjusted for maintenance of an arterial $p$CO₂ of 5.0–5.5 kPa. Ringer’s acetate solution in 10 ml/kg boluses and Dobutamine was administered if necessary for maintenance of a mean perfusion pressure above 65 mmHg and SvO₂ above 60%.

Intranasal cooling was maintained for 180 min in both groups. Thereafter, the process was reversed and warm saline was circulated through the intranasal balloons for another 180 min in order to rewarm the pigs. The temperature of the perfusate was adjusted to 2°C higher than the recorded brain temperature at all times during rewarming. No external heating was applied. Brain and oesophageal temperatures, haemodynamic variables and intracranial pressure were recorded.

Study III
The experiment began with the two or three single voxel MRS acquisitions at the baseline temperature. The baseline measurements were continued by six high-spatial-resolution MRSI scans. Selective cooling of the brain was then started and relative temperature changes were monitored by MRSI. Fifteen MRSI acquisitions were typically repeated with a period of ~75 seconds after beginning of the cooling. The experiment finished with two or three single voxel MRS measurements. Two successive experiments were performed on each pig. The first cooling of the brain was stopped after ~30 minutes. Passive rewarming of the pigs was then allowed. A temperature plateau was achieved after ~120 minutes. The brain temperature was usually lower by about ~0.3°C with respect to the baseline level after rewarming. The second series of measurements and brain cooling was performed in the same way as the first one. Altogether, twelve cooling experiments were performed with the six pigs.

High-spatial resolution MRSI was applied to measure relative changes and temperature maps of the brain. The proposed technique was verified using the water and vegetable oil phantom and calibrated against the temperature measured by the fiber-optic probes. Singel voxel spectroscopy (SVS) using an internal reference to NAA, choline (Cho) and creatinine (Cr) spectral lines measured the absolute temperature at baseline and at the end of cooling. This technique was also calibrated against the fiber-optic probes. Two successive experiments (cooling-rewarming-cooling) were performed on each pig.
Statistics

In study I and III values are expressed as mean ± standard deviation (SD). In study II the changes from baseline to 180 minutes and the changes from 180 minutes to 360 minutes were expressed as the slopes for each pig and compared between groups with the un-paired t-test. Data levels at 180 and at 360 min were compared between the groups with an un-paired t-test or an analysis of covariance model with the group as the factor and baseline value as the covariate. The relationship between brain temperature and oesophageal temperature at 180 and at 360 minutes were compared between groups with an analysis of covariance model. In study III standard linear regression was performed to evaluate the relationship between water chemical shift and temperature. P < 0.05 was considered statistically significant.

Clinic study – study IV

Study design

This was an open, non-randomised study. The research project was performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects and was approved by the local ethical review board.

Inclusion criteria

Ten healthy volunteers (9 males) over the age of 18 were originally included. One volunteer discontinued his participation early on due to an allergic reaction caused by surgical tape used in the fixation of the balloon catheters and was replaced with a reserve. Median volunteer’s age was 22 (range, 21-62 years).

Exclusion criteria

The exclusion criteria were a history of repeated sinusitis (more than three episodes during the last year), known allergy to lidocaine, known history of coagulopathy, known pregnancy and smoking.

Monitoring and testing

The volunteers were continuously monitored with ECG (Datex-Ohmeda, S/5, Finland). Blood pressure was measured and recorded every tenth minute (Datex-Ohmeda, S/5, Finland).

The body temperature of the first five volunteers was measured continuously using MR-compatible fibre-optic probe (Biomedical Lab Kit, Luxtron,
Santa Clara, USA) placed in the volunteer's axillae. However, this method was recognized as less reliable due to unstable sensor contact and a tendency of the probe to dislodge. The temperature readings could not be interpreted. The body temperature of the last five volunteers was therefore measured in the rectum using a digital thermometer (Microlife AG, Switzerland). Measurements were taken before and after the cooling by the volunteers themselves. In addition, the finger/forearm temperatures were monitored by MR-compatible fiber-optic probe (Biomedical Lab Kit, Luxtron, Santa Clara, USA) during the cooling. Cortisol levels were measured before, during and after the cooling.

An ear, nose and throat (ENT) specialist examined the nasal cavities before insertion and after removal of the balloon catheters.

Volunteers performed the Mini Mental State Examination (MMSE) before and after the cooling. The MMSE consists of 11 questions and was designed to assess the cognitive status. Afterwards, the volunteers completed a short questionnaire about their experience.

The cooling device and circuit was similar with that used in the experimental studies I-III. We used intranasal balloons catheters customized for human nose size and form (Arrotek, Co. Sligo, Ireland).

All MR measurements were performed on a 1.5 T scanner (Intera, Philips Healthcare, Best, the Netherlands) using a quadrature receiver head coil. Relative brain temperature was computed from the positions of the water spectral lines in each pixel (voxel). Phase maps used in phase-mapping method were computed from the first image record (TE = 6 ms) of the MRSI sequence.

Experimental Protocol

The cooling circuit was vented and rinsed through and the temperature of saline adjusted to 20°C while the volunteers were prepared for the procedure and took the MMSE test. Rectal temperature was then measured. Intravenous access was established and baseline blood sampling was performed. The ENT specialist inspected the nasal cavity with a fibroscope prior to introduction of intranasal balloons. Lidocaine gel was smeared on the balloons which were then inserted into the nasal cavity bilaterally. The body was covered with blankets until a subjective feeling of warm and comfort was expressed by the volunteer.

The experiment began with four MR baseline records. Intranasal cooling was then started. The MR measurements continued throughout the cooling period. Typically, 20 MRSI acquisitions in the axial and sagittal slices were repeated every 1.5 min after the cooling began.

The volunteers could stop the experiment at any time if they experienced too much discomfort. The experiment ended after 60 min and the catheters were removed. Rectal temperature was measured again and the ENT special-
ist made a second inspection of the nasal cavities. A second MMSE test and a short questionnaire about their experience were completed. At follow-up one week later the volunteers were asked about possible adverse effects and general feelings during the experiment.

Statistics
Data are presented as mean ± one standard deviation (SD). The mean temperature reduction was computed in the different volumes of interest.
Results

Study I
Temperatures

During the first 20 minutes after the start of the cooling procedure, the temperature in both cerebral hemispheres dropped from a mean temperature of 38.1°C ± 0.6°C to 35.3°C ± 0.6°C (Fig.4).

![Temperature decrease during the first hour after the induction of intranasal cooling.](image)

After 1 h of continuous cooling, brain temperature was 35°C ± 0.6°C and after six hours 34.7°C ± 0.9°C. The temperature of the right atrium decreased from 37.9°C ± 0.5°C to 36.2°C ± 1°C after six hours. The oesophageal temperature decreased during the same interval from 38.3°C ± 0.5°C to 36.6°C ± 0.9°C and the rectal temperature from 38.6°C ± 0.5°C to 37.1°C ± 1.1°C (Fig.5).
Fig. 5. Temperature variation under 6 h intranasal cooling

The brain-body temperature gradient peaked about 30 min after the initiation of cooling and could be maintained throughout the rest of the experiment. 
After an initial decrease in temperature with a mean temperature of 2.8°C ± 0.6°C during first 25-30 minutes of brain cooling, the brain temperature stabilised despite further cooling with the chilled saline. This was followed by a period where the brain temperature slowly decreased. A variation in pump flow of up to 50ml/min in this phase did not affect brain temperature. No difference was found in the temperature kinetics between right and left hemispheres.

The measured cooling effect of the system varied up to 30W, while the warming effect of the heating mattress varied between 30 and 40W.

Hemodynamics and physiologic parameters
No significant changes in mean arterial blood pressure were recorded. The heart rate dropped slowly after the induction of cold saline from 110 ± 20/min to 84 ± 14/min after an hour and then it stabilised. No arrhythmia was noted. No metabolic abnormalities were noted during the experiment.
Study II

Two pigs, one in each group, did not achieve ROSC. Another pig in the N-group died after about three hours after ROSC because of sudden refractory ventricular fibrillation. The baseline parameters were similar except for baseline temperatures, which were higher in the N-group [paper II, table 1].

Temperatures

A comparison of the slopes of the temperature curves showed that brain and body temperatures decreased at a similar rate in both groups (p = 0.48 for brain temperatures and p = 0.13 for body temperatures; Fig.6).

Brain temperatures continued to decrease until rewarming in both groups, and the maximum decrease in brain temperature was 3.8 ± 0.7°C in the N-group and 4.3 ± 1.5°C in the N+S-group (p= 0.47). When corrected for baseline variation, there was no difference in absolute brain temperatures between the groups after 180 min cooling. The maximum body temperature decrease was 3.6 ± 1.2°C in the N-group and 4.6 ± 1.5°C in the N+S group (p= 0.1)

The mean brain rewarming rate/ hour was 0.6 ± 0.1°C in the N-group and 0.2 ± 0.2°C in the N+S-group. During 180 min rewarming, the brain temperatures increased by 1.7 ± 0.6°C and 0.9 ± 2°C for the N- and N+S-group
respectively. The corresponding increases in body temperature were 0.9 ± 1.5°C and 0.6 ± 2°C (N and N+S group respectively; Fig.). Due to the spread of temperatures, when the slopes of the temperature curves were compared, no difference was found in the rewarming rate for either brain temperature (p = 0.14) or oesophageal temperature (p = 0.33). However brain temperature in the N-group was higher after rewarming (p=0.03) compared to the N+S-group.

**Haemodynamic parameters**

There were no differences in number of defibrillations and time to ROSC between groups. Seven pigs in the N-group and six in the N+S-group were defibrillated once, and two respectively three pigs twice (p = 0.62). Time to ROSC after cardiac arrest was 9.7 ± 1.4 min in the N-group and 9.4 ± 0.7 min in the N+S group (p = 0.68). All pigs received vasopressin during CPR and only one in the N-group needed one dose epinephrine. The dobutamine dose in N-group (4.7 ± 1.2 μg/kg/min) was higher than in N+S group (2.9 ± 1.2 μg/kg/min) (p= 0.045). The need for 10ml/kg Ringer acetate boluses in N-group (3.5 ± 2) was higher than in N+S-group (1 ± 0.8) (p= 0.003).

No differences were noted in the haemodynamic parameters between the groups during cooling or rewarming (Table) other than mixed venous oxygen saturation, which was lower in the N-group after both cooling and rewarming (p = 0.024, and p = 0.002 respectively) compared to N+S (Fig.7).

![Graph](image)

*Fig. 7. Mixed venous saturation (SvO2) variation during cooling and rewarming. N+S group: combined cooling induction; N-group: intranasal cooling alone; # cardiac arrest; *p<0.05.*
Intracranial pressure (ICP)

There was no difference in ICP after 180 min cooling between the groups. ICP was higher in the N-group compared to N+S after 180 min rewarming (25.2 ± 2.9 mmHg and 15.7 ± 3.3 mmHg respectively; p = 0.01) (Fig.8).

![Intracranial pressure during cooling and rewarming. N+S group: combined cooling induction; N-group: intranasal cooling alone; # cardiac arrest; *p<0.05.](image)

Metabolic parameters

The measured metabolic parameters were stable and within normal limits during the experiment in both groups (paper II, Table).

Study III

The mean temperature coefficient of the water chemical shift without internal reference was -0.0192 ± 0.0019 ppm/°C. This estimated coefficient was used to compute the temperature changes during cooling. A comparison of the temperatures measured by high-spatial resolution MRSI in the voxels V1, V2 and reference temperatures measured by fiber-optic probes is shown in Fig.9. Temperature changes inside the pig’s brain were measured with an accuracy of ± 0.5°C.
Fig. 9. Comparison of the reference invasive temperatures (thick line) and relative temperatures measured by high-spatial-resolution MRSI (thin line). Offset of relative temperatures was determined from difference between mean baseline temperatures. (a) Temperatures in the voxel V1 (right hemisphere). (b) Temperatures in the voxel V2 (left hemisphere).

The temperature map of the pig’s brain is shown in Fig.10. Intranasal cooling resulted in a temperature reduction at all sites, with a somewhat larger fall (−4.7°C) in the vicinity of the removed scalp.
Significant relationships (|r| > 0.90, P < 0.0001) were found among brain temperature and chemical shift differences between water line and Cho, Cr and NAA spectral lines obtained by single voxel spectroscopy.

Study IV

Volunteer no. 6 was at an early stage excluded due to allergic reaction to the surgical tape. He was replaced by another volunteer. Magnetic resonance measurements of volunteer no. 4 were discarded because of extreme movement artefacts. Volunteer no. 8 had only one intranasal balloon catheter because of a narrow nasal cavity on one side. Room temperature during experiments was 24.3 ± 0.5°C. The cooling effect of the balloons was 15.8 ± 6.4W.

After 60 min of intranasal cooling brain temperature reduction was - 1.7 ± 0.8°C as measured by MRSI and - 1.8 ± 0.9°C as measured by phase-mapping method. The rectal temperature of the last five volunteers decreased with - 0.5 ± 0.3°C. Fig.11 summarizes the relative temperature changes after 60 minutes of the cooling.
Fig.11. Temperature decrease after 60 min intranasal cooling. Volunteer’s no. 4 data was discarded because of extreme movement artefacts; volunteer no. 6 was replaced due to allergic reaction. Rectum temperature was measured in the last five volunteers. ΔT – temperature difference from the baseline; MRSI – magnetic resonance spectroscopic imaging;

Temperature mapping at the end of cooling showed a relatively uniform effect on intra-cerebral temperature reduction, i.e. in the limits of the estimated accuracy ± 0.5°C for both measurements methods. Fig.12 shows a representative example. The forearm-finger temperature difference varied between 5.3 ± 2.2°C at the beginning and 5.8 ± 1.6°C at the end of the experiment.
Blood pressure and heart rate remained stable during the experiment. Cortisol levels were within the normal laboratory range of 250 - 750 nmol/l.

All 10 volunteers reported feeling fairly calm during and after the experiment. They were alert during the cooling and gave the same scores for the MMSE tests before and after cooling. None reported shivering during cooling.

The ear, nose and throat examination revealed increased nasal secretion after the experiment in 9 out of 10 volunteers. Of these, 3 volunteers had a slightly increased red tone of the mucous membrane and 3 volunteers had small ulcers/rifts, but no bleeding, on the inferior concha after the cooling. None of these adverse events were considered severe. Of the 10 volunteers, one appreciated that the nasal balloons felt pleasant in the nose, 3 were indifferent and 6 rated them as unpleasant. None said that they were unbearable. Afterwards, 7 volunteers reported rhinorrhea of mild to moderate intensity.

Other adverse events reported when the volunteers were questioned seven days later were headache (4 cases) and dizziness (1 case) during the day of the experiment. Both events were considered by the volunteers to be of mild severity. All volunteers confirmed that they felt fully recovered at the follow-up seven days later.
Discussion

Brain and body temperatures

In all of the studies the brain temperatures decreased after initiation of cooling by cold saline circulated within intranasal balloons. Intranasal cooling induced a reduction in brain temperature under conditions of both normal circulation and after cardiac arrest. This effect was recorded in both pigs and human volunteers.

In studies I and III, intranasal cooling was applied in pigs with normal circulation. Brain temperature was rapidly lowered by 2-3°C in the first 30 min. The fast response was followed by a slowly descending phase which was more noticeable in study I where cooling was maintained for 6 h.

The first phase of cooling could be explained by several mechanisms. Local contact with the cold balloons could have induced conduction cooling in their vicinity. The inferior frontal lobe, the hypothalamus and posterior regions of the brainstem would have been the first to cool. The important local effect of the intranasal balloons on brain temperature was also observed in study II during rewarming when the brain temperature was the first to rise. On the other hand, pigs have carotid rete and therefore also the anatomical and physiological bases to induce physiologic selective brain cooling [61]. Cooled blood from the venous plexuses of nasal mucosa reaches the carotid rete, which functions like a heat exchanger. In addition, in pigs, arterial cooled blood could also reach the carotid rete via the ascending pharyngeal artery [105].

However, the presence of normal circulation further dissipated the effect and cooled the whole brain [paper III, IV, 106]. It seems that the presence of normal circulation had a double effect on cooling: first, by boosting the spread of the effect to the brain and second, by distributing the cooled blood to the body, which made further cooling of the brain dependent on core cooling. At this point, the rate of cooling slows and brain and body temperatures continue to decrease in a parallel fashion [paper I, 106]. This second phase is probably the result of a thermal equilibrium where heat removal and heat production are almost equal. The right atrium temperature probes documented the fast return of cooled blood from the nasal cavity to systemic circulation in study I. This temperature pattern was closely followed by the oesophageal recording. The change in rectal temperature lagged behind as
expected since this measurement site does not follow core temperatures changes when fast hypothermia is induced [88,107].

The influence of normal circulation on spreading and limiting the effect of intranasal cooling was also shown in a study by Boller et al., which found the same results [106]. Nasopharyngeal cooling was applied in condition of normal flow, no flow (cardiac arrest) and low flow (CPR situation). It was shown that the brain is preferentially cooled compared with the other compartments of the body but that this is limited under normal blood flow conditions. After the first drop in brain temperature the same parallel evolution of brain and body temperatures was observed.

The influence of circulation distributes the initial local effect in the brain and this was more noticeable in study III and IV, where temperature maps could be constructed. The high-spatial resolution MRSI technique accurately followed the invasive measurement and showed a uniform distribution of temperature reductions in the brain [paper III, IV].

The return of circulation induced fast cooling after cardiac arrest in pigs [paper II]. Brain cooling was followed by body cooling in both groups. The addition of cold fluid to intranasal cooling did not change the order of cooling and the magnitude of it. However, since in this study the body was not externally heated, the body temperatures dropped quite rapidly. The intravenous administration of cold fluid rapidly decreases core body temperature [48] but when the administration of cold fluid is interrupted, a spontaneous and rapid recovery of core temperature usually occurs [47,48]. In study II, continuous intranasal cooling provided the necessary heat exchange momentum to maintain core hypothermia. In a previous study performed in our laboratory [108] that used the same experimental protocol for inducing cardiac arrest, the maximal body temperature decrease was -1.6°C in the group that received an intravenous infusion of ice-cold (4°C) saline solution. In the present studies, body temperatures decreased by -3.6°C and -4.6°C in the intranasal and combined method group, respectively. As such, the intranasal cooling may have accentuated the effect of cold intravenous fluids.

Several factors could have influenced the possibility that the results in the experimental studies could be reproduced in humans. Firstly, in all of the experimental studies anaesthesia was maintained with pentobarbital. Data indicate that the brain–body temperature homeostasis under pentobarbital anaesthesia in rats is altered [109]. All general anaesthetics increase the threshold for sweating and decrease the thresholds for vasoconstriction and shivering [110]. Body warming is unable to fully compensate for anaesthesia-induced brain hypothermia and enhances the negative brain–body temperature differentials typical of anaesthesia. However, in the face of hypothermia, its role in decreasing cerebral metabolism might be concealed [111]. The effect of anaesthesia on thermoregulation can be used to externally control brain and body temperature and brain-body gradients [112]. Secondly, there are anatomical and physiological differences between pigs.
and humans that are important in this context. The baseline temperature in pigs and humans is different. Also, the brain weight to body weight ratio is 0.02/1 in a 70 kg human and 0.003-0.004/1 in a 30 kg pig. Importantly, the pig, in contrast to humans, has a carotid rete that may be a selective brain cooling system specific for the pig.

However, intranasal cooling with cold balloons did reduce the brain temperatures in healthy, unsedated volunteers [paper IV]. Variations in brain temperature during 60 minutes intranasal cooling was measured by two MR techniques. The high-spatial resolution MRSI technique calibrated both in vitro and in vivo in study III was used together with phase-mapping. Phase mapping is a more conventional method with shorter acquisition time but it also has a high sensitivity to interscan movements [92]. Temperature maps at the end of cooling reflected a uniform cerebral spreading of temperature reductions. The use of the calculated coefficient in study III enabled a short acquisition time per temperature point which permitted an accurate temperature follow-up within ± 0.5°C limit. A reduction in body temperature, measured rectally, was also recorded in this study.

A preferential cooling of the brain similar to that observed in the experimental studies is hard to prove since the absolute temperatures of the brain were not measured at all times and rectal temperature does not adequately reflect core cooling [paper I, 88,106]. However, local cooling and a subsequent systemic spread of cooling are plausible.

Local contact, heat exchange in the upper airways and cold venous inflow from the nasal cavity to the brain might explain the local decrease in brain temperature [53, 113]. Cerebrospinal fluid might be involved in the spread of the cooling effect [53].

However, during hypothermia the blood flow in the angularis oculi vein, which is theoretically responsible for a large part of selective brain cooling, reverses and flows via the facial and jugular veins to the heart, cooling the body [13]. The rate of heat removal from brain tissue can be estimated by the product of regional cerebral blood flows and the temperature difference between brain tissue and arterial blood [52]. Therefore, it is possible to increase heat removal by increasing cerebral blood flow and/or brain-body temperature differences. Usually, the brain-body temperature difference varies between 0.3°C [52] and 2.3°C [114]. Cold venous blood returning to the heart via the jugularis vein could increase the brain-body temperature difference and provide the necessary gradient for heat exchange. In study I, the reflux of cold blood in the right atrium was observed almost concomitantly with the initiation of intranasal cooling. A relative increase in cerebral blood flow would further contribute to the cooling effect. Data from our experiment could provide some evidence to support this mechanism.

In study IV, no shivering was reported and there was a possible vasoconstriction associated with intranasal cooling. Cooling is usually associated with shivering and reflex vasoconstriction, protective mechanisms that tend
to raise the body’s temperature [115]. The shivering threshold in supine patients is a decrease in core temperature by 0.37°C/hour [116]. It is interesting that although this value was attained, none of the volunteers reported shivering during intranasal cooling. Cutaneous cooling, which contributes 20% to control of vasoconstriction and shivering [117], was mitigated by covering the volunteers with blankets. The absence of shivering, which usually hinders cooling, might imply that the metabolic rate was unchanged.

A mean forearm-finger temperature difference higher than 4°C is interpreted as a sign of thermoregulatory peripheral vasoconstriction [118]. Higher differences were recorded both at the beginning and at the end of the present experiment, which makes a cooling-associated vasoconstriction [110] hard to prove. The level of stress experienced by the volunteers in the MR scanner could complicate the interpretation of these results [119, 120]. Therefore, it is hard to differentiate between cooling and stress-induced vasoconstriction in this study.

The results of study IV are comparable with those reported by Pretorius et al. during whole-head, facial and dorsal immersion in 17 °C water [121]. In their study, isolated facial cooling events induced body cooling rates of 0.47 ± 0.1 °C/hour. Similar to our results, heart rate and mean arterial pressure remained unchanged during face cooling. The volunteers remained alert throughout the experiment. Peripheral vasoconstriction was detected but no shivering was reported. The metabolic rate was unchanged, whereas an increase in heat loss through the exposed surface of the head was measured. These results were explained by the redistribution of circulation due to trigeminal activation and peripheral vasoconstriction. This produced a relative increase in blood flow to the head with enhanced heat loss, which was responsible for core cooling [121].

It is tempting to speculate that intranasal cooling with the cold balloons, similar to face cooling, exploited the same mechanisms. It could be hypothesized that an increased rate of heat loss through the head and via the nasal balloons reduced the temperature of the venous blood returning to the heart [paper I] and consequently reduced the arterial blood temperature. An increase in cerebral blood flow is elicited via the trigeminal nerve. Stimulation of the anterior ethmoidal nerve, a branch of trigeminal nerve that innervates the face and nasal passages, triggers the trigeminal cardiac reflex [122]. The activation of this reflex produces reflex peripheral vasoconstriction and cerebrovascular vasodilatation and induces mechanisms of neuroprotection [123, 124]. This reflex is included in a group of reflexes defined by oxygen-conserving effects [122]. An activated oxygen preservation mechanism may explain the absence of shivering in the presence of cooling. Trigeminal activation is also implicated in migraine pain pathways [125] and could thus be responsible for the post-cooling headache reported by four of the volunteers. Further evidence for the existence of a reflex basis associated with the intranasal cooling with balloons was possibly provided by the results obtained in
volunteer 8, where only one balloon was present. The existence of a trigeminal reflex was also described in animals such as cats, rabbits and rats [126-128]. It is possible that the intranasal balloons in pigs made use of the same pathways to induce their effects, at least when normal circulation was present.

The haematogenous spread of cooling might explain the relatively uniform intra-cerebral distribution of temperature reductions [paper III, IV, 73, 129]. There was an inter-individual variation in brain temperature reductions. This is not so surprising if one considers several factors. Firstly, while the baseline body temperature is closely regulated around a balance point [130], it is also highly individual [131]. Secondly, the mean brain-body temperature difference in normothermia varies between 0.3°C [52] and 2.3°C [114]. In a study on normothermia in 18 healthy volunteers, we found a brain-body temperature difference of 1.3 ± 0.4°C when brain temperature was measured non-invasively [132]. Differences in the baseline temperatures and brain-body temperatures, and a variable cooling efficiency, could explain the different results. Thirdly, no sedation was used. Sedation alters the inter-threshold zone and renders the body poikilothermic [115]. The use of sedation might have produced more predictable results after thermal manipulation [115].

This study lacked a control group with no cooling intervention. However, body temperatures can rise during MR procedures [133], which mean that the temperature reductions detected in our volunteers were due to the cooling method.

In summary, direct local cooling and locally mediated systemic effects may explain the reduction in brain temperature induced by intranasal cooling with saline at 20°C circulating within the balloons. This method induces an increase in the brain-body temperature difference via the return of cold venous blood to systemic circulation, augmenting cerebral blood flow via trigeminal nerve activation. These conditions could increase the rate of heat removal from the brain and explain the uniformity of the cooling effect. The reflex induced by intranasal cooling may induce a state of oxygen conservation that explains the absence of shivering when normal circulation is present.

**Haemodynamic effects**

The intranasal cooling via cold saline circulating within balloons applied in subjects with normal circulation produced minimal circulatory changes. In study I, a reduction in heart rate was observed after cooling was initiated, similar to an effect previously described [134-136]. Mild hypothermia has been shown to depress the arterial baroreflex and to decrease the heart rate with an increase in stroke volume, an effect that might be beneficial in resuscitation.

In study II, after cardiac arrest, intranasal cooling was studied both alone and associated with intravenous cold fluids. The early initiation of hypo-
Hypothermia might explain the similar success rates of resuscitation for both groups [137, 138]. Intranasal cooling was beneficial even after prolonged cardiac arrest, where it dramatically improved the ROSC rate and reduced CPR duration [139]. The administration of cold intravenous fluids and intranasal cooling were both tested pre-hospital in human studies with good results [17, 46, 140]. These methods proved to be feasible, safe and improved the neurological outcome.

Hypothermia was rapidly induced in both groups. The haemodynamic parameters monitored did not significantly differ between the groups. The one exception was that of mixed venous oxygen saturation, which was lower in the group with only intranasal cooling during both cooling and rewarming. In the post-ROSC period, the pigs in the intranasal cooling group needed more fluid boluses and a higher dobutamine dose compared to the combined method group.

It was previously shown that the left ventricular ejection function was improved after nasopharyngeal cooling only [137, 141, 142], or by endovascular cooling in a model of myocardial ischaemia [143]. In the same study by Yannopoulos et al. [143], the infusion of cold saline during early CPR was shown to augment the post-ROSC global myocardial dysfunction due to volume loading. On the other hand, the systemic ischaemic-reperfusion response described post-cardiac arrest is sepsis like and includes intravascular volume depletion, impaired vasoregulation and impaired oxygen delivery and utilization, which may respond to early induced treatment [144]. Good myocardial function was also shown in humans after cardiac arrest and cold intravenous fluid-induced mild hypothermia [145]. However, the patients studied needed large amount of fluids during the post-resuscitation period despite a positive fluid balance.

Since none of the other variables differed, we presume that in the intranasal cooling group mixed venous oxygen saturations were lower due to an increased rate of metabolism and heighten peripheral oxygen extraction [146]. This may mean that the oxygen-conserving mechanism described [122-124] was not working in this setting or that its effect is limited to the brain. Intranasal cooling was applied early, before the return of circulation. It can be speculated that under this circumstance cold intranasal balloons only have a local brain cooling effect and do not activate trigeminal reflexes. This need not mean that the local cooling effect is not neuroprotective after cardiac arrest.

However, it seems that the heart and circulation after cardiac arrest could benefit from the induction of hypothermia by intranasal cooling and one early dose of intravenous cold fluids with a continued intranasal maintenance of hypothermia. There is also a need for more research in order to chart the early and late effects of intranasal cooling via cold saline circulating within intranasal balloons on myocardial contractility and circulation after cardiac arrest.
Intracranial pressure

Hypothermia decreases the intracranial pressure (ICP) after traumatic brain injury [32,36,147,148]. In study II, ICP was decreased after cardiac arrest in both cooling groups. The reduction in ICP was parallel to the reduction in brain and body temperatures. The administration of only cold intravenous fluids after cardiac arrest in pigs in a similar experimental model to ours did not reduce ICP [149]. This effect was explained by the volume of fluid administered as well as the possible occurrence of vasogenic or cytotoxic brain oedema [149,150]. However, the rapid infusion of cold saline controlled the temperature in selected brain-injury patients without affecting ICP [151].

During rewarming, the ICP increased more in the intranasal cooling group compared to the combined cooling group. The risk of an increase in ICP after rewarming was also previously described after traumatic brain injury [152]. A rapid rewarming can lead to impaired vascular reactivity with vasodilatation and a rebound increase in intracranial pressure [153]. Data on rewarming after hypothermia for cardiac arrest were lacking, but inspired by studies in traumatic brain injury patients, the current recommendations suggest a rewarming rate of less than 0.5°C/hour [154]. In study II, the rate of active rewarming in the intranasal cooling group was higher than that recommended. In the same group, brain temperatures were higher after rewarming and this may have influenced the results. However, there is data to show that temperatures over 37°C after hypothermia alter the cerebrovascular reactivity but do not raise ICP if the rate of rewarming is low [155]. In the combined cooling group, a resistance to rewarming via the intranasal balloons was observed that was probably due to deep core hypothermia induced by the cold fluids. The result was a slower rate of rewarming. This might indicate that the rate of rewarming was more important to the rise in ICP in this experiment than the level of absolute temperature.

Other factors may have played a role in the observed effect of fast rewarming. In the intranasal cooling group, due to circulatory instability the amount of fluids was larger and this may have decreased intracranial compliance [156]. The fluids were also at room temperature, which may have further contributed to the increase in temperature. In study III, after 30 min of intranasal cooling, rewarming was passive and a return to baseline levels occurred after approximately 120 min. This means that after short-term intranasal cooling, the passive rewarming rate reached in pigs was about 1°C/hour. No measurements of intracranial pressure were made in study III.

The possible associated reflex nature of the cooling response after intranasal cooling with cold balloons during normal circulation makes the occurrence of deep core cooling short-lived and unreliable. One may presume that after induction with intranasal balloons the cooling should be maintained, either continuously (intranasal) or by other methods in order to avoid the risks associated with a fast rewarming rate.
Future perspective

The early application of intranasal cooling balloons in association with intravenous cold fluids after cardiac arrest will be further studied in a pilot feasibility study. This will provide more data regarding the use of this technique in humans.

The application of intranasal balloons for neuroprotection after stroke and brain injury would be a very interesting research area where the vascular and metabolic effects of intranasal cooling might be studied.

The nasal mucosa is a complex structure containing specific receptors [157-159]. A part of them are mechanically-sensitive receptors that respond to pressure and to temperatures in the range of 8-22°C and over 42°C. Central connections via trigeminal or glossopharyngeal nerves relay stimuli to a vast array of neuronal populations in the brain and brain stem. The activation of these zones induces several reflexes with local, mucosal, and systemic importance, such as the trigeminal cardiac reflex [122-125]. The presence of the intranasal cold balloons could activate many of these pathways and induce potentially beneficial but also deleterious effects, such as migraine and sinusitis [160]. This could be an interesting path to explore.

An organism is a complex thermodynamic system that has one output, the temperature. This makes a certain temperature level an attractive therapy goal easy to attain. But in the studies discussed, a recurring problem was the sometimes large spread of temperatures, both at baseline and after the induction of cooling. This is a widely encountered problem [131] that is usually explained by measurement errors and/or so-called individual factors. As such, a specific range of temperatures set as goal for hypothermia treatment might be too rudimentary to treat a unique individual. Instead of using the temperature in a “no” or “yes” judgement one can nowadays study this phenomenon in-depth. Temperature curve complexity was studied and it was shown that the reduction of temperature’s complexity response reflect an injury of the biological system and could predict survival in critical ill patients [161,162]. The practical application of non-linear dynamic theories to every day diagnostic and treatment decisions is an immense research area just waiting to be explored. When manipulating the body’s thermoregulation in an effort to restore order where chaos emerged, these thoughts seem even more relevant.
Conclusions

Intranasal cooling with balloons circulated with cold saline may induce preferential cerebral hypothermia under conditions of normal circulation as well as after cardiac arrest [paper I-IV].

Intranasal cooling with balloons circulated with cold saline can also be used to maintain and control hypothermia under conditions of normal circulation as well as after cardiac arrest [paper I, II, IV].

Body temperature, while affected by the intranasal cooling, can be maintained higher than the brain temperature by external heating [paper I].

Systemic effects are minimal and possibly protective (no shivering) when intranasal cooling is applied under conditions of normal circulation [paper I, IV].

Intranasal cooling with balloons circulated with cold saline may enhance the effect of intravenous cold fluids after cardiac arrest [paper II].

Intranasal cooling with balloons circulated with cold saline associated with intravenous cold saline after cardiac arrest in pigs seems to provide additional physiological stability [paper II].

Intranasal balloon cooling alone or combined with intravenous cold fluids reduces intracranial pressure after cardiac arrest [paper II].

The intranasal balloons can also be used to induce rewarming [paper II].

The effects on brain temperature, induced by cooling methods, can be studied with the help of magnetic resonance techniques [paper III, IV].

Intranasal cooling with balloons circulated with 20°C saline was well tolerated by awake and unsedated volunteers and the local complications were minimal after one hour experiment [paper IV].
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References

30. Hess ML, Manson NH. Molecular oxygen: friend and foe. The role of the oxygen free radical system in the calcium paradox, the oxygen paradox and ischemia/reperfusion injury. J. Mol. Cell Cardiol 1984; 16:969–85;


123. Reis DJ, Golanov EV, Galea E, Feinstein DL. Central Neurogenic Neuroprotection: Central Neural Systems That Protect the Brain from Hypoxia and Ischemia. Ann N Y Acad Sci 1997;835:168-86;


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