

Title: Brain Temperature, Perfusion and Metabolism under Thermo-Neutral Condition and with Induced Hypothermia

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PhD Thesis

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Thesis ~32,500 words.

Declaration

I, Sachiko Iwata, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Contribution

I, Sachiko Iwata performed surgical procedures, instrumentation, physiological data collection and provided intensive care for all animal experiments with my collaborators; in the clinical study, in which physiological data were collected from newborn infants, I was in charge of echocardiographic and temperature data collection. I also performed the data analysis for all data related to this current thesis, and pursued manuscript writing with support and suggestions by my supervisors and collaborators.

Abstract

Background:

Therapeutic hypothermia following perinatal hypoxia-ischaemia improves survival and neurological functioning at 18 months of age. Precise brain temperature control commenced shortly after birth may improve the beneficial effects.

Aims:

The aim of this thesis was (i) to clarify associations of regional brain temperatures with the body weight, ambient temperature, cerebral perfusion and oxygen metabolism under normothermia and induced hypothermia; and (ii) to develop safe and reliable cooling device for pre-hospital cooling.

Methods:

1. Regional brain temperatures were monitored in 14 anaesthetised newborn piglets with normothermia, whole body cooling and selective head cooling.
2. The rectal temperature (T_{rectal}), non-invasive brain temperatures (zero-heat flux method), relative superior vena-cava (rSVC) flow (rSVC flow; echocardiography) to the brain weight, and the cerebral metabolic rate of oxygen relative to rSVC flow (CMRO_2 index; measured using near-infrared spectroscopy), were obtained in 32 newborn infants.
3. Eleven anaesthetised newborn piglets were cooled to 33-34°C 2-26 h after hypoxia-ischaemia using either water bottles or phase changing material (PCM), which works as a heat buffer at 32°C.

Results:

1. In the piglet model, brain temperatures were positively correlated with the body weight under normothermia, whole body cooling and selective head cooling, the influence of which was prominent under selective head cooling.
2. In the normothermic newborn infant, rSVC flow and CMRO_2 index were positively coupled between each other. A greater rSVC flow was associated with higher superficial brain temperatures; brain temperatures were independent of the CMRO_2 index. A cooler ambient temperature was associated with a greater temperature gradient between the scalp surface and the body core.

3. In the piglet model, therapeutic hypothermia was induced and maintained with both water bottles and PCM; the latter provided more stable cooling within the target range.

Conclusions:

Brain cooling was more efficient with lower body weight due to greater head surface area to volume ratios; body-size adjustment may be required to accomplish consistent regional temperatures. In healthy newborn infants under thermo-neutral temperature, cerebral perfusion and ambient temperature, but not cerebral metabolism, were significantly associated with brain temperatures. PCM cooling provided stable body temperatures, which may substitute for electronic cooling devices during transportation.

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Abbreviations

aEEG - amplitude-integrated electroencephalogram

ANOVA - analysis of variance

CBF - cerebral blood flow

CBV - cerebral blood volume

CMRO₂ - cerebral metabolic rate of oxygen

FiO₂ - fraction of inspired oxygen

Hb – haemoglobin

LVO - left ventricular output

MRI - magnetic resonance imaging

NIRS - near-infrared spectroscopy

PCM - phase changing material.

rSVC flow - superior vena cava flow corrected to 100 g brain mass

SpO₂ – percutaneous arterial oxygen saturation

SD - standard deviation

SVC - superior vena cava

TOI - tissue oxygenation index

T_{brain-25} and T_{brain-15} - brain temperatures measured using a zero-heat flux thermometer with probe diameters of 25 mm and 15 mm respectively

T₅, T₁₀, T₁₅ and T₂₀ – invasive brain temperatures 5, 10, 15 and 20 mm respectively from the brain surface measured using a multi-channel fibre-optic probe

T_{scalp} - forehead scalp skin temperature measured using a non-contacting infrared thermometer

T_{rectal} - rectal temperature

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Chapter 1

Introduction

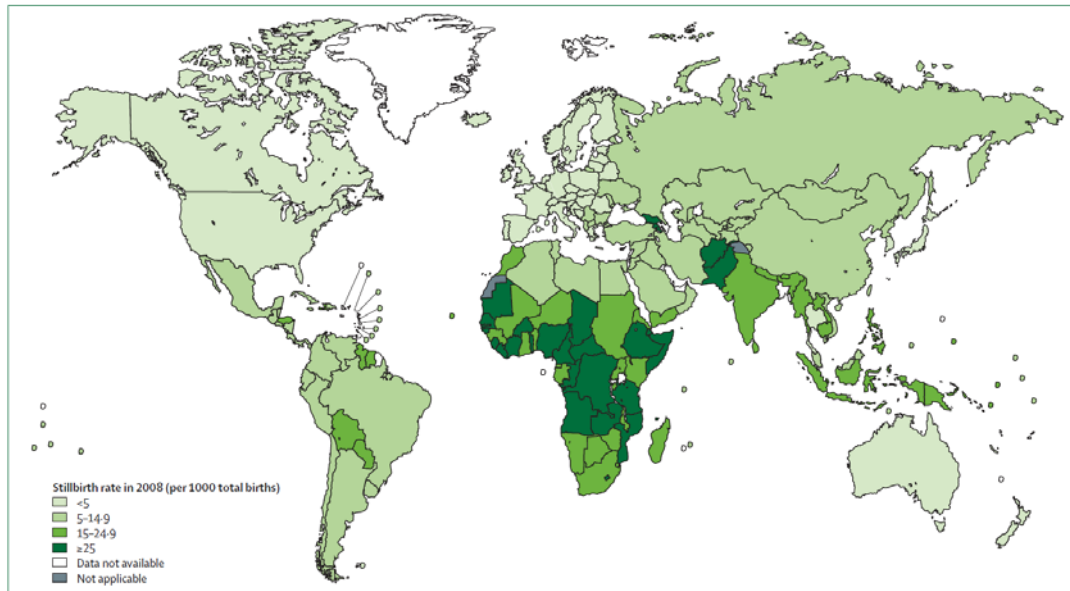
1.1 Impact of perinatal hypoxia-ischaemia

1.1.1 Global impact of perinatal hypoxia-ischaemia and neonatal encephalopathy

Acute neonatal encephalopathy following perinatal hypoxia–ischaemia is a major cause of neonatal death in developing countries, where approximately 23% of annual neonatal deaths worldwide are attributed to perinatal hypoxia–ischaemia (1).

Perinatal hypoxia–ischaemia remains a common causative of neonatal death and permanent neurological deficit even in developed countries, where the incidence of moderate to severe neonatal encephalopathy is 1 to 2 per 1000 term births in the UK and other parts of the developed world (2, 3).

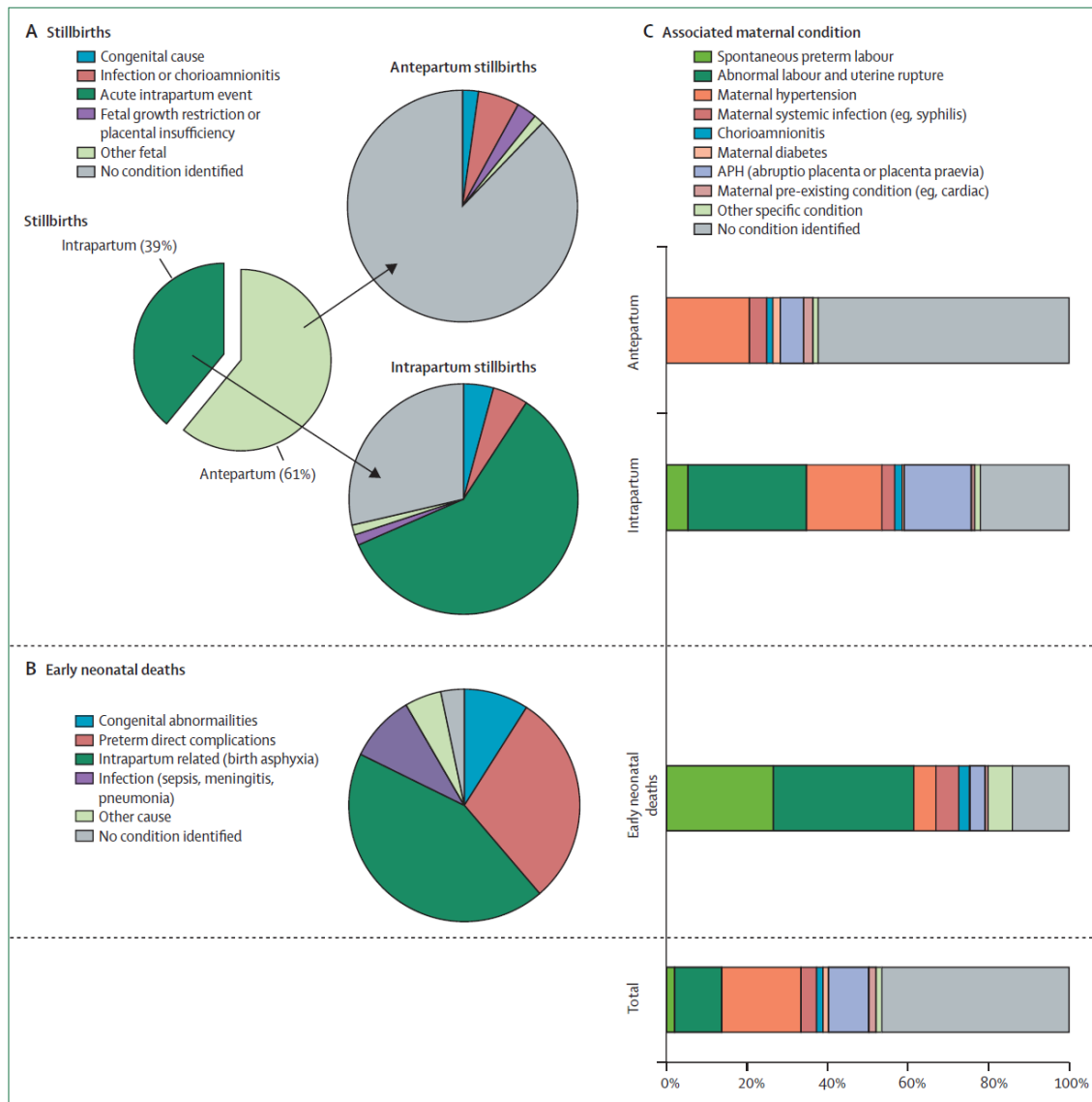
Figure 1.1: Country variation in third-trimester stillbirth rates in 2008



The rate of stillbirth in developing countries remain much higher compared to the rate in developed countries.

From “Stillbirths: Where? When? Why? How to make the data count?” by Lawn JE, Blencowe H, Pattinson R, Cousens S, Kumar R, Ibiebele I, Gardosi J, Day LT, Stanton C; Lancet’s Stillbirths Series steering committee. Lancet. 2011 Apr 23;377(9775):1448-63.

Figure 1.2: Stillbirths, early neonatal deaths and causatives



Antepartum stillbirths, intrapartum stillbirths, and early neonatal deaths with fetal (A) or neonatal (B) causes and associated maternal conditions (C). Data based on 19,976 stillbirths and 8562 neonatal deaths in South Africa, 2008–09. Data from Medical Research Council Maternal and Infant Health Care Strategies Research Unit.⁷²

From “Stillbirths: Where? When? Why? How to make the data count?” by Lawn JE, Blencowe H, Pattinson R, Cousens S, Kumar R, Ibiebele I, Gardosi J, Day LT, Stanton C; Lancet's Stillbirths Series steering committee. *Lancet*. 2011 Apr 23;377(9775):1448-63.

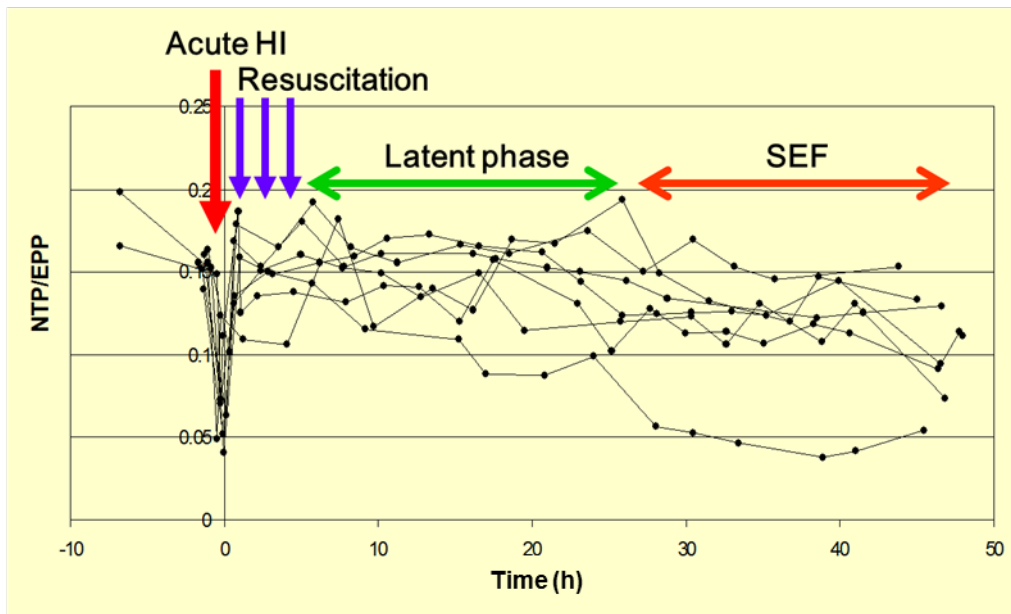
Abbreviation: APH, antepartum haemorrhage.

The outcome associated with neonatal encephalopathy varies according to the type, depth and duration of hypoxic-ischaemic events as well as the maturational state of the infant's brain. Infants with mild neonatal encephalopathy generally recover well without apparent neurological impairments (4, 5). In contrast, subjects with moderate to severe neonatal encephalopathy are at significant risks for neonatal mortality or survival with serious neurodevelopmental impairments; it is this group of infants who benefit from neuroprotective treatments, with a reduction in the risk of death or disability and increased survival free of disability at 18-24 months (6-10). The favourable outcome at 18-24 months is associated with favourable outcomes at 6-7 years (11, 12).

1.1.2 Mechanism of cerebral injury following perinatal hypoxia-ischaemia

Brain injury after transient hypoxia–ischaemia is an evolving process (13): severe hypoxia–ischaemia causes acute energy depletion to the cerebral tissue, leading to immediate cell death to a fraction of neuronal and glial cells. Successful resuscitation may help restore cerebral energy metabolism, however, reperfusion/reoxygenation may additionally precipitate complex biochemical events, which eventually lead to secondary energy failure evolving after 8-30 h of resuscitation (14), and some additional delayed neuronal death (15-19).

Figure 1.3: Acute hypoxia-ischaemia and evolution of secondary energy failure



NTP (mainly adenosine triphosphate) relative to EPP with time in the piglet model of asphyxia encephalopathy. Following successful resuscitation, cerebral high energy phosphate transiently recovers to the normal level (latent phase), which is followed by SEF after 8-30 h.

Abbreviation: HI, hypoxia-ischaemia. SEF, secondary energy failure. EPP, exchangeable high-energy phosphate. NTP, Nucleotide triphosphate.

Experimental studies suggested that neuroprotective treatments are likely to be beneficial only when they are applied before the overt evolution of secondary energy failure (13). A study in a piglet model of neonatal hypoxia–ischaemia demonstrated that the greater the acute energy deficit, the sooner the evolution of secondary energy failure, highlighting the importance of early initiation of treatments for severely asphyxiated subjects (20).

1.2 Hypothermic neuroprotection for neonatal encephalopathy

1.2.1 Mechanisms of hypothermic neuroprotection

A range of neuroprotective treatments have been proposed by basic scientists, the number of which is still increasing every year (21, 22). However, no pharmacological treatment has thus far been confirmed to be clinically neuroprotective in the newborn infant, except for magnesium sulphate, which is administered antenatally in the preterm infant (23). In contrast, therapeutic hypothermia has demonstrated exceptional neuroprotective properties in both experimental and clinical settings.

Figure 1.4: Injury cascade following perinatal hypoxia-ischaemia

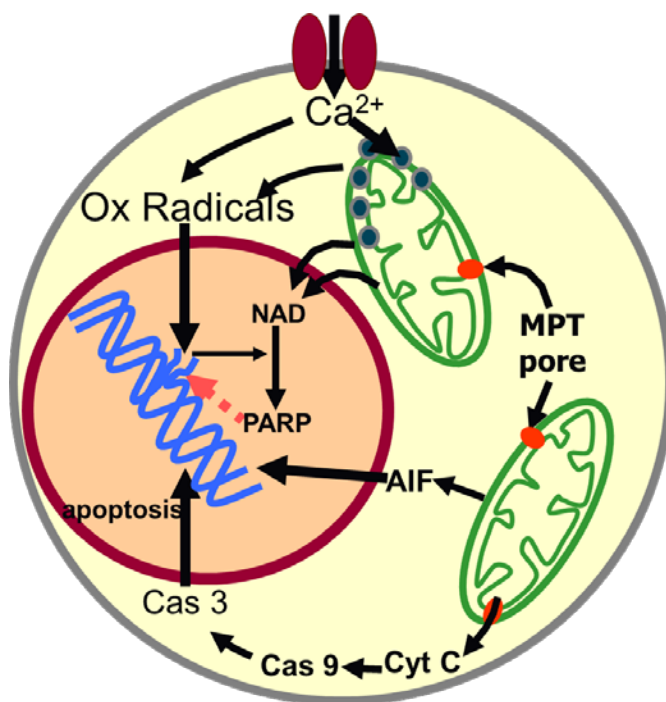


Diagram depicting the deleterious injury cascade following hypoxic-ischaemic stress. Even after successful resuscitation, cytotoxic neuronal injury persists resulting in acute necrosis and delayed apoptosis in the tissue. Hypothermia is known to ameliorate most of these degenerative reactions.

Abbreviations: AIF, apoptosis inducing factor. Ca, calcium. Cas, caspase. Cyt C, cytochrome c. MPT, mitochondrial permeability transition. NAD, nicotinamide adenine dinucleotide. Ox Radicals, oxygen radicals. PARP, Poly (ADP-Ribose) Polymerase.

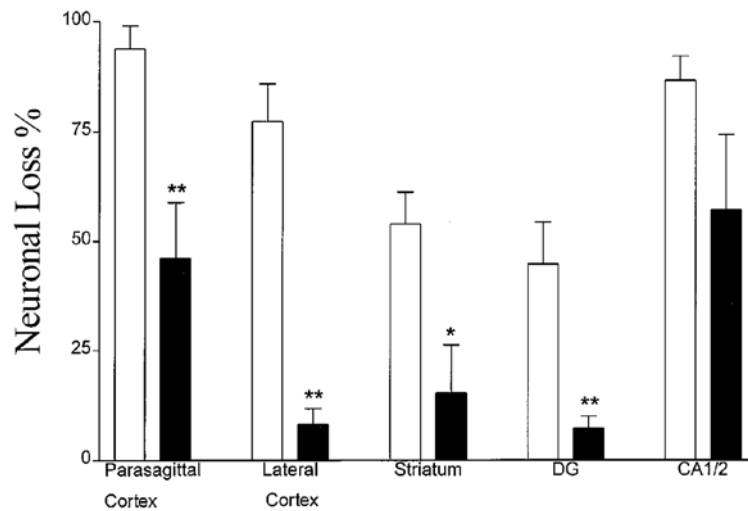
Unlike some pharmacological treatments targeting specific stages within the injury cascade, the neuroprotective effect of therapeutic hypothermia covers a wide range of deleterious reactions (24). Therapeutic hypothermia reduces the metabolic rate of the cerebral tissue by approximately 4–7% for 1°C temperature reduction (25-27), decreases the release of glutamate and other excitotoxic neurotransmitters (28, 29), attenuates the activity of N-methyl-D-aspartate receptors (30), reduces the production of nitric oxide and oxygen radicals (30-32) and contributes to decreasing intracranial pressure (33, 34).

1.2.2 Neuroprotective effect of therapeutic hypothermia in preclinical models

In preclinical models, mild to moderate therapeutic hypothermia (temperature 32-34°C) reduces secondary brain injury and improves behavioural outcome when applied immediately after transient hypoxia–ischaemia (35-37).

There is also evidence to suggest that significant long-term neuroprotection occurs in experimental models even with a delay of 2-6 h between hypoxia–ischaemia and the commencement of cooling (38-43).

Figure 1.5: Neuronal loss following fetal ischaemia with or without selective head cooling 6-72 h after resuscitation



Microscopic neuronal loss was assessed in different brain regions 5 days after ischemia. A significant overall reduction in neuronal loss was seen in fetuses treated with selective cerebral cooling (closed bars) compared with sham-cooled fetuses (open bars), except in the most severely affected field of the hippocampus, CA1/2.

From “Neuroprotection with prolonged head cooling started before postischemic seizures in fetal sheep.” by Gunn AJ, Gunn TR, Gunning MI, Williams CE, Gluckman PD. *Pediatrics*. 1998 Nov;102(5):1098-106.

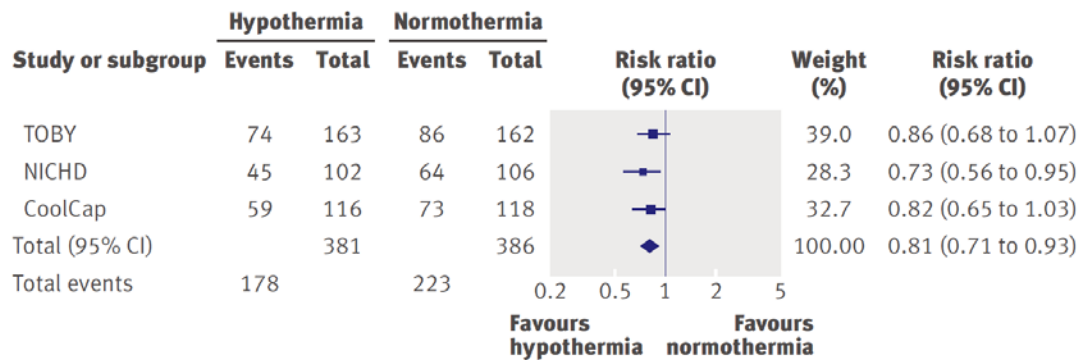
Abbreviations: DG, dentate gyrus. CA1/2, hippocampus zone 1 and 2.

1.2.3 Clinical trials of therapeutic hypothermia for neonatal encephalopathy

Based on the accumulated preclinical evidence and the safety consideration by several clinical pilot studies, two large-scale randomised controlled trials of therapeutic hypothermia were carefully designed and conducted in infants with moderate to severe neonatal encephalopathy. These clinical studies, using selective head cooling or whole body cooling, demonstrated that mild to moderate therapeutic hypothermia was

associated with significant reduction in death or severe disability at 18 months of birth, except in subjects who showed the most severe pattern on the amplitude-integrated electroencephalogram (aEEG) and were cooled using selective head cooling (8, 9). Further large-scale trials of therapeutic hypothermia using similar patient selection and cooling criteria to the former trials consistently confirmed the neuroprotective effect of therapeutic hypothermia (7, 10, 44, 45).

Table 1.1: Effect of therapeutic hypothermia on death or disability



Forest plot of the effect of therapeutic hypothermia compared with standard care (normothermia) on death or disability (“events”) from randomised controlled trials including the Total Body Hypothermia (TOBY) trial, the National Institute of Child Health and Human Development (NICHD) trial, and the CoolCap trial.

From “Neurological outcomes at 18 months of age after moderate hypothermia for perinatal hypoxic ischaemic encephalopathy: synthesis and meta-analysis of trial data.” by Edwards AD, Brocklehurst P, Gunn AJ, Halliday H, Juszczak E, Levene M, Strohm B, Thoresen M, Whitelaw A, Azzopardi D. *BMJ*. 2010 Feb 9;340:c363.

A meta-analysis based on the results of these clinical trials confirmed that therapeutic hypothermia commenced within 6 h of birth reduces risks for mortality, death or severe neurodevelopmental impairments, and neurological impairments in survivors at 18

months corrected age (46). More recent meta-analysis conducted by the Cochrane Neonatal Review Group further confirmed that the benefits of cooling on survival and neurodevelopmental outcome outweigh the short-term adverse effects such as sinus bradycardia and thrombocytopenia (47). Since 2010, therapeutic hypothermia in the tertiary centre setting has been recommended for near-term to term newborn infants with moderate to severe neonatal encephalopathy in “2010 International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science with Treatment Recommendations” (48) and was endorsed by the UK National Institute for Clinical Excellence (NICE) (49).

Although clinical evidence supporting the benefit of therapeutic hypothermia following perinatal hypoxia-ischaemia is robust, findings from clinical trials newly raised important questions around the optimal indication, window of opportunity, cooling modalities, target temperature and cooling duration. For example, in two large-scale randomised controlled trials of neonatal hypothermia, the primary hypothesis, or the amelioration of death or severe neurodevelopmental impairments by 18 months of age in cooled infants, has been refuted; the beneficial effect of therapeutic hypothermia has only been shown in a subgroup of infants (8) or in the secondary endpoint (7). Especially in a trial which used selective head cooling, the treatment appeared to be ineffective for infants with relatively smaller body weights or for ones who showed the most severe findings on the amplitude-integrated electroencephalogram at admission (50). Interestingly, the impact of the body size and the severity of encephalopathy on the efficacy of hypothermia was not observed in studies which used whole body cooling (7, 51). Although selective head cooling and whole body cooling have been demonstrated to provide similar beneficial effects, it is possible that current cooling modalities, especially selective head cooling,

might still be optimised by adjusting cooling temperatures for patients' clinical backgrounds and injury patterns. In addition, a number of infants who were randomised to normothermia in these trials developed fever greater than 38°C, which was related with unfavourable outcomes (7, 45, 50, 52). It is still unclear whether such fever was induced by suboptimal temperature control (e.g. excessive warming and sparse body temperature monitoring), or was spontaneously induced as a consequence of relatively more severe hypoxia-ischaemia. Further studies are required to identify patients who are most likely to benefit from therapeutic hypothermia, and to establish cooling protocols which provide optimal neuroprotection to infants with different injury patterns and clinical backgrounds.

1.3 Key factors required to optimise efficacy of therapeutic hypothermia

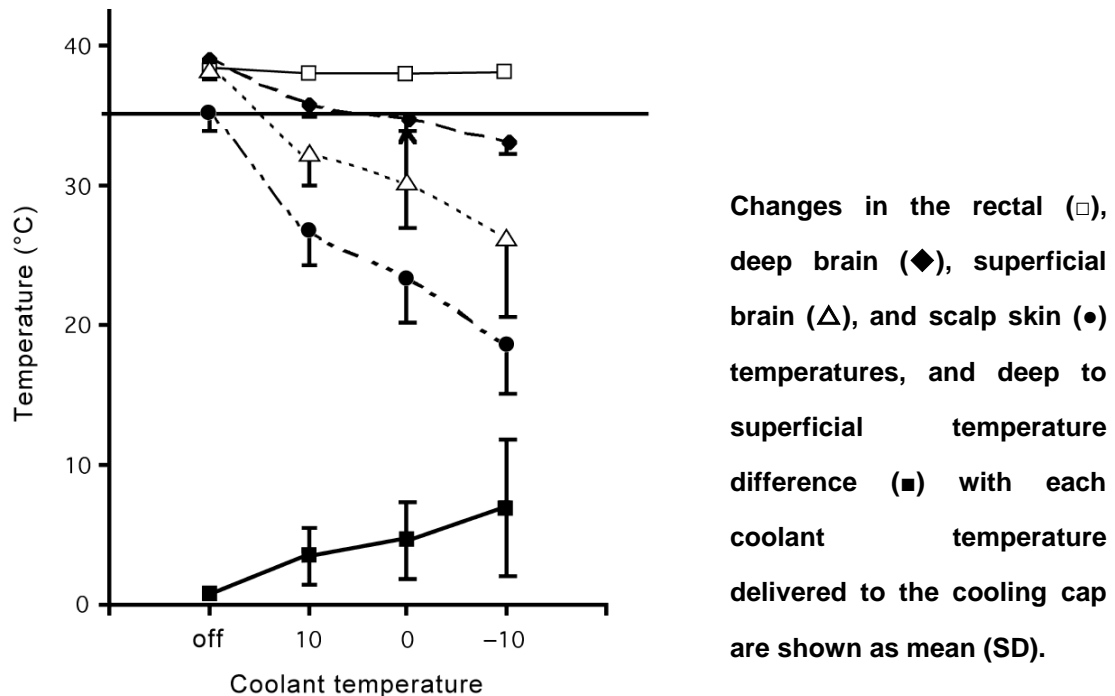
1.3.1 Newly raised questions around therapeutic hypothermia

Although therapeutic hypothermia has now been promoted into a standard of care for asphyxiated newborn infants, several important questions were raised relating to the optimal application of this treatment. There remains uncertainty about the precise temperature reduction, therapeutic time window, duration and method of cooling (whole body cooling and/or selective head cooling) that provides optimal neuroprotection (53). Furthermore, it is also unknown whether different regions or cell types in the brain are protected equally by the same fall in temperature. Although the currently accumulated clinical evidence suggests similar neuroprotective effects between whole body cooling (7) and selective head cooling (8), no study has compared the difference in the strength and type of neuroprotection provided by these cooling modalities.

1.3.2 Cooling modality and brain temperature distribution – normothermia and whole body cooling

As the first step towards studying what are the optimal temperatures for best neuroprotection in different brain regions, it is also important to understand the temperature gradients within the body and the brain with whole body and selective head cooling. From previous studies we know that, in newborn piglets at normal temperature, the deep brain temperature is similar to the rectal temperature (T_{rectal}) whereas, because of scalp heat loss, the superficial brain is up to 1°C cooler (54-56).

Figure 1.6: Temperature distribution under normothermia, whole body cooling and selective head cooling in the newborn piglet



From “Brain temperature in newborn piglets under selective head cooling with minimal systemic hypothermia.” by Iwata O, Iwata S, Tamura M, Nakamura T, Sugiura M, Ogiso Y. *Pediatr Int.* 2003 Apr;45(2):163-8.

Under whole body cooling, body temperature is reduced without altering subtle temperature gradients between body core temperatures and overall brain temperatures (57). The majority of experimental studies and clinical trials have used whole body cooling because of its simplicity in the temperature control and application (58).

1.3.3 Cooling modality and brain temperature distribution – selective head cooling

Selective head cooling has been used to theoretically minimise the risk for adverse systemic effects such as respiratory and cardiac suppression, sympathetic stimulation

and coagulation disorders (25, 59). Selective head cooling is induced by a cooling cap with simultaneous trunk warming thereby creating large differences between body core and both deep brain and superficial brain temperatures (55, 57, 58, 60).

Figure 1.7: Temperature distribution under normothermia, whole body cooling, selective head cooling and their combination

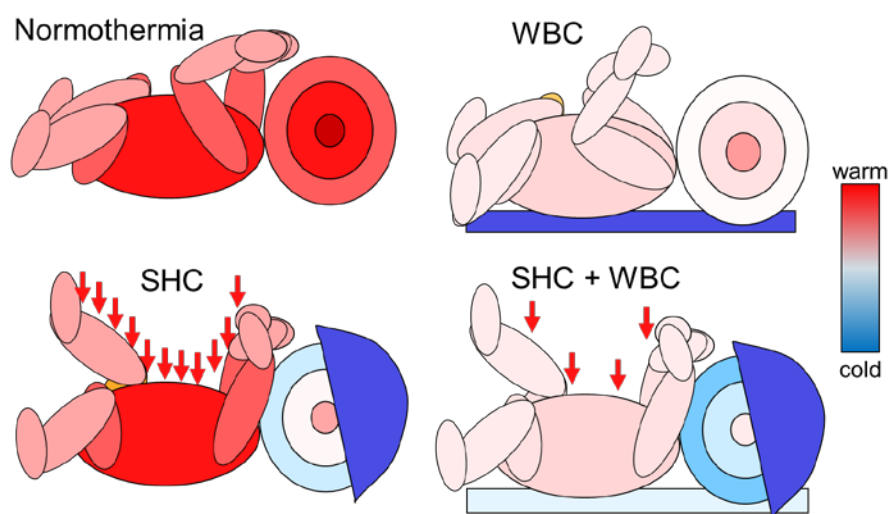
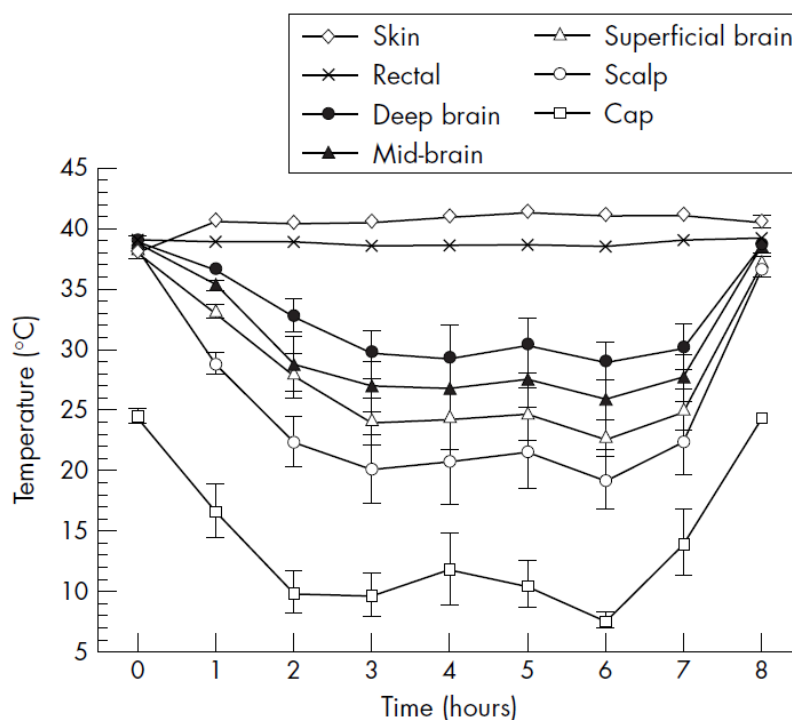


Diagram depicting the temperature gradients between body core, deep brain and superficial brain. Under normothermia and WBC, temperature gradients remain minimum, whereas SHC introduces substantial temperature gradients within the body and the brain. Abbreviations: WBC, whole body cooling. SHC, selective head cooling.

Temperature control with selective head cooling is more complex compared to whole body cooling because of the active thermal flow caused by simultaneous head cooling and body warming. Nevertheless, this cooling modality has been applied in two clinical trials (8, 44) expecting to accomplish moderate hypothermia in the deep brain structure while maintaining slightly higher core body temperature compared to whole body cooling.

Although little is known about the actual temperature distribution in the human infant under therapeutic hypothermia, several experimental studies have investigated the temperature of deep brain structures using invasive temperature monitoring (55, 57, 60). These studies demonstrated that selective head cooling applied to newborn piglets can lower deep-brain temperatures below the body core temperature. The temperature difference between the body core and the deep brain has been used as a measure of selective head cooling efficiency.

Figure 1.8: Highly efficient surface cooling of the brain while maintaining the normal core body temperature in the newborn piglet



Significant head cooling can be achieved while maintaining normothermia in the newborn piglet. Values are shown as mean (standard error).

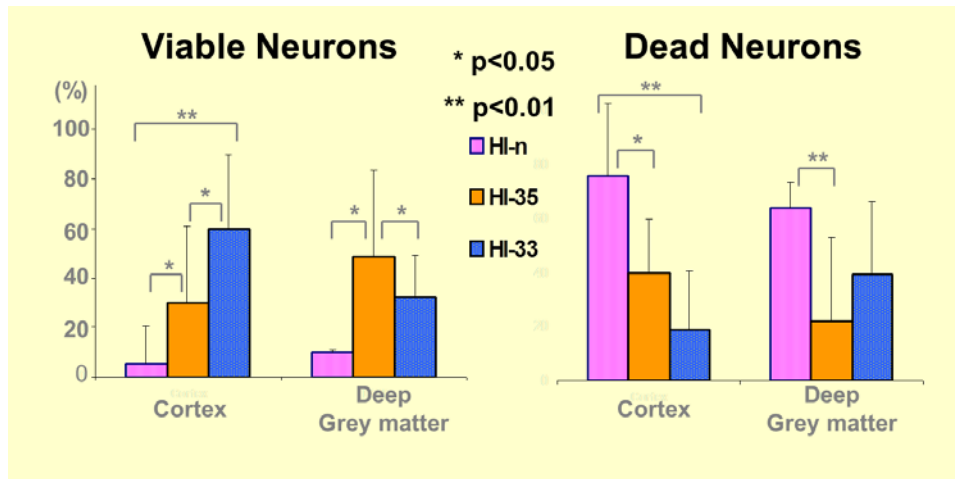
From “Significant head cooling can be achieved while maintaining normothermia in the newborn piglet.” by Tooley JR, Eagle RC, Satas S, Thoresen M. Arch Dis Child Fetal Neonatal Ed. 2005 May;90(3):F262-6.

In newborn piglets, using the cooling cap of relatively lower temperatures ~7 to 10°C, the deep-brain temperature was lowered up to 9°C below the T_{rectal} with the latter normal (56). Given that the deep grey matter is highly vulnerable to hypoxia-ischaemia in the term infant (61), it is encouraging that selective head cooling can also provide profound hypothermia to the deep brain structure. However, efficient surface cooling inevitably increases the intracerebral temperature gradient, resulting in very low superficial brain temperatures < 25°C (56). The safety and efficacy of deep hypothermia needs to be investigated, as evidence suggests that a temperature “threshold” exists below which point hypothermia is sub-optimal or even deleterious because of adverse effects of excessive cooling (25, 59, 62, 63).

1.3.4 Cooling modality and spatial protection pattern

In a clinical trial of selective head cooling, to optimise the balance between the efficacy and safety of surface cooling, the cooling cap of ~10 to 20°C was used in combination with mild systemic hypothermia to 34 to 35°C (8). However, the exact temperature distribution provided by this cooling method remains unknown in newborn infants. If hypothermic neuroprotection in a given cerebral region depends on the local tissue temperature, selective head cooling may provide spatial neuroprotection patterns different from those provided by whole body cooling. An experimental study in a piglet model of asphyxial encephalopathy reported that whole body cooling to 35°C provided deep-grey-matter dominant neuroprotection as opposed to 33°C which provided more profound tissue protection in the cortical grey matter but not in the deep grey matter (62).

Figure 1.9: Dependence of neuroprotection pattern on the cooling level in the piglet model of asphyxial encephalopathy



In the piglet model of transient hypoxia-ischaemia (HI), compared to normothermic subjects (HI-n), whole body cooling to 35°C (HI-35) provided modest cortical protection and favourable deep grey matter protection, whereas whole body cooling to 33°C (HI-33) led to optimal cortical protection and less deep grey matter protection.

From “Depth of delayed cooling alters neuroprotection pattern after hypoxia-ischemia.” by Iwata O, Thornton JS, Sellwood MW, Iwata S, Sakata Y, Noone MA, O'Brien FE, Bainbridge A, De Vita E, Raivich G, Peebles D, Scaravilli F, Cady EB, Ordidge R, Wyatt JS, Robertson NJ. *Ann Neurol.* 2005 Jul;58(1):75-87.

An observational study which assessed brain magnetic resonance imaging (MRI) of infants, who were cooled using either whole body cooling or selective head cooling, demonstrated that the latter cooling modality led to relatively more cortical-grey-matter dominant protection compared to whole body cooling, which provided global tissue sparing (64).

Table 1.2: MRI findings in representative brain regions and cooling modalities

Lesions	Noncooled (<i>n</i> = 52), % (<i>n</i>)	SHC (<i>n</i> = 14), % (<i>n</i>)	WBC (<i>n</i> = 20), % (<i>n</i>)
Mild BGT	5.8 (3)	21.4 (3)*	30 (6)*
Moderate BGT	23 (12)†	14.2 (2)	5 (1)
aEEG <6 h after birth	27.2 (6/22)†		
Severe BGT	59.6 (31)†	14.2 (2)	40 (8)
aEEG <6 h after birth	50 (11/22)†		
Severe WM	34.6 (18)	14.3 (2)	25 (5)
Minimal cortical lesions	17 (9)	28 (4)	20 (4)
Moderate cortical lesions	36.5 (19)	35.7 (5)	15 (3)
Severe cortical lesions	26.9 (14)*	0 (0)	25 (5)
Hemorrhage	28.8 (15)	43 (6)	40 (8)

* Statistically significant ($P < .05$) and results with P values within the text.

† Highly significant ($P < .001$) results with P values within the text.

Newborn infants who were cooled using SHC appeared to have relatively less severe cortical lesions, whereas WBC appeared to provide non-region-specific protection.

From “Mild hypothermia and the distribution of cerebral lesions in neonates with hypoxic-ischemic encephalopathy.” by Rutherford MA, Azzopardi D, Whitelaw A, Cowan F, Renowden S, Edwards AD, Thoresen M. *Pediatrics*. 2005 Oct;116(4):1001-6.

Abbreviations: SHC, selective head cooling. WBC, whole body cooling. BGT, basal ganglia and thalamus. WM, white matter.

Another clinical study with a large sample size reported that whole body cooling was associated with improved MRI findings in the deep grey matter, posterior limb of the internal capsule, and white matter, but not in the cortical grey matter (65), supporting the idea that surface cooling of the head may be beneficial for the protection of the cortical grey matter. Although it is likely that cooling modality affects the spatial pattern of neuroprotection derived from therapeutic hypothermia, further large-scale studies are required to identify the exact association between different cooling modalities and subsequent neuroprotection patterns, as another clinical hypothermia study suggested the association between whole body cooling and cortical protection (66).

It is well known that thermogenesis and heat exchange are highly dependent on physiological factors such as motion activity, sympathetic stimulation, cardiac function, cerebral perfusion, and body size (25, 67), suggesting that these factors need to be taken into consideration when monitoring brain temperature using core body temperature.

Figure 1 is a scatter plot showing the relationship between Esophag Temperature (°C) on the x-axis and Head Core Temperature (°C) on the y-axis. The x-axis ranges from 36.4 to 37.8, and the y-axis ranges from 36 to 39.5. Data points are labeled with station codes: OZK, PUS, SKA, FUS, FRA, FIN, TVA, VOL, and EGG. Two regression lines are shown: a solid line for stations OZK, PUS, SKA, FUS, FIN, TVA, VOL, and EGG, and a dashed line for stations FUS, FRA, and FIN. The solid line shows a positive correlation, while the dashed line shows a negative correlation.

hydrocephalus with myelomeningocele (FIN) and one patient with hydranencephaly (SKA)) had lower deep brain temperatures, and subjects with low cardiac output (♠: n=2 including one patient with hypoplastic left heart syndrome (FRA) and one patient with unrepairable complex cardiac malformation with ventricular fibrillation (EGG)) had higher deep brain temperatures, relative to the oesophageal temperature.

35

In newborn infants, who are undergoing therapeutic hypothermia, the proxy brain temperature is measured by the core body temperature from either a rectal or oesophageal probe (7-9). It is also possible that, compared to whole body cooling, such a variation in the regional brain temperature is greater under selective head cooling, by which simultaneous head cooling and body warming inevitably cause an active heat flow and a substantial temperature gradient.

1.3.6 Determination factors of regional cerebral temperature

Regional brain temperatures are determined by the balance between the local heat production and heat removal; the former is represented by the cerebral metabolic rate of oxygen (CMRO₂) whereas the latter depends on the cerebral blood flow (CBF) and heat dissipation through the scalp (68).

Figure 1.11: Determination factors of local tissue temperature

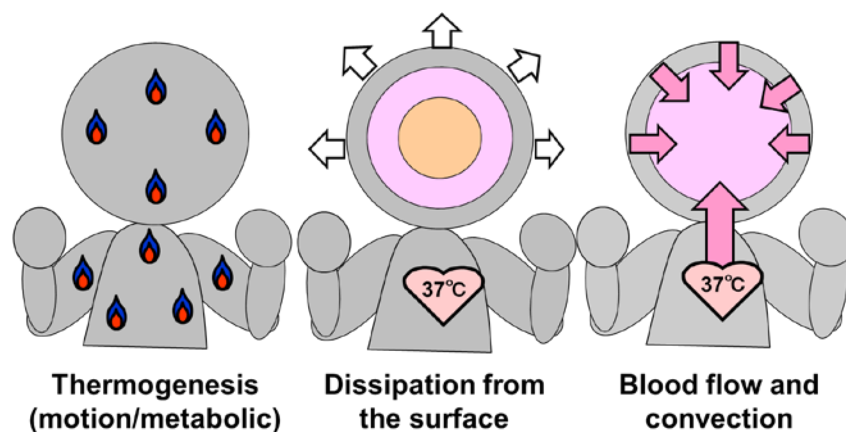


Diagram depicting elements which may determine the regional tissue temperature. Regional temperature levels are determined by the interplay between thermogenesis, heat distribution and dissipation to the ambient environment.

Previous studies in the newborn infant and newborn preclinical models identified factors that determine body/brain temperatures, such as the ambient temperature and humidity, and maturity and body size of subjects (69-71), however, associations between the brain temperature, metabolism and perfusion have not been fully investigated. In a clinical trial which used selective head cooling, the body weight of the infant was identified as an important contributing factor to the efficacy of therapeutic hypothermia; cooling was not as beneficial for infants with lower body weight as those with higher body weights when adjusted for the severity of encephalopathy (50). Pathological backgrounds or injury types characteristic to infants with relatively low birth weight might also be responsible for the body weight specific benefit of therapeutic hypothermia. However, it is also possible that the brain temperature distribution under selective head cooling may be significantly altered according to the body size, thus resulting in different protection pattern. Further understanding of factors modulating regional cerebral temperatures with whole body cooling and selective head cooling may be beneficial to improve the efficacy of therapeutic hypothermia.

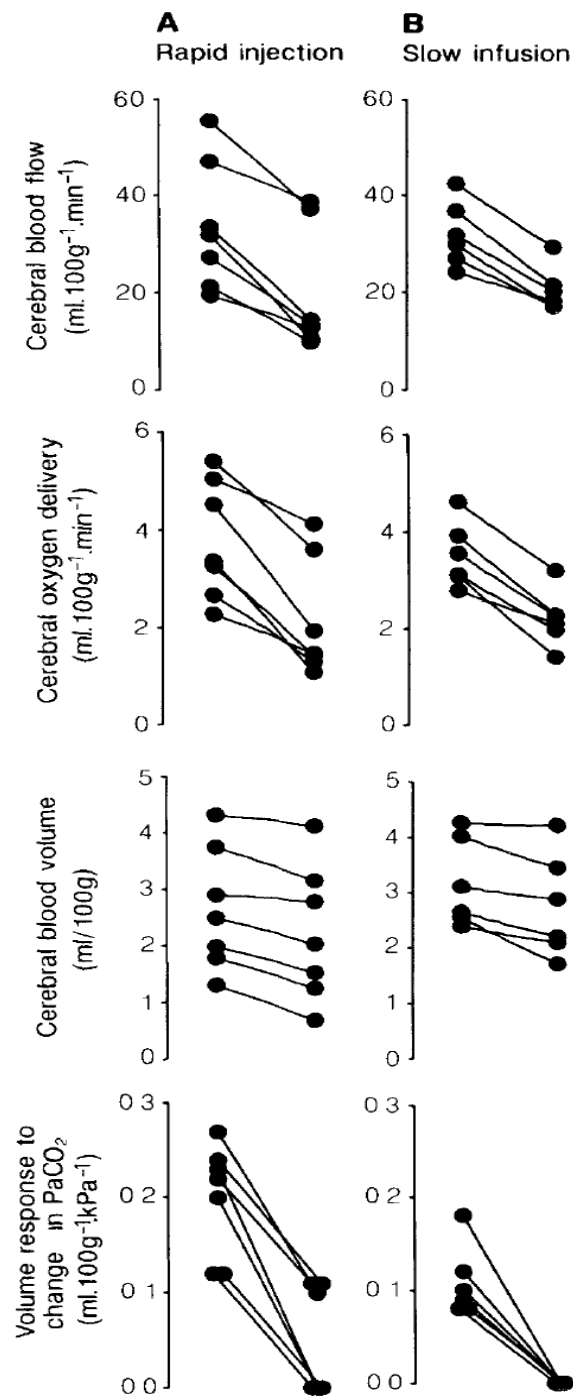
1.3.7 Techniques used to monitor brain perfusion and metabolism

As mentioned in the previous section, changes in cerebral perfusion and metabolism are both important factors to determine the regional brain temperature. However, the dependence of the local brain temperature on the cerebral perfusion and metabolism has not been fully investigated in the newborn infant, in part because of the lack in non-invasive cotside monitoring technique of these variables. Near-infrared spectroscopy (NIRS) for several years now has been used to assess and quantify non-invasively brain oxygenation and haemodynamic changes in newborn infants (72,

73). Using relatively safe tracers such as oxygen and indocyanine green, NIRS can also give crucial information about the cerebral perfusion (74, 75). However, these techniques may not be suitable for some infants, because oxygen bolus technique is inapplicable when infants are already on 100% oxygen or are well saturated in air; intravenous infusion of the dye may be unethical in healthy infants who do not have an intravenous cannula, as well as in very sick infants whose cardiac/hepatic function is severely impaired.

Figure 1.12:

**Cerebral circulation
variables before and after
indomethacin**



Significant reductions in circulation variables are demonstrated using NIRS before and after the injection of indomethacin.

From “Effects of indomethacin on cerebral haemodynamics in very preterm infants.” by Edwards AD, Wyatt JS, Richardson C, Potter A, Cope M, Delpy DT, Reynolds EO. Lancet. 1990 Jun 23;335(8704):1491-5.

However, recently NIRS has allowed the absolute quantification of brain tissue oxygenation and estimation of oxygen metabolism enabling direct and non-invasive observation of cerebral oxygen metabolism in the clinical setting (76-79).

As alternative non-invasive CBF markers, several techniques have been proposed using ultrasound Doppler velocimetry (80, 81). Of these, monitoring of the superior vena cava (SVC) flow has widely been accepted, based on its strong correlations with the flow velocity of cerebral arteries, cerebral tissue oxygenation, and the incidence of intra-ventricular haemorrhage in preterm infants (82-85).

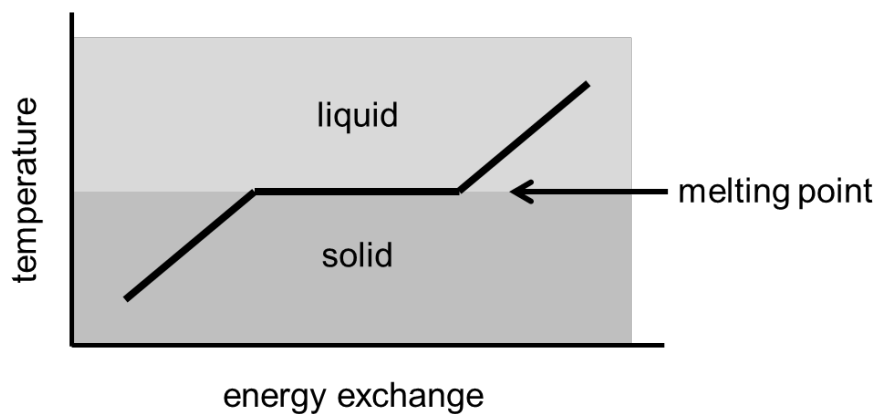
1.3.8 Other approaches to improve the efficacy of hypothermic neuroprotection

1.3.8.1 Pre-hospital cooling

In addition to the investigation for the precise brain temperature control to the specific level tailored for each patient and cerebral region, the concern of clinical investigators is focused on more practical strategies. In adult patients, pre-hospital and/or intra-arrest cooling induced by rapid intravenous administration of cold crystalloids or transnasal evaporative have been demonstrated to shorten the time interval required to accomplish target temperatures (86, 87). Pre-hospital cooling has also been adopted in clinical studies of asphyxiated newborn infants during transportation to the tertiary centre where standard cooling is provided. Unlike in adult patients, even passive cooling has been demonstrated to provide sufficient temperature reductions in the newborn infant (88-90). Although the benefit of early cooling administration has been demonstrated in preclinical studies, a considerable fraction of infants who underwent passive cooling in these clinical studies experienced excessive cooling under the target range for

therapeutic hypothermia at the time of admission (89, 90). Such an unfavourable temperature control consequential to passive cooling has been attributed primarily to the lack of continuous temperature monitoring during transportation, however, a heat buffering device, which can both initiate hypothermia and prevent excessive cooling may help improve the early temperature control of asphyxiated infants. Phase changing materials (PCMs) are passive heating and cooling substances, usually made of a salt hydride, fatty acid and ester or paraffin, such as octadecane (91). PCMs are solid at room temperature; when in contact with a warmer object, PCMs liquefy and absorb and store heat. Conversely liquid PCMs can solidify and give off heat, thus working as heat buffers and stabilising the temperature of whatever is in contact with them.

Figure 1.13: The theoretical heat buffering effect of PCM with a specific melting point related to energy exchange



Schematic diagram showing the heat buffering mechanism of PCMs. When heat is absorbed in solid PCM at a consistent rate, the PCM temperature will increase linearly until it reaches its melting point. The PCM temperature will remain at this temperature until it all melts; during this period PCM will absorb a larger amount of heat thus acting as a heat buffer and stabilising the temperature of whatever is in contact with them.

Abbreviation: PCM, phase changing material.

For example, vests made from PCM are used by firemen, athletes, soldiers and surgeons working in hot environments (92); PCMs also have applications in industry and housing where they smooth temperature fluctuations (93). Thus, a mattress made of PCM blocks may provide safe cooling initiation and its maintenance during transportation of infants to tertiary cooling centres.

1.3.8.2 Add-on therapies to therapeutic hypothermia

Although pharmacological agents have failed to demonstrate their benefit in clinical trials (21), attempts to boost the neuroprotective effect of therapeutic hypothermia using additional pharmacological treatments have yielded increasing clinicians' concern (53). Recent basic studies have provided a range of treatments targeting new injury cascades, such as pro-apoptotic reactions, activation of α_2 -adrenergic receptors, and post-resuscitation intracellular alkalosis, the last of which is caused by the overdrive of Na⁺/H⁺ exchangers in the neuronal plasma membrane (22). New therapeutic options targeting these injury cascades may be expected to ameliorate the neuronal injury in combination with or even independent of therapeutic hypothermia. Conventional pharmacological agents, such as magnesium sulphate and phenobarbital, might not be neuroprotective when used as the monotherapy because of their limited protective effect or of short therapeutic time window after hypoxia-ischaemia. Given that hypothermia itself has been reported to expand the window of opportunity for other neuroprotective interventions (20, 94), these conventional, and hence relatively safer options, may still increase the beneficial effect of therapeutic hypothermia.

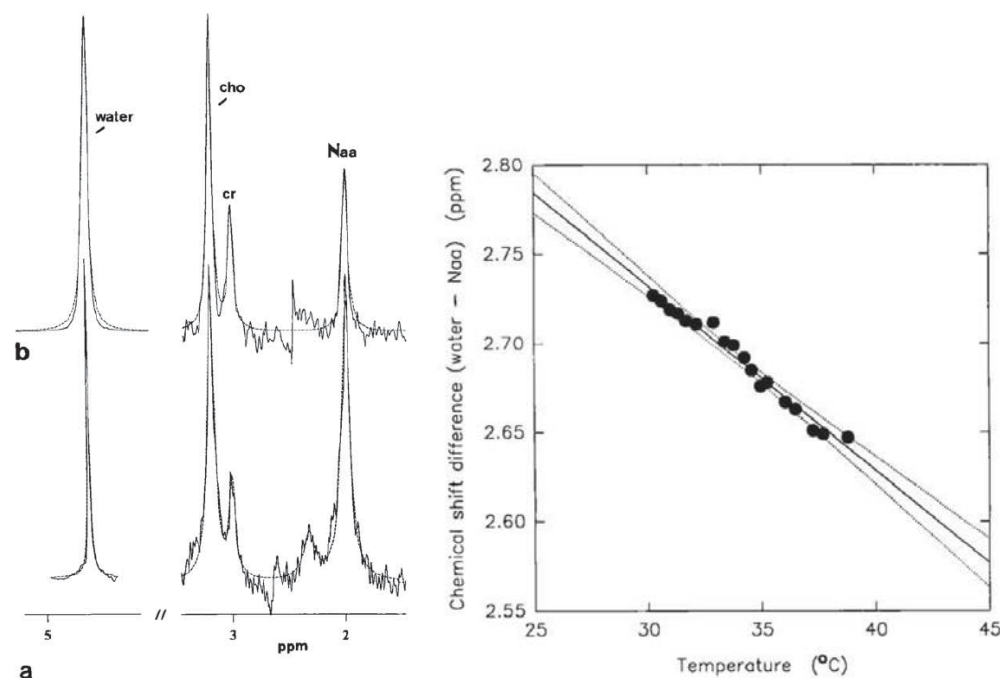
1.4 Cerebral temperature monitoring in preclinical and clinical settings

1.4.1 Techniques used to monitor brain temperature

Despite the increasing demand for continuous brain temperature monitoring, the utility of direct brain temperature monitoring technique is still of limited value in the clinical practice. Unlike in the animal model, direct insertion of invasive temperature probes is unethical especially in the newborn infant because of the considerable complications such as direct brain damage, haemorrhage and infection secondary to the probe insertion. Under normothermia and whole body cooling, deep brain temperature is similar to the core body temperatures, thus allowing the use of rectal or oesophageal temperatures as a surrogate for the deep brain temperature (57). However, under selective head cooling, estimation of the brain temperature is challenging for both the superficial and deep brain structures. So far, the nasopharyngeal temperature is considered to be an optimal, non-invasive surrogate for the deep brain temperature based on the observation in the large animal model (57); accuracy of nasopharyngeal temperature monitoring as a surrogate for the deep brain temperature needs to be assessed in the clinical case. For the purpose of indirect estimation of the core brain temperature, the zero-heat flux technique has been used in the human newborn infant (67, 95). This technique enables the estimation of the tissue core temperature by nullifying the heat flux from the scalp surface using a piece of heat insulator tightly attached over the scalp skin and a temperature probe; for more precise temperature estimation and swift equilibrium of the temperature reading, more advanced thermometers accomplish zero-heat-flow condition using thermal-compensation probes (96). However, these techniques have a limitation in the use under selective head cooling, because these methods are likely to warm up the superficial part of the brain to

erase the relatively large temperature gradient created by selective head cooling, thus potentially interfering efficient cooling. Brain tissue temperature measurement is also available using advanced bioengineering techniques, such as the multi-frequency microwave radiometer (97) and the water chemical shift of the proton magnetic resonance spectroscopy (98).

Figure 1.14: Estimation of local brain temperature using ^1H magnetic resonance spectroscopy

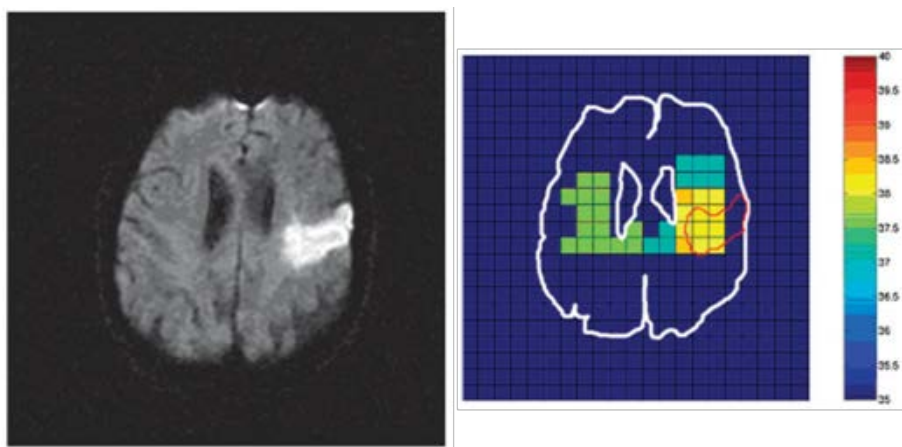


An in vivo calibration piglet spectrum (left-a) and a human brain spectrum (left-b) obtained using ^1H magnetic resonance spectroscopy single-shot temperature estimation techniques. Relationship between the difference in chemical shifts between water and the methyl resonance of N-acetylaspartate, and tympanic brain temperature in the newborn piglet.

From "The estimation of local brain temperature by in vivo ^1H magnetic resonance spectroscopy." by Cady EB, D'Souza PC, Penrice J, Lorek A. Magn Reson Med. 1995 Jun;33(6):862-7.

Especially the latter can provide 3-dimensional temperature maps, which could be used for the validation of other surrogate temperature monitoring techniques. These techniques may help enable tailored temperature control in future with further validation in the newborn infant.

Figure 1.15: Temperature maps in an adult brain with ischaemic lesion



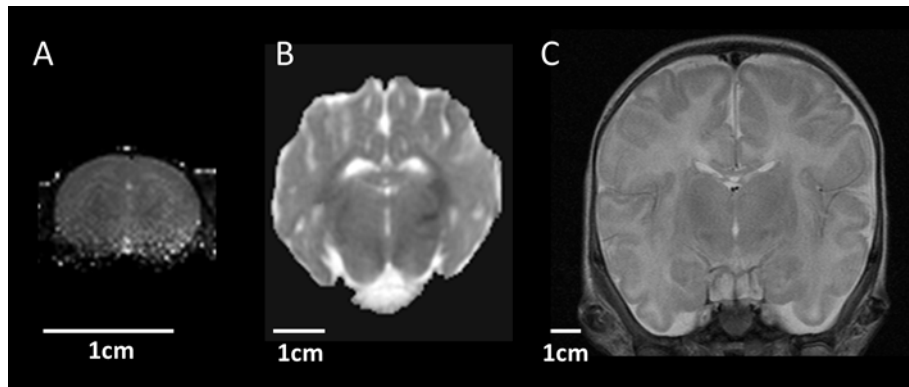
Maps of voxel temperatures relative to the diffusion weighted imaging for a patient. Diffusion weighted imaging shows an ischaemic lesion in the left temporoparietal cortex (left panel). Colour maps of regional temperatures per voxel superimposed on a line drawing of the diffusion weighted imaging lesion outline for the same patient (right panel). From “Measurement of brain temperature with magnetic resonance spectroscopy in acute ischemic stroke.” by Karaszewski B, Wardlaw JM, Marshall I, Cvorovic V, Wartolowska K, Haga K, Armitage PA, Bastin ME, Dennis MS. *Ann Neurol*. 2006 Oct;60(4):438-46.

1.4.2 Utility of the piglet model for the investigation of brain temperature distribution

Newborn piglets have been widely accepted as a translational model suitable to assess the temperature distribution under therapeutic hypothermia (55-57, 60). A normal

term-born piglet has greater body weight, greater brain to body volume ratio, and a more mature brain compared to rodents (99, 100).

Figure 1.16: Coronal MRI of newborn mouse pup, piglet and human brains



Coronal slices demonstrating the difference in the size and anatomical structure of the brain from different species. The brain of the newborn piglet, although still smaller compared to the human brain, is likely to allow more practical estimation of cerebral temperature distribution under different intrinsic and extrinsic conditions.

There still remain substantial differences in the size, proportion and anatomical structure of the brain and the surrounding tissue between human infants and piglets, and hence, caution is still required to interpret the findings in the piglet model to the human newborn infant. Nevertheless, findings from these preclinical studies have helped determine the target core body temperatures for the clinical trials, which are likely to accomplish sufficient cooling levels in the deep brain structure, and further fine-tuning of therapeutic hypothermia and additional neuroprotective treatments may benefit from the model especially when a procedure needs to be assessed in a clinically relevant setting by incorporating multiple physiological factors.

Chapter 2

Aims and Hypothesis

2.1 Aim

1. To investigate the temperature distribution and its associated factors under induced hypothermia in the newborn piglet
2. To highlight the associations between the ambient temperature, body size, cerebral metabolism, perfusion and body/brain temperatures in healthy newborn infants using non-invasive techniques
3. To establish a non-electronic cooling device, which provides safe and stable cooling in low resource settings or during transport to a tertiary cooling centre

2.2 Hypotheses

1. In the piglet model under induced cooling, brain temperatures are associated with the body size.
2. In healthy normothermic newborn infants, brain temperatures are associated with the ambient temperature, body size, cerebral blood flow and metabolism.
3. In the normothermic newborn infant, brain perfusion and metabolism are positively coupled between each other to suffice the tissue energy demand.
4. Using a mattress made of PCM, whole body cooling can be initiated and maintained with more stable body temperatures compared to water bottles.

Chapter 3

Determinants of regional cerebral temperatures in newborn piglets under selective head or whole body cooling

3.1 Methods

3.1.1 Summary

This chapter presents the first part of the study. The aim was to prove the hypothesis that, in the piglet model, brain temperatures are dependent on the body size under induced cooling. Temperature distribution in the brain and body was investigated under normothermia, whole body cooling and selective head cooling.

- Ethical approval of this study

Experiments were performed under UK Home Office Licence in accordance with UK guidelines.

3.1.2 Subject preparation

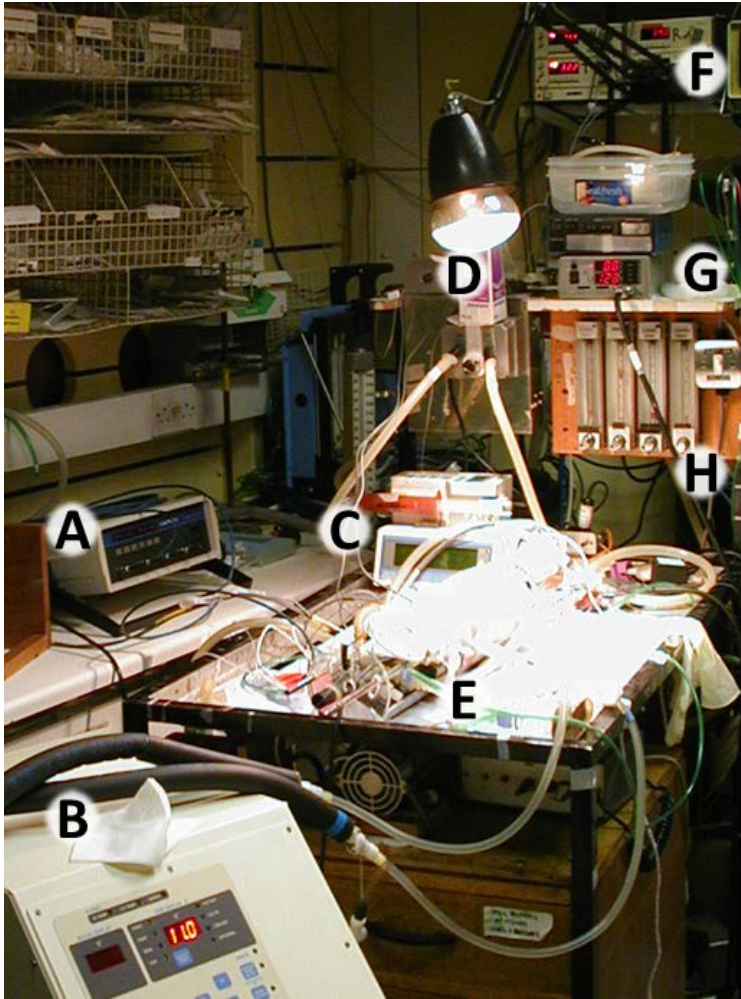
Within 24 h of birth, 14 healthy Large-White piglets born at term were randomly selected for whole body cooling (4 females and 3 males, mean body weight (standard deviation (SD)), 1.46 (0.44) kg) or selective head cooling (3 females and 4 males, 1.40 (0.32) kg).

3.1.3 Anaesthesia and surgical procedures

Piglets were sedated with intramuscular midazolam (0.2 mg/kg), and the percutaneous arterial oxygen saturation (SpO₂) was monitored using a pulse oximeter (8600 FO; Nonin Medical, Plymouth, MN, U.S.A.). Five percent isoflurane was given as the initial concentration through a facial mask during the insertion of the tracheostomy tube. Anaesthesia was maintained by the combination of isoflurane (3% during the surgical procedures and 2% for the rest of the procedures), nitrous oxide, and a continuous infusion of fentanyl (5-10 µg/kg/h). After the insertion of the tracheostomy tube, piglets

were ventilated mechanically with ventilator settings adjusted to maintain PaO_2 and PaCO_2 levels at 8 to 13 kPa and 4.5 to 6.5 kPa, respectively. The blood gas was analysed allowing for the temperature correction to the T_{rectal} of the subject (pH-stat). We preferred this method to the alpha-stat (blood samples analysed at 37°C and reported without correction regardless of the patient's body temperature) to monitor blood gas tensions and their physiological effects at the patient's body temperature. Although both the pH-stat and alpha-stat have widely been used in clinical studies (8, 45), there is a trend that the pH-stat is more frequently used in young patients including newborn infants and children, presumably because of the increased risk of hypocarbia and subsequent reduction in CBF when the alpha-stat is used (101, 102). An umbilical venous catheter was inserted for the continuous infusion of maintenance fluids (10% dextrose, 60 ml/kg/day), morphine, and intravenous injections of antibiotics (50 mg/kg benzylpenicillin and 2.5 mg/kg gentamicin, every 12 h). An umbilical arterial catheter also was inserted to enable continuous monitoring of the heart rate and arterial blood pressure, and intermittent blood sampling to measure PaO_2 , PaCO_2 , pH, electrolytes, glucose, and lactate. Bolus infusions of colloid (Gelofusin; Braun Medical, Emmenbrücke, Switzerland) and dopamine infusions (5-15 $\mu\text{g/kg/min}$) were used as required to maintain the mean arterial blood pressure greater than 40mm Hg. During surgery, T_{rectal} was maintained normothermic for the newborn piglet ($38.5 \pm 0.5^\circ\text{C}$). Following surgery, the piglet was positioned prone (head immobilized) in a plastic cylindrical pod.

Figure 3.1: Surgical preparation of the piglet model



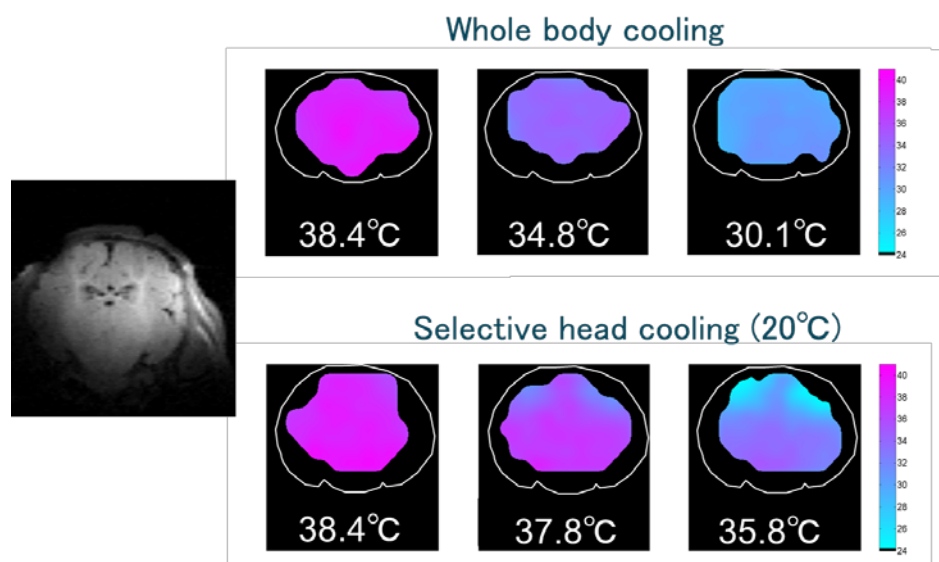
Surgical preparation of the piglet model under general anaesthesia using isoflurane. Physiological variables were continuously monitored as in the clinical intensive care setting. The core body temperature was controlled using a cooling blanket, which was circulated by temperature controlled water.

A: multi-channel fibre-optic thermometer, B: electronic cooling machine, C: end-tidal capnometer, D: radiant heater, E: piglet inserted within a plastic pod, F: polygraph for heart rate, blood pressure and body temperatures, G: pulse-oxymeter and H: ventilator and gas blender.

3.1.4 In vivo temperature measurement

These studies coincided with the calibration of magnetic resonance regional temperature measurements: apart from the period when the animal was allowed to recover and to be stabilised after surgical procedures (~30 - 60 min), all in-vivo temperatures were measured within a 7 Tesla MR scanner.

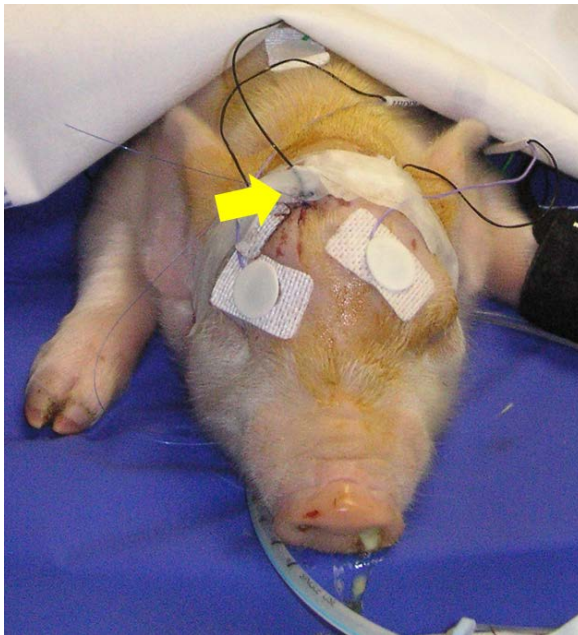
Figure 3.2: Temperature maps using magnetic resonance spectroscopic imaging techniques in a representative piglet



Scout magnetic resonance imaging (left) and representative maps of brain temperature under whole body cooling (right, upper panel) and selective head cooling (right, lower panel), showing remarkable temperature distributions specific to both cooling modalities and cooling levels. Rectal temperatures are shown at the bottom of temperature maps. From “Cerebral Temperature Mapping by Magnetic Resonance Spectroscopy in a Model of Total Body and Selective Head Cooling.” by Thornton, J. S. Cady, E. B. Shanmugalingam, S. Ordidge, R. J. Iwata, O. Iwata, S. Wyatt, J. S. Robertson, N. J. Pediatric Academic Societies Annual Meeting, Washington DC, USA. 2005:p2681.

Temperature probes were calibrated between 20 and 40°C using a water bath and standard mercury thermometer. Over this temperature range, all probes showed temperature measures within 0.1°C of the standard thermometer. A thermistor probe (Arbo N44-91, Kendall, Powell, TN, U.S.A.) was inserted to 5 cm depth from the anal sphincter to measure T_{rectal} . To measure the brain temperature 5, 10, 15 and 20 mm from the brain surface (T_5 , T_{10} , T_{15} , and T_{20} respectively), a multi-channel fibre-optic probe (MR compatible, SMM, Luxtron Corporation, Santa Clara, CA, U.S.A.) was inserted 23 mm subdurally into the striatum via a right-cranial burr-hole 1 cm lateral to and 1 cm posterior to the bregma.

Figure 3.3: In-vivo temperature monitoring

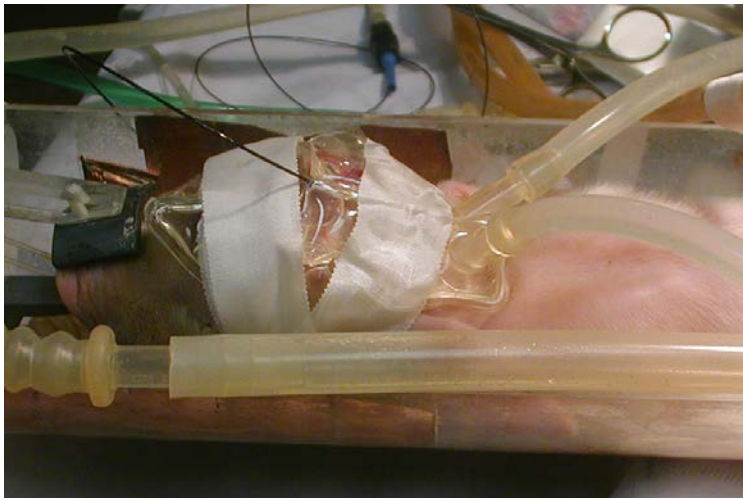


Brain temperatures between 5 and 20 mm from the brain surface were monitored using a multi-channel fibre-optic probe inserted towards the striatum (yellow arrow).

The dural aperture was minimised to reduce the leakage of the cerebrospinal fluid and air inflow. After the insertion of the brain temperature probe, the scalp was sutured, and the probe was tightly fixed to the scalp surface using the surgical tape. The anatomical

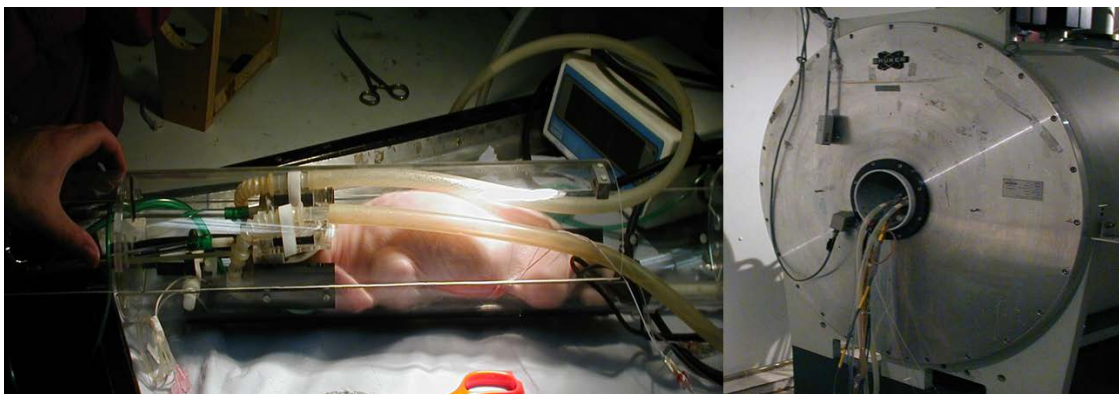
location of the probe was ascertained retrospectively using MRI, and then, with post-mortem brain slices, however, to minimise traumatic brain injury and haemorrhage associated with instrumentation, the probe was positioned once only.

Figure 3.4: Cooling device used for selective head cooling in the piglet model



A cooling cap was attached to the head of the animal to minimise the free space between the cap and the scalp skin.

Figure 3.5: Insertion of the pod into the bore of the magnetic resonance scanner



The data acquisition was performed within a specially designed plastic pod, in which animals were mechanically ventilated under general anaesthesia (left). The pod was then inserted into the bore of the magnetic resonance scanner (right).

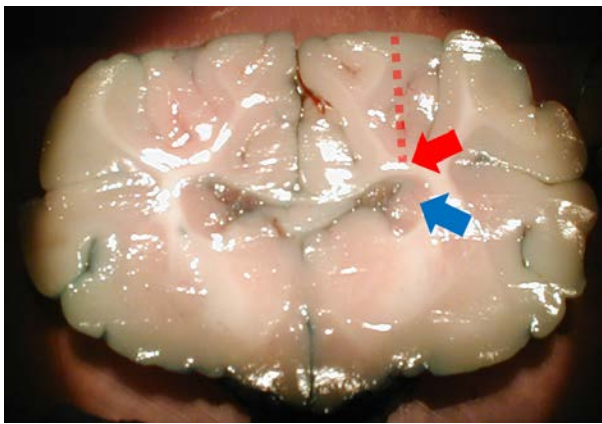
After the surgical procedures, piglets were positioned prone within a plastic pod; the scalp of the animal was firmly immobilised below the surface coil; the pod was inserted into the bore of the magnet. T_{rectal} was varied using a mattress applied over the ventral and dorsal trunk, through which temperature-adjustable water circulated (K20, Haake, Thermo Electron Corporation, Waltham, MA, U.S.A.). For subjects which underwent selective head cooling, in addition to the mattress, a flexible plastic cap, through which temperature-controlled water circulated (Cool Care System, Olympic Medical, Seattle, WA, U.S.A.), was positioned so as to contact the entire scalp above the orbit and nostril midpoint, infraorbital margin, occipital torus, and the midpoint between the mandibular angle and external auditory meatus. The cap covered a similar relative scalp area for all head sizes.

3.1.5 Experimental protocol

Temperature values were recorded every minute from the brain and every 15 min from the pod interior, coolants and rectum. After normothermic stabilisation and baseline temperature measurements, whole body cooling or selective head cooling were commenced. For whole body cooling, mattress water temperature was decremented to attain each successive target T_{rectal} of 36.5, 34.5, 32.5, and 30.5°C. For selective head cooling, measurements were made firstly with T_{rectal} normothermic and the cooling-cap in situ but void of coolant. The cap temperature was then adjusted sequentially to 20, 15, and 10°C whilst keeping T_{rectal} normothermic by increasing mattress temperature applied to the trunk. Then the mattress temperature was lowered to maintain T_{rectal} at 34.5°C, and cap temperatures of 10, 15 and 20°C were applied again. The final measurements were made with no circulating cap water (whole body cooling at T_{rectal}

34.5°C without selective head cooling). Temperature stability was defined as T_{rectal} within 0.5°C of the target temperature and both this and regional brain temperatures varying by less than 0.2°C for 15 min. It generally took 2 to 4 h for each target T_{rectal} until the establishment of stable brain and body temperatures for each setting.

Figure 3.6: Detection of the position for the temperature probe end



A coronal slice of the brain which was harvested, perfusion fixed, post fixed, and sliced with the thickness of ~5 mm. Broken line, the trace of the probe; Red arrow, the end of the probe; the blue arrow, the caudate head towards which the probe was inserted.

After each experiment the animal was humanely sacrificed by injecting an overdose of intravenous pentobarbitone and the brain was perfusion fixed using phosphate saline buffer and 4% paraformaldehyde. The brain was then dissected out, post-fixed using 2% paraformaldehyde, and sliced to 5 mm thickness. The distance from the probe tip to the centre of the caudate-nucleus was measured to assess the influence of the body size on the location and the temperature reading at the deep brain structure. Haematoxylin and eosin staining was used to examine slices for lesions associated with the temperature probe insertion.

3.1.6 Data analyses

Relative temperature values of deep brain to rectal ($T_{\text{rectal}} - T_{20}$), rectal to superficial brain ($T_{\text{rectal}} - T_5$) and deep brain to superficial brain ($T_{20} - T_5$) temperatures were calculated in order to self-correct for inter-subject and intra-subject body core temperature variations. Physiological data were inspected for normality and equality of variance. Repeated measures analysis of variance was used to examine the effects of magnetic field on the heart rate, mean arterial blood pressure, and brain temperatures. Primary outcomes were the linear body weight dependences of the absolute and relative cerebral temperatures for each temperature setting. In addition to the univariate general linear model, multivariate analysis was used to elucidate the contribution of the putative brain temperature modulators, such as the body weight, target T_{rectals} and target cap temperatures. Post-hoc analyses employed the Bonferroni test and p-values were corrected for multiple comparisons. For clarity, tabulated temperatures were corrected to the mean body weight of the whole body cooling group or selective head cooling group using the multivariate technique.

3.2 Results

3.2.1 Summary

In this chapter, the results of the first part of the study “determinants of regional cerebral temperatures in newborn piglets under selective head or whole body cooling” are presented. Under normothermic condition, a lower body weight of the piglet led to a lower superficial brain temperature ($p < 0.01$). Deep brain to superficial brain and rectal to superficial brain temperature gradients increased with decreasing body weight (both $p < 0.05$). When whole body cooling was applied, a lower body weight led to a lower superficial brain temperature and a higher rectal to superficial-brain temperature gradient ($p < 0.05$ and $p < 0.01$ respectively). Under selective head cooling, a lower body weight was associated with lower temperatures in the superficial brain and deep brain ($p < 0.01$ and $p < 0.05$ respectively), whereas a lower body weight led to greater rectal to deep brain, rectal to superficial brain, and deep brain to superficial brain temperature gradients ($p < 0.05$, $p < 0.01$ and $p < 0.05$ respectively). Compared with selective head cooling alone, superimposition of whole body cooling (34.5°C) reduced all regional temperatures (all $p < 0.001$), however, the temperature gradients were not affected.

3.2.2 Associations between regional cerebral temperatures and the body weight

3.2.2.1 Intracranial temperature gradient and body weight

Results are shown as mean (SD) unless otherwise stated. Significance was assumed for $p < 0.05$.

3.2.2.2 Temperature probe location and trauma

One piglet which underwent selective head cooling was excluded from analysis because the probe tip was > 1 cm from the caudate nucleus, leaving the selective head cooling group consisting of 2 females and 4 males with body weight 1.45 (0.31) kg. Otherwise, the probe tip was 0.4 (0.2) cm and 0.5 (0.3) cm deeper than the head of the caudate nucleus in whole body cooling and selective head cooling piglets respectively (overall 0.4 (0.3) cm), and this distance was independent of the body weight in the entire population and both cooling groups. No cerebral macroscopic or microscopic lesions were discovered apart from a little blood along the probe path.

3.2.2.3 Physiological variables under normothermia, whole body cooling and selective head cooling

Two piglets which underwent whole body cooling developed severe hypotension and bradycardia at target T_{rectal} 30.5°C; ventilator failure terminated one experiment in a piglet which was assigned to selective head cooling before collecting data at target T_{rectal} 34.5°C: physiology data for the quoted T_{rectal} targets in these 3 piglets were excluded from analysis.

The heart rate, mean arterial blood pressure, and T_{rectal} were invariant with respect to both the 7 Tesla magnetic field and magnetic resonance data acquisition: these were 38.3 (0.9) °C, 161 (25) min⁻¹, and 60.3 (11) mmHg respectively before entering the magnetic resonance scanner and 38.2 (1.1) °C, 163 (26) min⁻¹, and 60.7 (12) mmHg shortly after entry.

In the whole body cooling group, the heart rate was reduced at 34.5°C (134 (28) min⁻¹, n = 7, p < 0.05), 32.5°C (122 (29) min⁻¹, n = 7, p < 0.01) and 30.5°C (91 (20) min⁻¹, n = 5, p

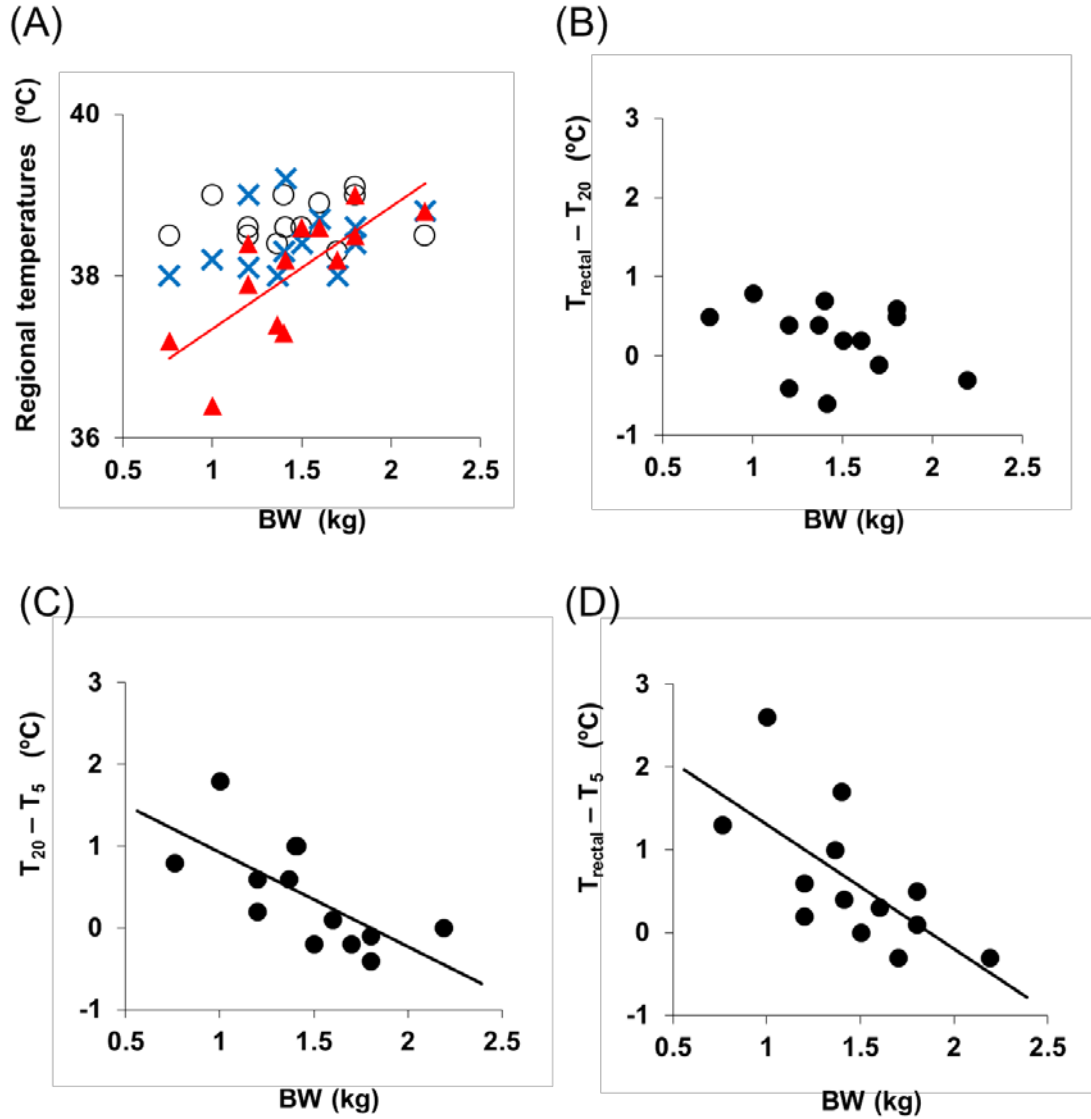
< 0.001) compared to normothermia ($176 (25) \text{ min}^{-1}$); mean arterial blood pressure was altered only at 30.5°C ($36 (6) \text{ mmHg}$; $n = 5$, $p < 0.05$) compared to normothermia ($48 (9) \text{ mmHg}$). The heart rate and mean arterial blood pressure were unaffected by selective head cooling alone ($n = 6$). During selective head cooling with whole body cooling ($n = 5$, target $T_{\text{rectal}} 34.5^{\circ}\text{C}$), the mean arterial blood pressure was invariant from the value under the normothermic condition; however, the heart rate was reduced with cap cooling off ($141 (15) \text{ min}^{-1}$, $p < 0.001$) and on ($144 (17) \text{ min}^{-1}$ at 20°C , $139 (19) \text{ min}^{-1}$ at 15°C , and $139 (25) \text{ min}^{-1}$ at 10°C , all $p < 0.01$) compared with baseline (normothermic T_{rectal} and cap cooling off, $196 (26) \text{ min}^{-1}$).

3.2.2.4 Temperature distribution under normothermia

($n = 13$, univariate analysis)

Under normothermic T_{rectal} before the commencement of active cooling, T_5 , T_{10} , and T_{15} were linearly correlated with the body weight ($p = 0.003$, 0.006 and 0.030 respectively) (See Fig. 3.7 (A)), however, T_{20} was not dependent on the body weight. $T_{\text{rectal}} - T_{20}$ was $\sim 0^{\circ}\text{C}$ and was not dependent on the body weight (See Fig. 3.7 (B)). In contrast, relative brain temperatures of $T_{20} - T_5$ and $T_{\text{rectal}} - T_5$ were both negatively associated with the body weight ($p = 0.010$ and 0.011 respectively) (See Fig. 3.7 (C)-(D)).

Figure 3.7: Associations between rectal temperature, regional brain temperatures and the body weight under normothermic condition



(A) neither T_{rectal} (\circ) nor T_{20} (deep brain temperature; \times) depended on the body weight; however, T_5 (superficial brain temperature; \blacktriangle) decreased as the body weight fell ($p < 0.01$). (B). $T_{\text{rectal}} - T_{20}$ was $\sim 0^\circ\text{C}$ and was not dependent on the body weight. (C) $T_{20} - T_5$ and (D) $T_{\text{rectal}} - T_5$ both increased linearly as body weight decreased over the experimental range (both $p < 0.05$). Regression lines are from univariate analyses with $p < 0.05$ (shown in red for T_5 in panel (A)).

3.2.2.5 Temperature distribution under whole body cooling

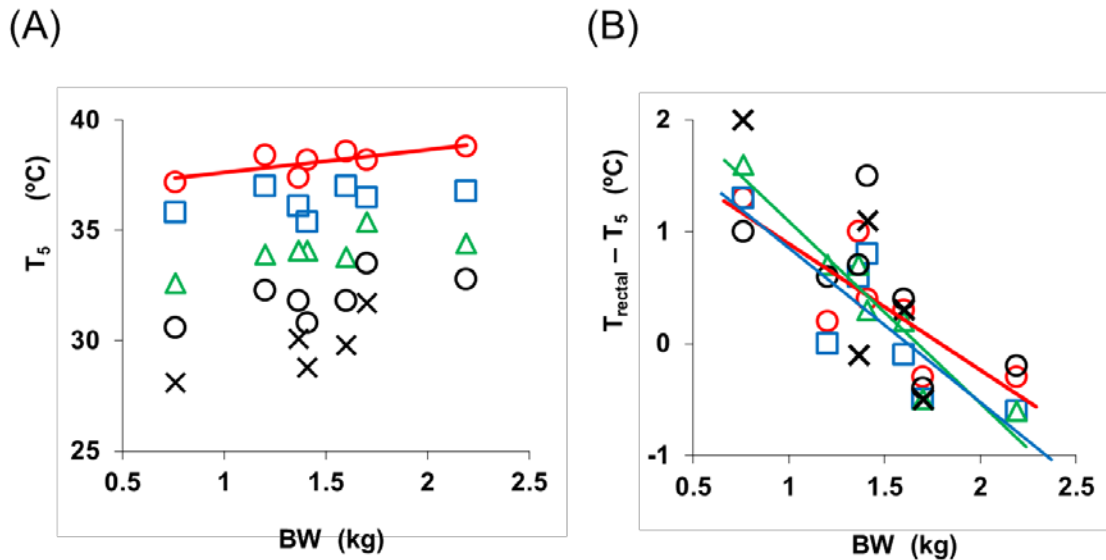
(n = 7, multivariate analysis)

With whole body cooling, a lower target T_{rectal} was associated with a lower T_{20} , T_{15} , T_{10} , and T_5 (all $p < 0.001$) (See Fig. 3.8 (A) and Table 3.1). Relative temperatures of $T_{\text{rectal}} - T_{20}$, $T_{20} - T_5$, and $T_{\text{rectal}} - T_5$ were independent of target T_{rectal} . A lower body weight was associated with a lower T_5 and a higher $T_{\text{rectal}} - T_5$ ($p = 0.034$ and 0.008 respectively) (See Fig. 3.8 (B)). T_{10} , T_{15} , T_{20} , $T_{\text{rectal}} - T_{20}$ and $T_{20} - T_5$ were not dependent on the body weight.

Table 3.1: Regional and relative temperatures for whole body cooling at each target rectal temperature (°C, mean (standard error); n = 7; corrected to the mean body weight of 1.46 kg)

Temperature measures	Target T_{rectal}			
	38.5 °C	36.5 °C	34.5 °C	32.5 °C
T_{rectal}	38.5 (0.1)	36.6 (0.2)	34.4 (0.2)	32.5 (0.2)
T_5	38.1 (0.2)	36.4 (0.2)	34.0 (0.2)	31.9 (0.3)
T_{10}	38.3 (0.2)	36.6 (0.2)	34.5 (0.3)	32.2 (0.3)
T_{15}	38.4 (0.1)	36.6 (0.2)	34.4 (0.2)	32.4 (0.3)
T_{20}	38.5 (0.2)	36.8 (0.2)	34.5 (0.2)	32.6 (0.2)
$T_{\text{rectal}} - T_{20}$	0.0 (0.2)	0.2 (0.1)	0.1 (0.2)	0.1 (0.1)
$T_{20} - T_5$	0.4 (0.1)	0.4 (0.2)	0.5 (0.2)	0.6 (0.3)
$T_{\text{rectal}} - T_5$	0.4 (0.1)	0.2 (0.2)	0.3 (0.1)	0.5 (0.2)

Figure 3.8: Associations between brain temperatures and the body weight with whole body cooling



(A) T_5 and (B) $T_{rectal} - T_5$ at target T_{rectal} of 38.5 (○), 36.5 (□), 34.5 (△), 32.5 (○), and 30.5°C (X). Regression lines are from univariate linear regression analyses with $p < 0.05$: in panel (A) for normothermia (red); and in panel (B) red - normothermic, blue - target T_{rectal} 36.5°C, and green - target T_{rectal} 34.5°C.

3.2.2.6 Temperature distribution under selective head cooling without whole body cooling

(n = 6, multivariate analysis)

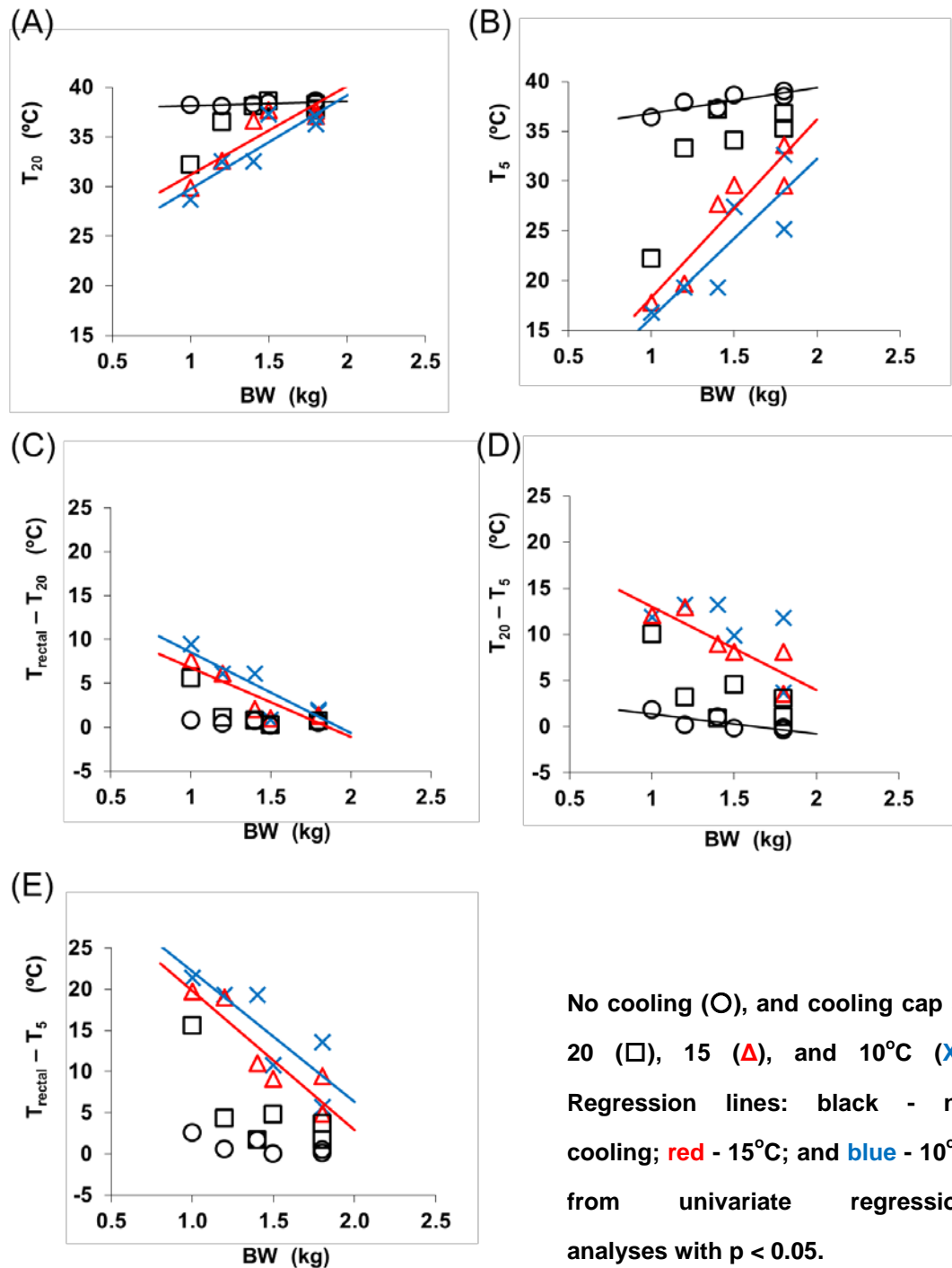
As the temperature of the cooling cap decreased, T_5 ($p = 0.004$), T_{10} ($p = 0.002$), T_{15} ($p = 0.001$), and T_{20} ($p = 0.001$) all fell, whereas relative temperatures of $T_{rectal} - T_{20}$ ($p = 0.001$) and $T_{rectal} - T_5$ ($p = 0.006$) both increased ($T_{20} - T_5$ not significant) (See Table 3.2 and Figs. 3.9 (A)-(E)). A smaller body weight was linearly associated with a lower T_5 ($p = 0.007$), T_{10} ($p = 0.019$), T_{15} ($p = 0.023$), and T_{20} ($p = 0.017$) (See Figs. 3.9 (A)-(B)), whereas relative temperatures of $T_{rectal} - T_{20}$ ($p = 0.020$), $T_{20} - T_5$ ($p = 0.029$) and $T_{rectal} - T_5$ ($p = 0.009$) were inversely correlated with the body weight (See Figs. 3.9 (C)-(E)). A

lower cap temperature and a smaller body weight were synergistically associated with even a lower T_{10} ($p = 0.019$), T_{15} ($p = 0.007$), and T_{20} ($p = 0.005$) (See Fig. 3.9 (A)), and even a greater relative temperature of $T_{\text{rectal}} - T_{20}$ ($p = 0.006$) (See Fig. 3.9 (C)).

Table 3.2: Regional and relative temperatures for selective head cooling with normothermic rectal temperature (°C, mean (standard error), $n = 6$, corrected to the mean body weight of 1.45 kg)

Temperature measures	Cooling Cap			
	off	20°C	15°C	10°C
T_{rectal}	38.9 (0.1)	38.5 (0.2)	38.5 (0.2)	38.5 (0.1)
T_5	38.0 (0.2)	33.2 (1.7)	26.3 (1.0)	23.4 (1.4)
T_{10}	38.2 (0.1)	34.4 (1.7)	29.3 (1.2)	27.0 (1.5)
T_{15}	38.4 (0.1)	36.2 (1.0)	33.2 (0.9)	31.5 (1.1)
T_{20}	38.3 (0.0)	36.9 (0.7)	35.3 (0.7)	34.1 (0.7)
$T_{\text{rectal}} - T_{20}$	0.5 (0.1)	1.5 (0.6)	3.2 (0.6)	4.4 (0.7)
$T_{20} - T_5$	0.4 (0.2)	3.8 (1.1)	8.9 (0.8)	10.6 (1.3)
$T_{\text{rectal}} - T_5$	0.9 (0.3)	5.3 (1.6)	12.2 (1.1)	15.0 (1.5)

Figure 3.9: Associations between regional and relative brain temperatures and the body weight for selective head cooling without whole body cooling



3.2.2.7 Temperature distribution under selective head cooling with superimposed whole body cooling

(n = 5, multivariate analysis)

Due to the interruption of the data collection in one piglet (see 3.2.2.3 Physiological variables under normothermia, whole body cooling and selective head cooling), only the data from 5 piglets were analysed. Compared to selective head cooling alone, superimposition of whole body cooling (target T_{rectal} 34.5°C) reduced all regional cerebral temperatures (all $p < 0.001$), however, relative temperatures of $T_{\text{rectal}} - T_{20}$, $T_{20} - T_5$ and $T_{\text{rectal}} - T_5$ were not affected (See Table 3.3).

Table 3.3: Regional and relative temperature differences for selective head cooling with target rectal temperature of 38.5°C and 34.5°C (°C, mean (standard error), p; n = 5, corrected to the mean body weight of 1.45 kg)

Temperature measures	Temperature difference with target T_{rectal} 38.5 vs. 34.5°C
T_{rectal}	4.0 (0.1), $p=0.000$
T_5	4.3 (0.7), $p=0.010$
T_{10}	3.5 (0.6), $p=0.012$
T_{15}	4.0 (0.5), $p=0.003$
T_{20}	4.0 (0.4), $p=0.002$
$T_{\text{rectal}} - T_{20}$	0.0 (0.4), NS
$T_{20} - T_5$	-0.8 (0.4), NS
$T_{\text{rectal}} - T_5$	-0.8 (0.8), NS

Pair-wise comparison by paired t-test with Bonferroni correction

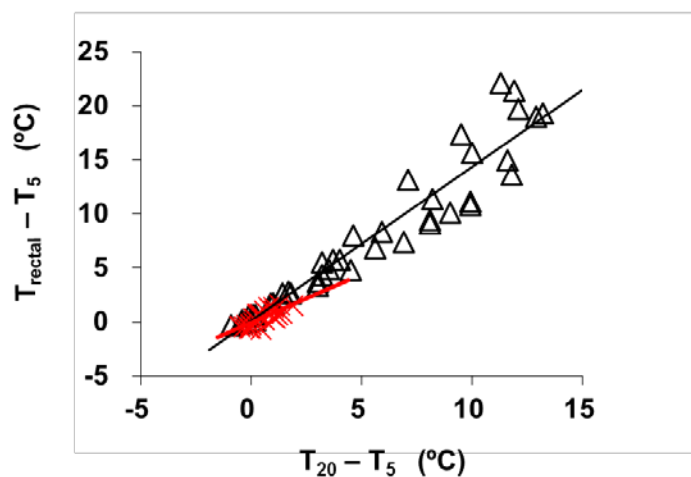
Abbreviation: NS, not significant.

3.2.2.8 Relationships between relative temperatures

(univariate analysis)

When temperature measurements at all target cooling levels were incorporated, there were significant linear relationships between $T_{\text{rectal}} - T_5$ and $T_{\text{rectal}} - T_{20}$, and between $T_{\text{rectal}} - T_5$ and $T_{20} - T_5$ for whole body cooling ($p = 0.020$ and $p < 0.001$ respectively), and between all the relative temperatures with selective head cooling (all $p < 0.001$) (See Fig. 3.10, data only shown for $T_{\text{rectal}} - T_5$ and $T_{20} - T_5$).

Figure 3.10: Relationships between relative temperature of $T_{\text{rectal}} - T_5$ and $T_{20} - T_5$



When whole body cooling (X) and selective head cooling (Δ) measurements at all cooling temperatures were separately incorporated, $T_{20} - T_5$ (intracerebral temperature gradient) correlated linearly with $T_{\text{rectal}} - T_5$ (rectal to superficial brain temperature gradient) for both cooling modalities (both $p < 0.001$). Temperature gradients were considerably greater for selective head cooling. Regression lines: red – whole body cooling; black – selective head cooling.

3.3 Brief Discussion

3.3.1 Summary

We have proved the hypothesis that brain temperatures are associated with the body size under induced cooling in the piglet model. The findings of this study are discussed in this chapter.

- Key Findings from this Study:

Brain cooling under whole body cooling, selective head cooling, or even normothermia was more efficient with lower body weight, presumably due to greater head surface area to volume ratios. The association between brain temperatures and the body weight was more prominent with selective head cooling compared to whole body cooling and normothermia, suggesting that infants with low body weight may be at an increased risk of excessive cooling. Cooling may require adjustment to the body weight to accomplish consistent regional temperatures and optimal neuroprotection while undergoing therapeutic hypothermia.

- What is already known and what our study adds:

What is already known:

- Both whole body cooling and selective head cooling can ameliorate death or severe neurodevelopmental impairments at least to 18 months old in infants with moderate to severe neonatal encephalopathy.
- Cooling was inefficient to several groups of infants, such as most severely asphyxiated infants defined by amplitude integrated encephalogram, and infants whose body weight was less than the quartile of the entire population.

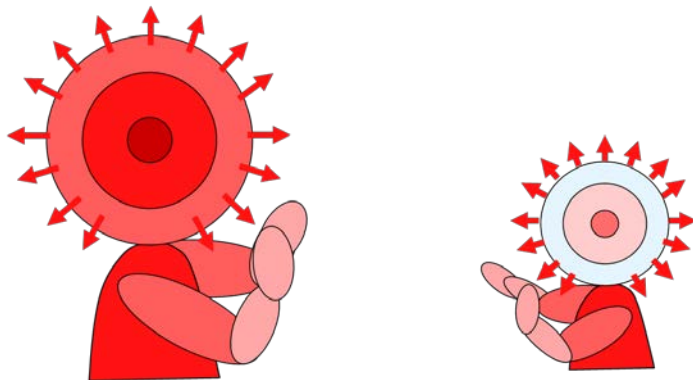
What our study adds:

- Brain cooling, including whole body cooling, selective head cooling, and even natural cooling with normothermic condition, was more efficient with smaller body size, suggesting that smaller subjects are at increased risks to be overcooled.
- Temperature differences between the body core, deep brain and superficial brain were positively coupled between each other with selective head cooling, suggesting that efficient surface cooling is inevitably accompanied by a large intracerebral temperature gradient, resulting in excessive cooling in the superficial brain.

3.3.2 Regional cerebral temperature and body weight

Brain temperature is determined by metabolic heat production, heat delivery or removal via the CBF, and heat exchange at the body surface through convection, radiation and evaporation (54). The contribution of each factor varies with specific physiological and pathological conditions, such as cardiac output, spontaneous activity, sleep – wake status, ambient temperature and humidity, and maturation of the skin. In this study, we have demonstrated that, even with normothermia, regional brain temperatures were associated with the body weight. At first glance this may be counter-intuitive. However, heat is continuously lost from the scalp, the amount of which depends on the head surface-area to volume ratio. In a simple sphere model, this ratio is inversely proportional to the radius and, hence, larger for smaller spheres.

Figure 3.11: Heat dissipation from the scalp surface: simulation in a sphere model



Cartoon depicting the influence of the head size to the heat dissipation from the scalp surface. As the head of the newborn infant is regarded as a sphere, a smaller head is likely to lose more heat from the scalp surface compared to a larger head, because of the greater surface to volume ratio.

Since heat loss may be proportional to head surface area and generation proportional to brain volume, smaller piglets may have more efficient heat dissipation and lower cerebral temperatures. We observed even more prominent associations between regional brain temperatures and the body weight of the animal with selective head cooling, which might explain the patient specific efficacy of therapeutic hypothermia using this modality (50). Caution is still required, because the coupling between brain temperatures and the body weight can in part be explained by the maturation-dependent cerebral metabolism i.e. more matured (and hence larger) subjects are likely to have higher cerebral metabolism than their peers (79, 103), resulting in greater local thermogenesis and higher brain temperature. However, given that experimental piglets are generally born within a few days of term (115 days), the difference in the body size may reflect the nutritional state during the fetal period rather than that in maturation (104). Future studies need to address the variation and dependence of cerebral metabolism and perfusion on the body size in our piglet model.

3.3.3 Clinical Implications: Normothermic low birth weight infants

Term low birth weight infants are likely to have lower superficial brain temperatures even when systemically normothermic. It is possible that lower regional brain temperatures in low body weight infants (and also higher regional brain temperatures in large body weight infants) could contribute in part to the regional pathophysiological characteristics of neonatal encephalopathy which are specific to particular gestational ages and body sizes (105). Future experimental and clinical studies should explore relationships between the spatial pattern of brain injury, body weight and regional brain temperatures.

3.3.4 Clinical Implications: Selective head cooling or whole body cooling

Increasing evidence indicates the existence of a “threshold” temperature below which the balance between protective and injurious effects of therapeutic hypothermia is weighted towards the latter (25, 58, 62, 63). In a clinical trial which used selective head cooling with mild whole body cooling to 34.5°C, subgroup analysis revealed that cooled infants with the birth weight of less than the quartile of the entire population had significantly worse outcome compared to their peers in the hypothermia group. Multivariate analysis demonstrated that cooling, lower encephalopathy grade, and lower birth weight were associated with better outcomes, whereas there was a significant interaction between treatment and birth weight, such that larger infants showed a lower rate of favourable outcomes in the normothermic group but greater improvement with cooling (50). In our study population which underwent selective head cooling, at each temperature for the cooling cap, the brain of animals with smaller body weight was cooled more efficiently; this relationship was most apparent in the superficial brain. We speculate that, in the clinical trial of selective head cooling (50), the infants with lower body weight did not benefit from cooling because excessive cooling upset the beneficial effect of the treatment (63, 106-108).

Table 3.4: Influence of the body weight on the outcome of the infants in a randomised controlled trial of selective head cooling

Parameter	<i>P</i>	OR (95% CI)
Treatment	.0009	0.28 (0.13–0.59)
Encephalopathy grade, 3 vs 1 or 2	<.0001	4.13 (2.08–8.2)
Birth weight, <25th vs ≥25th percentile	.0045	0.27 (0.11–0.67)
Birth weight–treatment interaction	.0026	6.67 (1.94–22.84)

A multivariate, binary, logistic regression analysis for the primary outcome incorporating birth weight and an interaction term for birth weight and treatment was performed (*N* = 218).

In a multivariate analysis, cooling, lower encephalopathy grade and lower birth weight were associated with better outcomes. Larger infants ≥25th percentile showed a lower rate of favourable outcomes in the control group but greater improvement with cooling. From “Determinants of outcomes after head cooling for neonatal encephalopathy.” by Wyatt JS, Gluckman PD, Liu PY, Azzopardi D, Ballard R, Edwards AD, Ferriero DM, Polin RA, Robertson CM, Thoresen M, Whitelaw A, Gunn AJ; CoolCap Study Group. *Pediatrics*. 2007 May;119(5):912-21.

Our results also suggest that cerebral to rectal and deep brain to superficial brain temperature gradients were tightly coupled between each other. In a study which used the newborn piglet, Tooley et al., demonstrated that the deep brain temperature can be lowered up to 9°C while maintaining normal T_{rectal} , using selective head cooling with the cap temperature below 10°C (56). The authors proposed that such a selective head cooling strategy could be used for preterm human infants. However, it is important to point out that very efficient selective head cooling will simultaneously produce an excessively low superficial brain temperature. Given that the safety and efficacy of prolonged deep hypothermia has not been investigated to the developing brain, it is

suggested that higher cap temperatures should be used in smaller infants to induce less profound superficial cooling.

It is well known that spatial injury patterns following perinatal hypoxia-ischaemia vary between infants (109). A study in a piglet model of asphyxial encephalopathy observed that the protection pattern of whole body cooling at 35°C and 33°C was spatially different, with the former providing deep grey matter dominant protection and the latter cortical grey matter dominant tissue sparing (62). Taken together, it is suggested that optimal neuroprotection may require region- and patient-specific cooling temperatures. Carefully tailored selective head cooling combined with whole body cooling may help “deliver” the optimal temperature for protection to the regional brain tissue, subsequently providing maximum tissue protection. Our results suggest that the body weight is a critical factor in optimising brain temperature control during therapeutic hypothermia, especially selective head cooling. Further studies are needed to determine the optimal regional temperatures which maximise neuroprotection for each injury pattern. Importantly, even with whole body cooling alone, a lower body weight was associated with a lower superficial brain temperature by up to ~2°C, implying that, without care, the brain tissue temperature of the cortical grey matter may be highly various between subjects, potentially resulting in different protection pattern in the superficial brain structure. Further studies using MRI in infants who underwent whole body cooling and selective head cooling are required to clarify the dependence of cortical grey matter protection on the infant’s body and head size.

3.3.5 Limitations of the Temperature study

The current study was conducted within the bore of the magnetic resonance spectrometer. The influence of magnetic resonance environment was, if any, similar throughout the study population. Nevertheless, the effect of a 7 Tesla magnetic field on the brain cannot be totally ignored; for example, even the data acquisition of MRI and magnetic resonance spectroscopy might give heat to the brain tissue (110). In our current study, physiological variables were similar before and after the insertion of the piglet's pod into the bore of the scanner. Of the magnetic resonance techniques used in our routine sequence, spectroscopic imaging supplied the highest power but less than half the clinical study limit (2 W/kg for the brain), which was defined for patients and volunteers undergoing clinical magnetic resonance diagnostic procedures (111); other methods within our routine, such as diffusion tensor imaging and single voxel magnetic resonance spectroscopy, generated less power. No significant physiological changes have been reported in humans up to 8 Tesla (112). In conclusion, the magnetic resonance environment was unlikely to have affected cerebral temperatures significantly in our current study.

The temperature probe was positioned consistently in the animal's brain except for a piglet in which the probe end was unacceptably dislocated, and the temperature data of which were excluded from the analysis. However, it is still possible that the deep brain temperature in a larger animal was underestimated (and hence $T_{\text{rectal}} - T_{20}$ was overestimated and $T_{20} - T_5$ was underestimated) especially with selective head cooling, because of the larger temperature gradient. However, for whole body cooling or selective head cooling, the depth of the temperature probe was independent of the body weight, suggesting the influence of minimal, if any, bias.

We related the cerebral temperature to the body weight rather than the head diameter or circumference, because the anatomy of the piglet's head rendered these measures inappropriate. Future studies should utilise the volumetric brain MRI technique and analyses which incorporate the scalp or brain surface.

Because of the limited number of subjects available for the current study, we were unable to include several important variables. For example, our current study subjects did not undergo hypoxic-ischaemic insult because hypoxia-ischaemia render intensive life support under deep hypothermia even more difficult. Careful interpretation of the current findings is required, because the pattern of CBF and brain metabolism may be specific to each subject, presumably depending on various factors such as the maturity, insult severity, extracerebral pathology, infection, and inflammation (16, 113, 114). These patient specific factors may in themselves engender intrinsic cerebral temperature modulations. In asphyxiated infants, the body temperature spontaneously falls shortly after the delivery ("behavioural hypothermia") (115). Lowering of the brain temperature after perinatal hypoxia-ischaemia has been observed in the newborn infant (67). However, there are reports of less efficient selective head cooling in the newborn piglet following hypoxia-ischaemia (60) and elevated core temperature in control asphyxiated infants in a clinical trial of whole body cooling (9, 60) suggesting the difficulty in incorporating the influence of hypoxic-ischaemic stress in the translational model.

Selective head cooling by itself may modulate regional CBF (55, 116) which in turn may alter cooling efficacy, because increased CBF can deliver greater amount of heat from the trunk. Laptook et al. observed reduced CBF concomitant with lower regional temperature in the newborn piglet (55) whereas Walter et al. reported increased

superficial CBF which they conjectured was consequential to vasoparalysis and subsequent loss of autoregulation (116). Such CBF re-distribution with selective head cooling might be further modified following hypoxia-ischaemia. Future studies have to address the regional brain temperature modulation consequential to a range of insult severities.

In addition to the further potential influencing factors of the regional brain temperature mentioned above, inter-species anatomical and maturational differences have to be taken into account before our results can be translated for application in human newborn infants. Compared to the human newborn infant, a normal term piglet has lower body weight, smaller brain to body volume ratio, a more mature brain, cranium and skin, and no anterior fontanelle (99). Associations between cerebral temperatures and the body weight under normothermia, whole body cooling and selective head cooling have to be urgently investigated in the human newborn infant using non-invasive techniques such as magnetic resonance cerebral temperature imaging (117).

3.3.6 Future investigations

Our results emphasise the importance of robust, cotside, non-invasive regional brain temperature measurements in the human infant. Nasopharyngeal temperature could provide a proxy for deep brain temperature (8, 57), however, its accuracy has not been fully validated. Also, our current study has highlighted the importance of monitoring the superficial brain temperature, although this appears to be technically more difficult to measure compared to the deep brain temperature. Although not for cotside use, we have recently used proton magnetic resonance spectroscopic imaging for cerebral

temperature mapping in the newborn piglet (117). Such a non-invasive, in-vivo technique could assess the association between regional brain temperatures on the body weight in the human infant.

3.3.7 Conclusions

Our study suggests that the body weight is tightly associated with regional brain temperatures under therapeutic hypothermia, and particularly, with selective head cooling: to avoid excessive cooling, a slightly higher cooling cap temperature may have to be considered in a smaller infant. Even with whole body cooling alone, the superficial brain temperature may be lower in a lighter infant with potential consequences for cortical neuroprotection. Lastly, in the infant who was born with a lower body weight and experienced perinatal hypoxic-ischaemic stress, spontaneously cooler superficial brain temperature (in spite of systemic normothermia under thermo neutral condition) may contribute to the pathophysiological characteristics specific to particular gestational ages and body sizes.

Chapter 4

Cerebral temperature, perfusion and metabolism under spontaneous body temperature drift in the newborn infant

4.1 Methods

4.1.1 Summary

This chapter presents the second part of the study. The aim was to prove the hypothesis that brain temperatures are dependent on the ambient temperature, body size, cerebral blood flow and metabolism, and that brain perfusion and metabolism are positively coupled to suffice the tissue energy demand, in the normothermic newborn infant. Thirty-two term born and near term born infants who were hospitalised at a tertiary neonatal intensive care unit but without critical general condition were studied using NIRS and ultrasound cardiography.

- Ethical approval of this study

This study was conducted under the approval of the ethics committee of Kurume University School of Medicine with written informed consent from a parent of each participating infant.

4.1.2 Study population

Thirty-two participating infants without major congenital anomalies (12 males and 20 females; post-natal age, 21 (17) days; post-conceptual age, 38.3 (2.6) weeks corrected age; body weight, 2616 (515) g; indicated as mean (SD)) were recruited from the newborn infants who were cared for at the special care unit of a tertiary neonatal intensive care unit (Kurume University Hospital, Fukuoka, Japan) between June 2009 and August 2009. These infants initially required medical care due to preterm birth (n = 18), transient feeding problem (n = 5), transient neonatal tachypnea (n = 4), gestational diabetes mellitus of the mother (n = 3), hypoglycaemia (n = 1) and multiple minor

anomalies ($n = 1$). However, by the time of the study, all infants were stable and healthy and were cared for in an open cot with the ambient temperature of approximately 25-6°C. A cotton blanket was placed over the limbs and the trunk in all infants.

4.1.3 Data collection

We examined the infants approximately 1 h after feeding when they are either asleep or calmly awake. To minimise the technical bias, data collection was conducted in the same order, and was completed within 20 min.

4.1.3.1 Temperature measurement

The data collection was undergone with the infant lying in the supine position (infants are not cared for in the prone position in the special care unit). The scalp temperature (T_{scalp}) was measured at the centre of the forehead using a non-contacting infrared thermometer (Thermofocus Pro, Technimed, Varese, Italy); temperature measurement was repeated three times and the median value was used. To measure the brain temperature, two thermal compensation thermistor probes connected to a dual channel zero-heat-flow tissue core thermometer (Coretemp, Terumo, Tokyo, Japan) were simultaneously applied to the centre of the forehead ($T_{\text{brain-15}}$, 15 mm in diameter) and the anterior fontanelle ($T_{\text{brain-25}}$, 25 mm in diameter); the diameter of the probe theoretically corresponds to the depth of the tissue, which the temperature reading reflects (118).

Figure 4.1: Mechanism of zero-heat flow tissue core thermometer

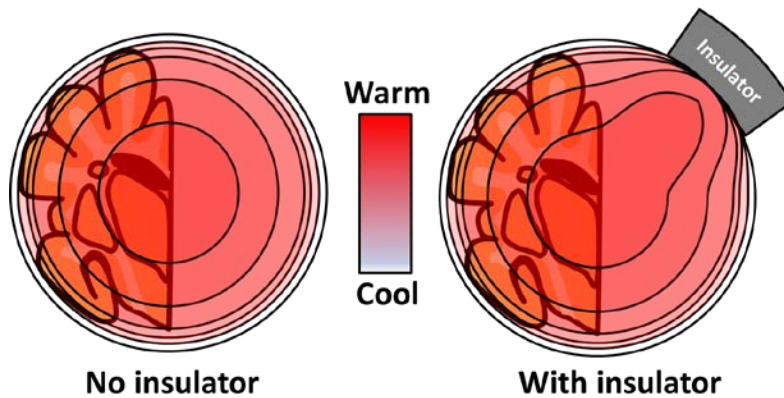


Diagram depicting the mechanism of zero-heat flux tissue core thermometer. Under physiological conditions, the deep brain temperature is slightly higher compared with the superficial brain, resulting in a modest thermal gradient within the brain (left). When an insulator or an equivalent thermal compensation probe is applied to the scalp surface, the heat dissipation is blocked, and the surface temperature starts increasing until it equilibrates to the deeper structure, the depth of which depends on the size of the insulator.

The temperature measurement was continued until the temperature readings equilibrated with the temperature drift of $< 0.05^{\circ}\text{C}$ over a minute for approximately 5 to 10 min. Finally the T_{rectal} was measured at 3 cm from the anal margin using a digital thermometer (C202, Terumo, Tokyo, Japan). The ambient temperature was measured beside the infant's cot using an ambient thermo-hygrometer (605-H1 Mini, Testo, Yokohama, Japan). In addition to these absolute temperatures, relative brain temperatures to T_{rectal} ($T_{\text{brain-25}} - T_{\text{rectal}}$, $T_{\text{brain-15}} - T_{\text{rectal}}$ and $T_{\text{scalp}} - T_{\text{rectal}}$, respectively) were calculated to self-correct for the inter-subject T_{rectal} variation.

4.1.3.2 Estimation of cerebral blood flow using ultrasound sonography

Echocardiographic data were obtained by a same examiner using an ultrasound scanner (iE33, Philips, Amsterdam, Netherland) and an 8-13 MHz vector array transducer. The SVC flow was measured by an established method (82) using the following formula.

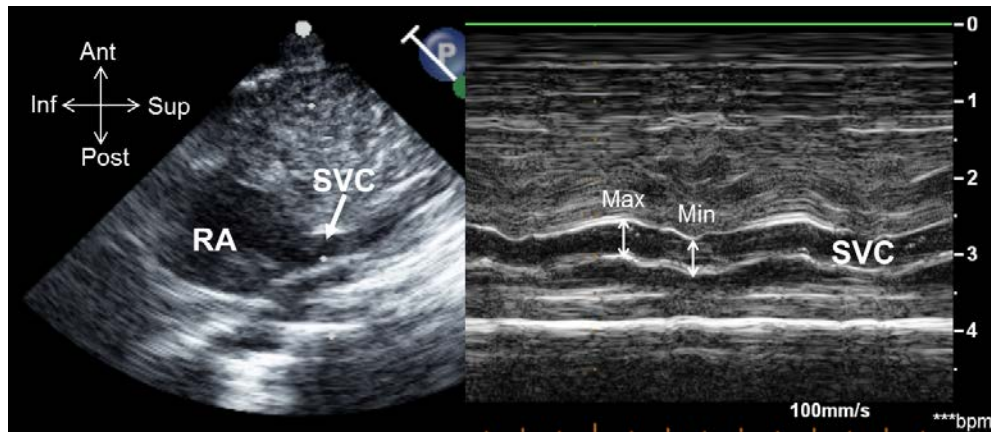
$$\text{SVC flow} = V_{\text{SVC}} \cdot \text{HR} \cdot \pi \cdot (\phi_{\text{SVC}}^2 / 4)$$

where V_{SVC} = velocity time integral of SVC in cm

HR = heart rate per a minute

ϕ_{SVC} = mean SVC diameter in cm

Figure 4.2: Estimation of superior vena cava diameter in a representative infant

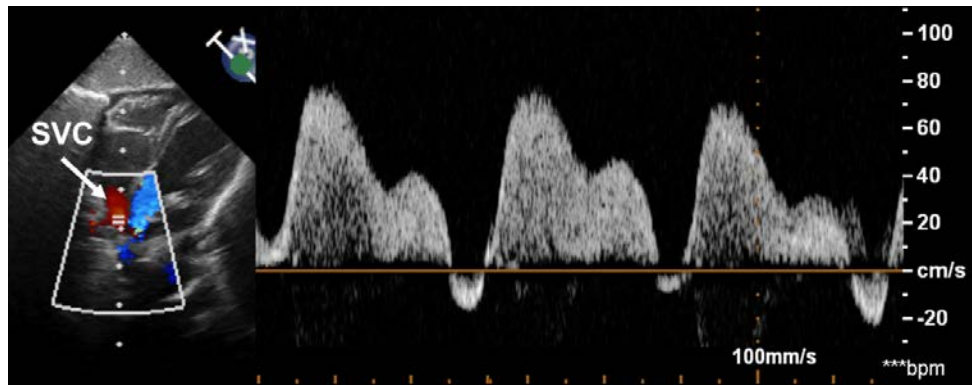


SVC scanned from the parasternal long axis view as it enters the RA. The maximum and minimum internal diameters were measured from an M-mode trace.

Abbreviations: SVC, superior vena cava. RA, right atrium. Max, maximum. Min, minimum.

Ant, anterior. Post, posterior. Sup, superior. Inf, inferior.

Figure 4.3: Measurement of velocity time integral for the superior vena cava flow in a representative infant



Colour Doppler mapping (red area) highlighting the flow up the SVC and into the right atrium (left panel) and SVC flow measured from the subcostal view (right panel). The SVC flow pattern is pulsatile with two peaks: the first associated with ventricular systole and the other with early ventricular diastole. Frequently, short periods of reverse flow associated with atrial systole is observed. The mean velocity of blood flow is calculated from the integral of the Doppler velocity tracings.

Abbreviation: SVC, superior vena cava.

SVC flow was corrected to 100 g brain mass (rSVC flow); the brain weight was estimated from the head circumference using an equation proposed by Dobbing and Sands (119).

$$\text{Brain weight} = \text{HC}^3/100 - 3000/2\text{HC}$$

where HC = head circumference in cm

The presence (and the diameter) of the patent ductus arteriosus was also assessed.

4.1.3.3 NIRS data acquisition

Simultaneously with the echocardiographic examination, another examiner underwent NIRS temporal profile data acquisition using a time-resolved NIRS system (TRS-10, Hamamatsu Photonics, Hamamatsu, Japan) and a probe fixed to the infant's forehead for the estimation of absolute cerebral tissue oxy-, deoxy- and total haemoglobin (Hb) concentrations.

This system employs three pulsed laser diodes with different wavelengths (760 nm, 794 nm and 836 nm) as the light source, which generates light pulses with pulse width of around 100 ps, pulse rate of 5 MHz and an average power of 30 μ W at each wavelength (see (120) for the detail of the system). A photomultiplier tube (H7422P-50MOD, Hamamatsu Photonics, Japan) for high sensitive detection and a signal processing circuit based on a time-correlated single photon counting method for high-speed time-resolved measurements are used. A single GI-mode optical fibre with a numerical aperture of 0.25 and a core diameter of 200 μ m is used for light incident to samples. A SI-mode optical bundle fibre with a numerical aperture of 0.26 and a bundle diameter of 3 mm is used to collect diffuse light from the samples. Prisms are mounted at the tip of each fibre respectively, and the direction of the light incident and collection is vertically bent along the fibre.

Figure 4.4: Mechanism of time resolved near-infrared spectroscopy

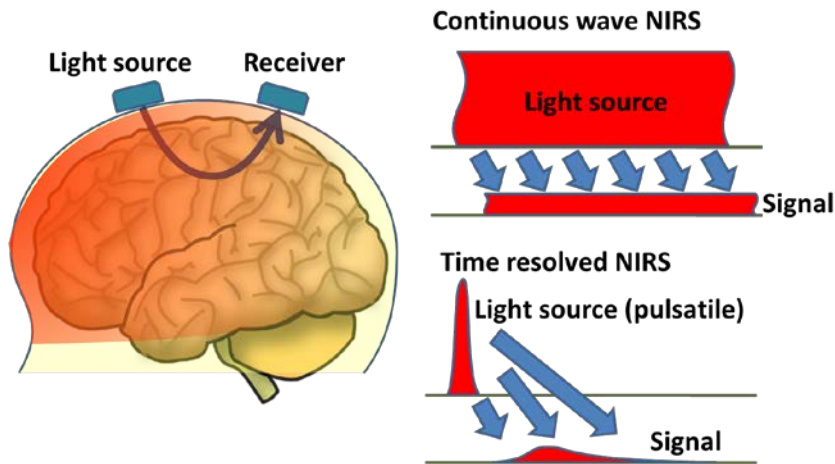


Diagram depicting the difference between continuous wave NIRS and time resolved NIRS. As the latter system can measure the time between the release of a photon and its arrival at the receiver, the path length of the photon can be calculated, which allows the estimation of the absolute haemoglobin concentration within tissue.

Abbreviation: NIRS, near infrared spectroscopy.

A rubber attachment is used for shielding the stray light except the incident light and fixing the incident and collection fibres on the surface of samples with the distance of 30 mm each other. The instrumental response of the system is measured by placing the incident fibre opposite the collection fibre with a neutral density filter between them. Applying the solution of photon diffusion equation for semi-infinite homogeneous media with zero boundary condition in reflectance mode, the values of the reduced scattering coefficients and the absorption coefficients are obtained using the non-linear least-square method considering the instrumental responses. Refractive index in the samples is assumed to be 1.37.

In this 700 nm to 900 nm wave length range, the light absorption $\mu_{a,\lambda}$ at wave length λ in sample can be expressed approximately by the following equation,

$$\mu_{a,\lambda} = \varepsilon_{HbO_2,\lambda} C_{HbO_2} + \varepsilon_{Hb,\lambda} C_{Hb} + \mu_{a,H_2O,\lambda}$$

where, $\varepsilon_{m,\lambda}$ is the molar extinction coefficient of substance m at wave length λ , C_m is the concentration of substance m and the water absorption in sample. After the subtracting the water absorption from $\mu_{a,\lambda}$, assuming that the volume fraction of the water content is constant, the concentrations of HbO₂ and Hb can be determined by solving the simultaneous equations at three wavelengths using the least-square method. Ten-second data acquisition was repeated five times by repositioning the probe each time to give mean values. The data quality was inspected retrospectively for their fitting into the photon diffusion equation and reproducibility before/after repositioning. The tissue oxygenation index (TOI) and cerebral blood volume (CBV) were further calculated using the following formulas.

$$TOI = [\text{oxy-Hb}] / ([\text{oxy-Hb}] + [\text{deoxy-Hb}])$$

$$CBV = ([\text{oxy-Hb}] + [\text{deoxy-Hb}]) \cdot MW_{Hb} \cdot 10^{-6} / (10 \cdot tHb \cdot 10^{-2} \cdot D_t)$$

where [] indicates Hb concentrations in μM

MW_{Hb} = molecular weight of Hb (64,500)

tHb = blood Hb concentration (g/dL)

D_t = brain tissue density (1.05g/mL)

SpO₂ values were recorded at the beginning, in the middle and at the end of the NIRS data acquisition using a pulse oximeter (Rad-8, Masimo, Irvine, CA, U.S.A.) attached to the infant's right hand; the mean value was used for the analysis. Cerebral venous oxygen saturation (SvO₂) was estimated using the method proposed by Tichauer and colleagues (121), which assumed that the relative contribution of venous and arterial blood to the total blood volume in the brain is approximately 3:1 (121, 122).

$$SvO_2 = 4/3 \cdot TOI - 1/3 \cdot SpO_2$$

As surrogate markers for cerebral metabolism, we also calculated the fractional oxygen extraction (FOE) and the cerebral metabolic rate index (CMRO₂ index) using following equations; we used rSVC flow as a surrogate marker of CBF. Because most participating infants were well saturated without additional oxygen, CBF measurement using the oxygen bolus technique was inapplicable. It was based on the assumption that the fraction of the total brain perfusion to the total SVC flow is similar or of limited variations between infants.

$$FOE = (SpO_2 - SvO_2) / SpO_2$$

$$CMRO_2 \text{ index} = 1.34 \cdot tHb \cdot rSVC \text{ flow} \cdot (SpO_2 - SvO_2)$$

where 1.34 (mL) = oxygen-binding capacity of Hb per gram

4.1.3.4 Clinical independent variables for cerebral temperature, perfusion and metabolism

Clinical information, such as the gestational age, body weight, post-natal age, head circumference at the time of the study, and the blood Hb concentration assessed within 3 days of the study, was obtained from the patient's record.

4.1.4 Data analysis

Absolute temperatures were compared between different sites using the paired t-test. Relationships between the absolute/relative temperatures, cerebral metabolism and perfusion, and other clinical and physiological variables were assessed using univariate linear regression analysis. For multivariate models, which explain the absolute and relative temperatures, we assigned up to 3 independent variables based on (i) our hypothesis that body/brain temperatures are determined by cerebral perfusion and metabolism (CMRO₂ index not included due to significant collinearity with the rSVC flow), and (ii) the result of univariate analysis (independent variables with p-values < 0.05 considered). The results from the statistical analysis were presented without correction for multiple comparisons because of the exploratory nature of the study.

4.2 Results

Values are shown as mean (SD) unless otherwise noted. Data from 3 infants were excluded because of poor fitting of NIRS data into the photon diffusion equation.

4.2.1 Summary

In this chapter, the results of the in-vivo measurement of the body/brain temperature, cerebral perfusion and metabolism are presented. In healthy newborn infants, brain temperature was associated with the ambient temperature and cerebral perfusion, but not the body weight; there was a trend that the relationship between cerebral perfusion and brain temperatures was more prominent towards the superficial brain. The rSVC temperatures were not associated with FOE and CMRO₂ index.

4.2.2 Temperature profile

T_{rectal} (37.0 (0.2) °C) was the highest of all temperature monitoring sites, which was followed by T_{brain-25} (36.7(0.2) °C), T_{brain-15} (36.2(0.2) °C) and T_{scalp} (34.7 (0.3) °C) (see table 4.1); significant differences were observed between T_{rectal} and T_{brain-25}, T_{brain-25} and T_{brain-15}, and T_{brain-15} and T_{scalp} respectively (all p < 0.001). A higher T_{rectal} was associated with a greater post-natal age, body weight and brain weight (all p < 0.01).

4.2.3 Echocardiographic observations

The ductus arteriosus was patent in 2 infants; the flow pattern was restrictive with the diameter of less than 1.0 mm in both cases. SVC flow relative to the body weight was 93.1 (35.6) mL/kg/min; no infant showed critically low SVC flow less than 40 mL/kg/min (123). The rSVC flow was not associated with any background clinical variables.

4.2.4 Haemoglobin data profile and its derivative indices

A greater post-natal age was associated with lower blood Hb concentration ($p < 0.001$), cerebral oxy-Hb concentration ($p < 0.01$), TOI ($p < 0.01$), and CMRO₂ index ($p < 0.05$), and greater CBV and FOE (both $p < 0.01$). A greater post-conceptual age was only associated with higher deoxy-Hb concentration ($p < 0.05$). A greater body weight showed weak correlations with greater deoxy-Hb concentration, CBV and FOE, and lower TOI and CMRO₂ index (all $p < 0.05$), whereas a greater brain weight was associated with greater CBV and FOE (both $p < 0.05$), and lower CMRO₂ index ($p < 0.01$).

Table 4.1: Data profile and their dependence on the clinical background variables

		Mean	SD	Pearson product moment correlation coefficient							
				post-natal age		post-conceptual age		body weight		brain weight	
Clinical background variables											
post-natal age	(days)	21.0	17.8	-	-	-	-	-	-	-	-
post-conceptual age	(weeks)	38.1	2.6	-	-	-	-	-	-	-	-
body weight	(g)	2556.0	503.3	-	-	-	-	-	-	-	-
brain weight	(mL)	323.5	55.5	-	-	-	-	-	-	-	-
Temperature measures		(°C)									
ambient temperature		25.7	0.9	-0.12		0.03		-0.22		-0.14	
T _{rectal}		37.0	0.2	0.56	**	0.17		0.53	**	0.52	**
T _{brain-25}		36.7	0.2	0.33		0.35		0.16		0.16	
T _{brain-15}		36.2	0.2	0.31		0.23		0.06		0.25	
T _{scalp}		34.7	0.3	0.37		0.16		0.03		0.21	
T _{brain-25} - T _{rectal}		-0.3	0.2	-0.23		0.20		-0.38	*	-0.37	
T _{brain-15} - T _{rectal}		-0.8	0.3	-0.08		0.13		-0.35		-0.12	
T _{scalp} - T _{rectal}		-2.3	0.3	-0.01		0.05		-0.37		-0.15	
Hb concentration, blood flow & derivatives											
blood Hb	(g/dL)	14.0	3.6	-0.75	***	0.01		-0.15		-0.30	
rSVC flow	(mL/100g/min)	72.7	26.1	-0.20		0.01		-0.22		-0.23	
oxy-Hb	(μM)	34.7	6.4	-0.61	***	0.22		0.13		-0.07	
deoxy-Hb	(μM)	18.2	2.7	-0.28		0.43	*	0.46	*	0.20	
TOI		0.65	0.03	-0.56	**	-0.20		-0.38	*	-0.36	
CBV	(mL/100g)	2.4	0.5	0.62	***	0.27		0.41	*	0.42	*
FOE		0.45	0.05	0.60	**	0.15		0.40	*	0.40	*
CMRO ₂ index	(mLO ₂ /100g/min)	5.7	2.1	-0.46	*	-0.09		-0.38	*	-0.56	**

* p < 0.05, ** p < 0.01 and *** p < 0.001

Abbreviations: SD, standard deviation. T_{rectal} , rectal temperature. $T_{\text{brain-25}}$ and $T_{\text{brain-15}}$, brain temperatures measured using a zero-heat flux thermometer with probe diameters of 25mm and 15mm respectively. T_{scalp} , forehead scalp skin temperature measured using non-contacting infrared thermometer. Hb, haemoglobin. rSVC flow, relative blood flow of the superior vena-cava. TOI, tissue oxygenation index. CBV, cerebral blood volume. FOE, fractional oxygen extraction. CMRO_2 , cerebral metabolic rate of oxygen.

4.2.5 Associations between cerebral temperature, perfusion and metabolism

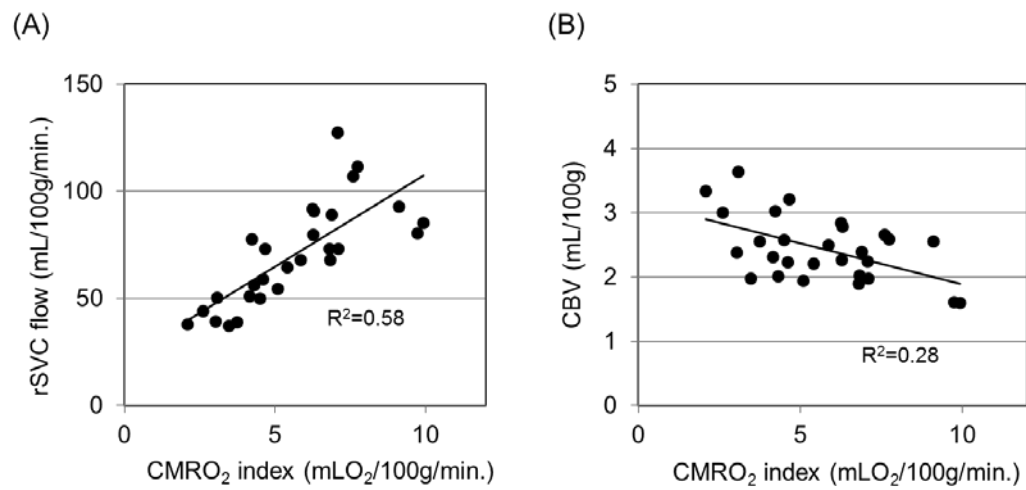
4.2.5.1 Univariate analysis

No significant association was observed between the rSVC flow and CBV. The $CMRO_2$ was positively correlated with the rSVC flow and was negatively correlated with CBV ($p < 0.001$ and $p < 0.05$, respectively) (Fig. 4.5). FOE was not associated with the rSVC flow and CBV. Linear correlations were observed between T_{rectal} , $T_{brain-25}$, $T_{brain-15}$ and T_{scalp} (Table 4.2 (a)). These temperature values were independent from the ambient temperature. A lower T_{rectal} was associated with higher TOI, smaller CBV and smaller FOE (all $p < 0.01$). A lower T_{scalp} was associated with lower rSVC flow and smaller CBV (both $p < 0.05$). Higher $T_{brain-25} - T_{rectal}$, but not $T_{brain-15} - T_{rectal}$ and $T_{scalp} - T_{rectal}$, was correlated with lower T_{rectal} (Table 4.2 (b)). Greater $T_{brain-25} - T_{rectal}$, $T_{brain-15} - T_{rectal}$ and $T_{scalp} - T_{rectal}$ were associated with the higher ambient temperature (all $p < 0.05$, Fig. 4.6 (A)). Greater $T_{brain-15} - T_{rectal}$ and $T_{scalp} - T_{rectal}$ were associated with a higher rSVC flow ($p < 0.05$ and $p < 0.01$, respectively; Fig. 4.6 (B)).

4.2.5.2 Multivariate analysis

A higher T_{rectal} was associated with greater FOE and CBV (both $p < 0.05$). A higher $T_{brain-25}$ and $T_{brain-15}$ were associated with a greater rSVC flow (both $p < 0.05$). A higher T_{scalp} was associated with a greater rSVC flow and CBV (both $p < 0.01$). Greater $T_{brain-15} - T_{rectal}$ and $T_{scalp} - T_{rectal}$ were associated with the higher ambient temperature (both $p < 0.05$). Greater $T_{scalp} - T_{rectal}$ was associated with a higher rSVC flow ($p < 0.01$).

Figure 4.5: Relationship between cerebral metabolism and haemodynamics

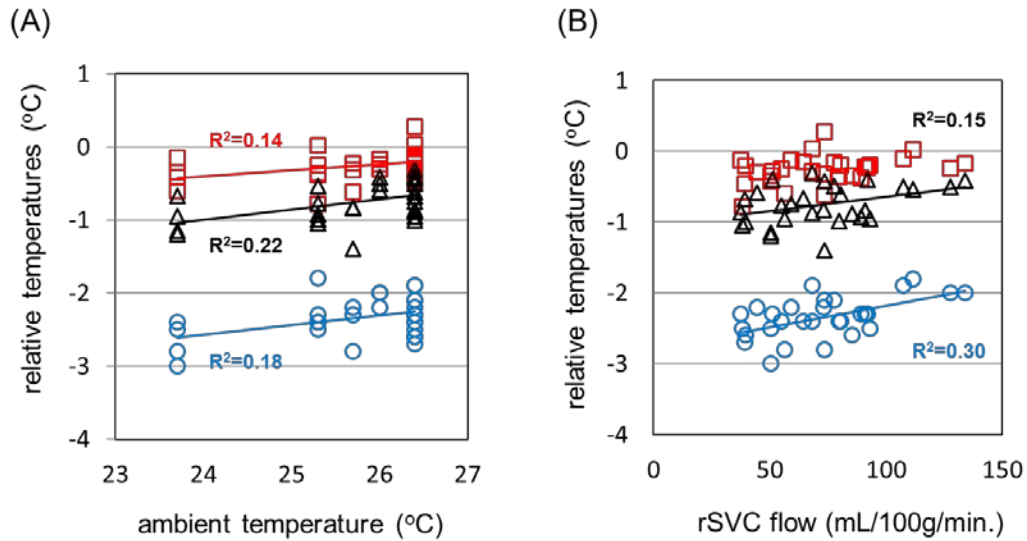


The CMRO₂ index was positively correlated with the rSVC flow (A) and was negatively correlated with CBV (B) ($p < 0.001$ and $p < 0.05$, respectively).

Regression lines are from univariate analysis.

Abbreviations: CMRO₂, cerebral metabolic rate of oxygen. CBV, cerebral blood volume.

Figure 4.6: Independent variables associated with relative brain temperatures



(A): Greater $T_{\text{brain-25}} - T_{\text{rectal}}$ (□), $T_{\text{brain-15}} - T_{\text{rectal}}$ (Δ) and $T_{\text{scalp}} - T_{\text{rectal}}$ (○) were associated with the lower ambient temperature (all $p < 0.05$). **(B):** Greater $T_{\text{brain-15}} - T_{\text{rectal}}$ and $T_{\text{scalp}} - T_{\text{rectal}}$ were associated with a higher rSVC flow ($p < 0.05$ and $p < 0.01$, respectively). R^2 values and regression lines were showed same colours with markers of relative brain temperatures, respectively: Regression lines are from univariate analysis with $p < 0.05$. Abbreviations: rSVC flow, relative flow of the superior vena cava. T_{scalp} , scalp temperature. T_{rectal} , rectal temperature. $T_{\text{brain-15}}$ and $T_{\text{brain-25}}$, brain temperatures obtained using zero-heat-flow tissue-core thermometer at the forehead and the anterior fontanelle, respectively.

Table 4.2: Relationships between cerebral perfusion, metabolism and temperatures

(a) Absolute temperature

Univariate analysis		Pearson product moment correlation coefficient			
Variables		T _{rectal}	T _{brain-25}	T _{brain-15}	T _{scalp}
Temperature measures					
ambient temperature		-0.19	0.17	0.28	0.26
T _{rectal}		-	0.54 **	0.51 **	0.46 *
T _{brain-25}		-	-	0.65 ***	0.67 ***
T _{brain-15}		-	-	-	0.81 ***
Hb, blood flow and derivatives					
rSVC flow		-0.05	0.27	0.30	0.47 *
TOI		-0.50 **	-0.34	-0.14	-0.16
CBV		0.52 **	0.31	0.21	0.39 *
FOE		0.51 **	0.29	0.17	0.14
CMRO ₂ index		-0.34	-0.05	-0.09	-0.05
Multivariate analysis		Standardised partial regression coefficient			
Variables		T _{rectal}	T _{brain-25}	T _{brain-15}	T _{scalp}
rSVC flow		0.14	0.40 *	0.40 *	0.59 **
CBV		0.43 *	0.30	0.22	0.45 **
FOE		0.44 *	0.32	0.21	0.17

(b) Relative brain temperature to T_{rectal}

Univariate analysis		Pearson product moment correlation coefficient			
Variables		$T_{\text{brain-25}} - T_{\text{rectal}}$	$T_{\text{brain-15}} - T_{\text{rectal}}$	$T_{\text{scalp}} - T_{\text{rectal}}$	
Temperature measures					
ambient temperature		0.38 *	0.47 *	0.42 *	
T_{rectal}		-0.46 *	-0.20	-0.23	
$T_{\text{brain-25}}$		0.50	0.33	0.34	
$T_{\text{brain-15}}$		0.17	0.75 ***	0.51 **	
T_{scalp}		0.23	0.56 **	0.76 ***	
Hb, blood flow and derivatives					
rSVC flow		0.34	0.38 *	0.55 **	
TOI		0.16	0.23	0.20	
CBV		-0.21	-0.16	0.05	
FOE		-0.22	-0.21	-0.23	
CMRO ₂ index		0.31	0.18	0.21	
Multivariate analysis		Standardised partial regression coefficient			
Variables		$T_{\text{brain-25}} - T_{\text{rectal}}$	$T_{\text{brain-15}} - T_{\text{rectal}}$	$T_{\text{scalp}} - T_{\text{rectal}}$	
ambient temperature		0.30	0.40 *	0.36 *	
rSVC flow		0.25	0.29	0.50 **	
CBV		-0.08	-0.05	-0.09	
FOE		-0.08	-0.01	0.24	

* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$

Abbreviations: T_{rectal} , rectal temperature. $T_{\text{brain-25}}$ and $T_{\text{brain-15}}$, brain temperatures measured using a zero-heat flux thermometer with probe diameters of 25mm and 15mm respectively. T_{scalp} , forehead scalp skin temperature measured using non-contacting infrared thermometer. Hb, haemoglobin. rSVC flow, relative blood flow of the superior vena-cava. TOI, tissue oxygenation index. CBV, cerebral blood volume. CMRO_2 , cerebral metabolic rate of oxygen.

4.3 Brief Discussion

4.3.1 Summary

Our hypothesis which was based on the findings in the experimental setting was refuted. Unlike anaesthetised animals undergoing temperature monitoring in a relatively cooler environment, brain temperatures in the newborn infants were associated with ambient temperature, but not body size. Brain temperature was associated with cerebral perfusion, particularly in the superficial brain; although brain perfusion and metabolism were positively coupled between each other, the variation in cerebral metabolism had no significant impact on regional brain temperatures. Thus far, CBF has been predominantly linked with heat removal from the deep brain tissue. However, our data suggest that CBF may have an alternative role to deliver heat to the superficial brain. The findings of this study are discussed in this chapter.

- Key Findings from this Study

We were able to obtain information about cerebral metabolism and perfusion in healthy newborn infants using two non-invasive bedside techniques: NIRS and echocardiography. In these healthy infants, cerebral perfusion and ambient temperature were significantly associated with brain temperatures. Cerebral perfusion was most closely associated with superficial, not deeper brain temperatures. A cooler ambient temperature was associated with a greater temperature gradient between the scalp surface and the body core. Further studies are required to assess the influence of cerebral metabolism and perfusion to regional brain temperatures in low-cardiac output conditions, fever and with therapeutic hypothermia.

- What is already known and what our study adds

What is already known:

- Bedside monitoring of cerebral metabolism and perfusion has been established using NIRS and tracers such as oxygen and indocyanine green.
- In uninjured brain, tissue perfusion is tightly adjusted according to the change in cerebral energy metabolism to suffice the tissue demand for energy substrates.
- Brain temperature is determined by the balance between cerebral metabolism, CBF and heat dissipation through the scalp.
- In newborn preclinical and clinical studies, ambient temperature and humidity, maturation of skin, and body size have been recognised as important influencing factors to the body/brain temperatures.

What our study adds:

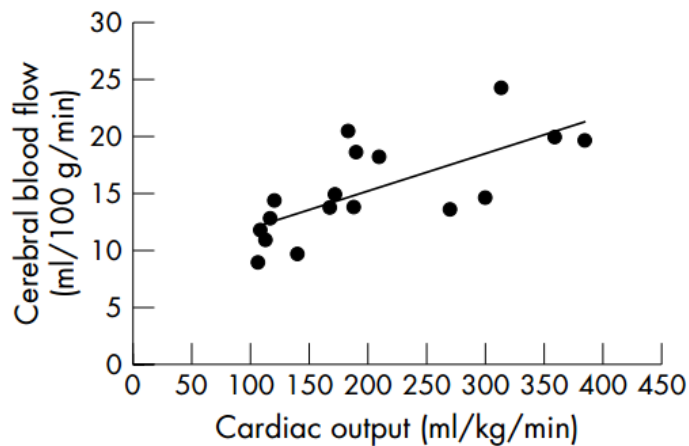
- Using NIRS and echocardiography, but not tracers, cerebral metabolism and perfusion were estimated in the newborn infant.
- In healthy newborn infants under normal ambient conditions, a greater rSVC flow was associated with higher superficial brain temperatures, suggesting a role of cerebral perfusion to deliver heat to the superficial brain.

4.3.2 Non-invasive, bedside monitoring of cerebral metabolism and perfusion

Recent advances in biophysical probes provided a range of useful techniques to monitor the cerebral oxygen metabolism and perfusion. As for the use in the newborn infant, NIRS, which is available at the cotside, has an outstanding advantage to other methods, such as MRI, magnetic resonance spectroscopy and positron-emission computed tomography. However, NIRS still requires clinically unnecessary tracers in measuring CBF, which may have restricted the extensive use of this technique. In our current study, by combining NIRS and echocardiography, we were able to obtain surrogate markers of $CMRO_2$ and CBF without using tracers.

Although the obtained data showed relevant correlations with other physiological variables, it has to be noted that our method is based on several assumptions. For example, the fraction of CBF relative to SVC flow was assumed to be similar between subjects. According to a study in newborn infants, which used indocyanine green pulse dye densitometry, the relationship between left ventricular output (LVO) and CBF was estimated as: $CBF = 0.03 \cdot LVO + 8.71$ (124). Given that SVC flow is approximately 52% of LVO in term infants (82), CBF in our study population is estimated to be 12.9 (1.5) mL/(100g brain weight)/min, which corresponds to the reported CBF values in newborn infants. However, unlike the tracer bolus technique which gives the regional CBF value, our method can only provide the surrogate for the global CBF, suggesting its limitation under pathologically altered CBF distributions (125).

Figure 4.7: Correlation between cardiac output and cerebral blood flow

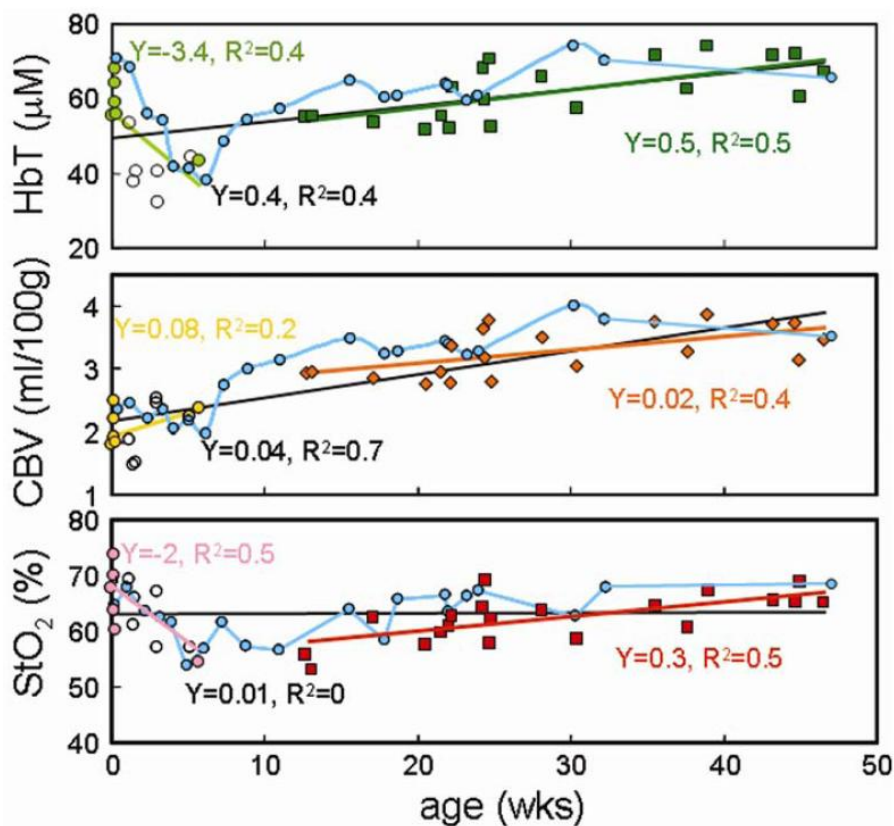


Cerebral blood flow was measured by multichannel near infrared spectroscopy and pulse dye densitometry using indocyanine green in 19 newborn infants. The linear regression line is described by $CBF = 0.03 CO + 8.71$, with a correlation coefficient of 0.70 and $p = 0.002$.

From “Cerebral distribution of cardiac output in newborn infants.” by Kusaka T, Okubo K, Nagano K, Isobe K, Itoh S. Arch Dis Child Fetal Neonatal Ed. 2005 Jan;90(1):F77-8.

Our data suggested post-natal decrease in oxy-Hb and TOI, and increase in CBV and FOE, highlighting the influence of growth-related increase in cerebral venous blood pool and the post-natal progress in anaemia (126).

Figure 4.8: Cerebral haemodynamic change after birth



From top to bottom: HbT, CBV, and StO₂ as a function of age measured in the frontal region in all infants. The straight lines are linear regressions from 0 to 6, 12 to 50, and 0 to 50 weeks. Open circles correspond to premature babies. Cyan circles are results for a baby measured longitudinally 20 times from 0 to 11 months. Y and R² for each regression line are reported on the graphs with the same color as the regression line.

From “Assessment of infant brain development with frequency-domain near-infrared spectroscopy.” by Franceschini MA, Thaker S, Themelis G, Krishnamoorthy KK, Bortfeld H, Diamond SG, Boas DA, Arvin K, Grant PE. *Pediatr Res.* 2007 May;61(5Pt1):546-51.

Abbreviations: HbT, total-haemoglobin concentration. CBV, cerebral blood volume. StO₂, tissue oxygen saturation.

In contrast, the CMRO₂ index showed a weak negative association with the postnatal age. Although previous studies have demonstrated gradual increases in CMRO₂ with

age (79), its timing and rate of change varied considerably between studies, suggesting that the range of the post-natal age for our subjects might be too narrow to demonstrate the growth-related change in cerebral metabolism.

In our current study, we used time-resolved NIRS, which instantly provides absolute Hb concentrations and CBV. Several studies have proposed the estimation of CBF from CBV using a formula: $(CBV/CBV_0) = (CBF/CBF_0)^G$, where G is the Grub's exponent, and CBV_0 and CBF_0 are baseline values. In our current study, we did not observe any association between the rSVC flow and CBV, presumably because this technique has only been validated for dynamic intra-subject CBF changes induced by substantial hypercapnia and hypocapnia (79, 127, 128). Future studies need to investigate whether subtle intra-subject differences in CBF can be extrapolated from CBV.

4.3.3 Brain temperature and monitoring

Non-invasive brain temperature monitoring using the zero-heat-flux method was initially proposed by Fox and Solman (129), and it was later introduced to the newborn infant (67, 95). Of various sizes and types of zero-heat-flux thermometers, Matsukawa and colleagues validated the same system with ours, but with a full-size sensor element (43mm in diameter), and reported that the temperature reflected the tissue of 18-38mm from the surface (96). Because the full-size probe was too large for the infant's forehead, we used small-sized probes with the diameters of 15 mm and 25 mm, expecting to monitor the temperature of the superficial brain tissue with approximately 10 to 20mm depths rather than the core structure. This assumption was relevant to the finding that the temperature showed gradual reduction from T_{rectal} to $T_{\text{brain-25}}$, $T_{\text{brain-15}}$, and then, T_{scalp} ,

as the core brain temperature of the newborn species is higher than other core body temperatures (67, 130).

4.3.4 Associations between body size, ambient temperature and brain/body temperatures

Previously, we found that a smaller body size was associated with a cooler brain temperature in a newborn piglet model (see chapter 2). Based on this observation, we hypothesised that a similar relationship between brain temperatures and the body size are observed in the newborn infant. However, this hypothesis was refuted in the current study, which recruited healthy newborn infants; no relationship was observed between brain temperatures and the body weight. In the previous experimental study, the rectal temperature of the piglet was tightly controlled to the target level; in addition, animals were anaesthetised throughout the experiment, which is likely to suppress the spontaneous temperature control and variation in the body temperature. In contrast, in the current clinical study, there was a greater variation in rectal temperature, which showed positive association with the body weight of infants. Given the positive correlation between brain temperatures and rectal temperature, the lack of relationships between brain temperatures and the body size might be explained by the greater variations in subjects' background factors (e.g. ambient temperature, gestational age and postnatal age) and their interactions between each other. For example, more mature (and hence generally larger) infants respond to both excessively warm and cold environments more efficiently by altering metabolic thermogenesis, vasomotor regulation and sweating, thus compromising the association between regional brain temperatures and other variables (69, 131, 132). In addition, unlike the experimental

model in the previous study, infants in the current study were insulated by cotton clothes and blanket, which may cause scalp-surface-predominant heat exchange with the ambient air.

4.3.5 Associations between metabolism, perfusion and brain temperature

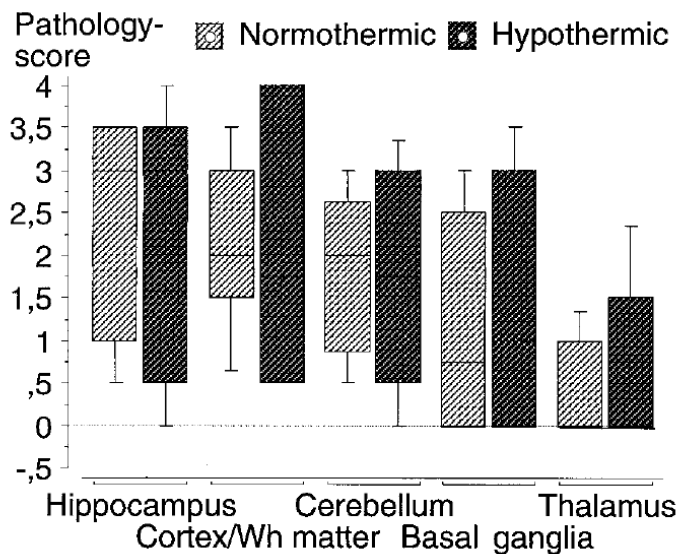
To promote intact survival following high-risk birth, it is essential to maintain cerebral perfusion appropriate to its demand, because of the little storage of energy substrates within the brain (133). Recent clinical studies in asphyxiated newborn infants suggest that mild to moderate therapeutic hypothermia is neuroprotective, whereas even modest hyperthermia is deleterious (52, 134). However, thus far, few studies have addressed the associations between the body/brain temperature, metabolism and perfusion in the newborn infant. Based on a small number of clinical cases, it was reported that newborn infants with diminished cerebral metabolism due to the underlying brain pathology, such as hydrocephalus and cerebral asphyxia, have low brain temperatures, whereas infants with cerebral hypo-perfusion due to hypo-plastic left heart syndrome have relatively higher brain temperatures (67). In addition to the local temperature regulation in the brain, systemic thermogenic response to cold stress has been well described in the newborn species (135, 136). Referring to these findings, we initially estimated that, in healthy newborn infants, spontaneous body/brain temperature reduction may be observed in association with reduced systemic/cerebral metabolism.

Contrary to our estimation, associations between the cerebral metabolism and the brain temperature were not recognised, which might be explained by the limited effect of cerebral metabolism to the brain temperature under a physiological condition (137). Instead, the rSVC flow showed the most prominent association with the brain

temperature in our subjects: the greater the cerebral perfusion was, the higher the brain temperature was. This finding may be initially counterintuitive, because the blood temperature is higher in the jugular vein than in the carotid artery (138), and hence, cerebral perfusion generally contributes to the heat removal from the brain tissue (67, 68, 137). However, the superficial brain is spontaneously cooled by the environment, and is cooler than the arterial blood, suggesting that, under a physiological condition, an increase in CBF may lead to simultaneous increase in the superficial brain temperature and decrease in the deep brain temperature. A similar phenomenon, or “the temperature-shielding effect of blood flow”, has been suggested from a mathematical model and in a small animal model, where CBF was supposed to protect the brain from “extracranial cold” to penetrate deep brain structures (68). Although we did not monitor the core brain temperature, the positive association between brain temperatures and rSVC flow became weaker towards deep brain structures.

Further investigations are required to assess the influence of cerebral metabolism and perfusion to regional brain temperature by incorporating a range of clinical conditions, such as low-cardiac output, high fever, neonatal encephalopathy, therapeutic hypothermia, and sedation, which may determine the optimal therapeutic temperature distribution within the injured brain.

Figure 4.9: Loss of hypothermic neuroprotection in unsedated newborn piglets



Pathology scores (and interquartile range) were invariant between cooled subjects (24 h to 35°C followed by 48 h of normothermia) and normothermic subjects (T_{rectal} at 39°C for 72 h). It was speculated that the stress of shivering and feeling cold interfered with the previously shown neuroprotective effect of hypothermia.

From “Twenty-four hours of mild hypothermia in unsedated newborn pigs starting after a severe global hypoxic-ischemic insult is not neuroprotective.” by Thoresen M, Satas S, Løberg EM, Whitelaw A, Acolet D, Lindgren C, Penrice J, Robertson N, Haug E, Steen PA. *Pediatr Res.* 2001 Sep;50(3):405-11.

It was also interesting that T_{scalp} , which was measured using a non-contacting infrared thermometer, showed relatively higher correlations with rSVC flow compared with other brain temperatures. This observation may merely reflect a greater bias to the indirect temperature estimation using thermo-compensation probes compared to the direct scalp-skin temperature measurement using infrared light. However, given that the perfusion of the cortical grey matter is much higher than that of subcortical white matter

(139), T_{scalp} might more precisely reflect subtle changes in the blood flow and metabolism in the superficial brain structure compared to other methods.

4.3.6 Limitation of the study

Our current study is a cross-sectional observational study, and hence, our speculations derived from inter-subject correlations of variables have to be confirmed in future studies, which incorporate repeated measures within subjects. As previously discussed, our non-invasive monitoring technique of cerebral metabolism and perfusion is based on the assumptions that haemodynamic information derived from NIRS is predominantly obtained from intra-cerebral blood flow, and that, several factors, such as the cerebral arterial/venous blood contribution and regional CBF distribution, are consistent or of limited variation between subjects. In addition, our method estimated global CBF, which would not be suitable to identify spatially heterogeneous CBF distributions (125). Hence, our non-invasive monitoring method might be less accurate compared to direct, invasive monitoring modalities. Because these variables were compared with body/brain temperatures, which had a limited variation range between 34.1 and 37.4°C, caution against false-positive/negative observations is required.

Newly developed techniques such as the one we describe and near-infrared diffuse correlation spectroscopy, which allow the estimation of local CBF without tracers need validation (140). In addition, we were unable to study newborn infants shortly after birth; during this time more dynamic changes in thermo-regulative variables might be observed. These limitations have to be noted in interpreting our current findings to the clinical practice.

4.3.7 Future investigations

Considering the rapid increase in the number of newborn infants who undergo therapeutic hypothermia as a standard of care for neonatal encephalopathy (141), it is essential to optimise the detailed protocol of this treatment to increase the number of responders to the treatment. Findings from our previous preclinical study suggested that, even under therapeutic hypothermia, which used a solid cooling protocol, the temperature value delivered to the local brain tissue is highly heterogeneous according to physiological background factors such as the body weight (see chapter 2). Although we observed significant relationships between regional brain temperatures, ambient temperature and cerebral perfusion, we did not demonstrate a relationship between brain temperature and body size. This differs to our experimental study in anaesthetised piglets. Future studies need to assess the relationship between the body size, cerebral metabolism/perfusion and regional brain temperatures by including larger dynamic changes in intrinsic and extrinsic temperature regulation, such in newborn infants shortly after birth and those undergoing therapeutic hypothermia.

In addition to the variation in response to cooling, inconsistency in the cooling procedures may further increase the range in the regional brain temperature and the corresponding level of tissue protection. Currently, therapeutic hypothermia for asphyxiated newborn infants are generally conducted using the protocols designed for the large scale clinical studies (142), which is compliant to the recommendation in 2010 Consensus on Science with Treatment Recommendations. However, the detail of cooling modalities and supportive treatments, such as the temperature of the cooling cap and the type of sedation provided, are highly heterogeneous between institutions

(143). To yield the optimal brain protection by therapeutic hypothermia, especially when selective head cooling is used, temperatures for the cooling device may have to be adjusted according to systemic/cerebral perfusion and metabolism. Future studies have to address exact relationships between clinical background factors, cooling modalities, regional brain temperatures, brain perfusion and metabolism, subsequent protection level of the brain tissue, and the long term neurodevelopmental functioning. Translational large animal models and bridging biomarkers such as magnetic resonance spectroscopic imaging and NIRS are likely to play important roles in providing swift feed back to the clinical practice.

4.3.8 Conclusions

Cerebral oxygen metabolism and perfusion were monitored in newborn infants using two non-invasive techniques. In these healthy infants, cerebral perfusion and ambient temperature showed significant correlation with brain temperatures. Cerebral perfusion was most closely associated with superficial, nor deep brain temperatures. Further studies are required to assess the influence of cerebral metabolism and perfusion to regional brain temperature in low-cardiac output conditions, fever and with therapeutic hypothermia.

Chapter 5

**Induction and maintenance of therapeutic hypothermia using
either commercial water bottles or a “phase changing material” mattress
to facilitate safe and efficient pre-hospital cooling**

5.1 Methods

5.1.1 Summary

This chapter presents the third part of the study. The aim was to prove the hypothesis that a temporal, non-electronic device provides safe and stable cooling by the time of admission to a tertiary cooling centre. To assess the utility of two low-tech, low-cost cooling devices, eleven anaesthetised newborn piglets were observed between 2-26 h after hypoxia-ischaemia with the target T_{rectal} of 33 to 34°C.

- Ethical approval of this study

This study was conducted under UK Home Office licence in accordance with UK guidelines.

5.1.2 Subject preparation

Eleven male Large-White piglets (mean body weight (SD), 1.73 (0.17) kg) were studied within 24 h of birth.

5.1.3 Anaesthesia and surgical procedures

Animals were surgically prepared under general anaesthesia, and received intensive life support and continuous physiological monitoring including arterial oxygen saturation throughout the experimental procedure as previously described (see chapter 3.1.3). Briefly, general anaesthesia was induced using isoflurane (5% through a facial mask during the insertion of the tracheostomy tube, ~3% during surgery and 1.5-2.5% thereafter). Animals were mechanically ventilated through the tracheostomy tube to maintain normal PaCO_2 and PaO_2 . Maintenance fluid, continuous morphine infusion

and antibiotics were given via the umbilical venous catheter. Heart rate, blood pressure, and blood gases were monitored via an umbilical arterial catheter. Both common carotid arteries were encircled by vascular occluders (OC2A, In Vivo Metric, Healdsburg, CA, U.S.A.). The aEEG (BRM2 Brain Monitor, Brainz Instrument, Manukau, New Zealand) was continuously monitored using 2 each right and left (1.5 cm lateral from the sagittal suture) recording electrodes (1 cm and 3.5 cm posterior from the bregma) and a reference electrode. Following surgery, piglets were positioned prone on an open incubator covered with a polytrianfoam mattress. The T_{rectal} was monitored (Arbo N44-91, Kendall, Powell, TN, U.S.A.) at 6 cm from the anal sphincter, which was initially maintained normothermic for the newborn piglet ($38.5 \pm 0.5^{\circ}\text{C}$) using cotton blankets over the dorsal trunk.

5.1.4 Transient hypoxia-ischaemia and assessment of insult severity

After stable normothermia was achieved (defined as a T_{rectal} fluctuation $< 0.2^{\circ}\text{C}$ for at least 30 min without modifying blanket use), piglets were exposed to transient hypoxia-ischaemia before the initiation of cooling, because perinatal hypoxia-ischaemia is known to alter CBF and cerebral metabolism (14, 144), which may further affect regional brain temperatures. The fraction of inspired oxygen (FiO_2) was reduced to 12% and simultaneously the carotid artery occluders were inflated. A fixed insult protocol was used for induction (10 min with FiO_2 12%) and maintenance (15 min with FiO_2 16%); this was based on the average induction time and the total insult duration required to induce 70% NTP deprivation and moderate cerebral injury with 30-40% cortical neuronal death at 48 h in our previous study performed using phosphorous-31 magnetic resonance spectroscopy (62). After this period resuscitation was commenced by deflating the

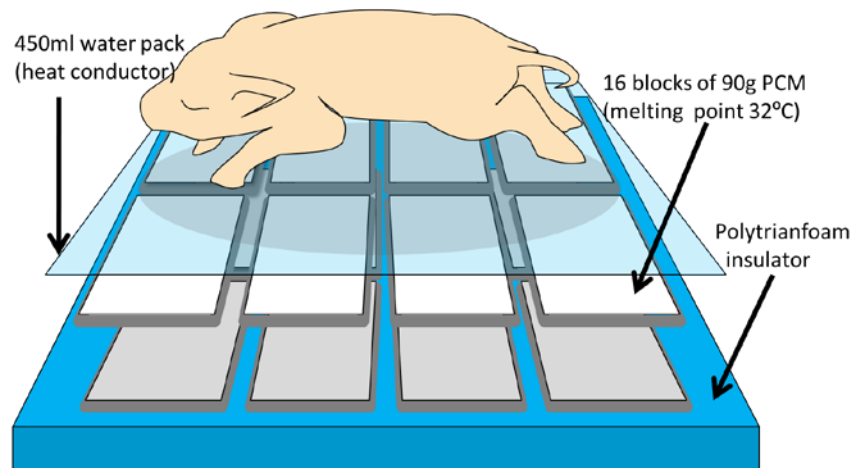
balloon occluders and increasing FiO_2 ; normal arterial oxygen saturation was maintained thereafter. The insult severity and the short term cerebral functional outcome were assessed using the upper and lower margin of aEEG at the commencement of and 2, 14 and 26 h after resuscitation and at the completion of rewarming.

5.1.5 Preparation of cooling devices

Piglets were randomly assigned to 2 groups: (i) therapeutic hypothermia using a PCM mattress (n = 6, body weight 1719 (163) g) or (ii) therapeutic hypothermia using water bottles (n = 5, 1749 (189) g). For the PCM mattress, 90 g of PCM with a melting point of 32°C (Climsel 32, Climator, Skövde, Sweden: latent-heat 54 Wh/kg, specific-heat 1 Wh/kg/°C) was packaged in a laminate pack. Sixteen PCM packs were built into 2 layers of 4 × 2 PCM sheets, with the upper surface covered by a thermal conductor (a plastic bag filled with 450 ml water, See Fig. 5.1 (A)). The mattress (total-weight 1940 g) was preserved at 25°C. For water bottle cooling, 2 commercially available water bottles (Boots, Nottingham, UK: efficient cooling surface ~22 × 30 cm per each, See Fig. 5.1 (B)) were filled with 1 liter each of tepid tap water adjusted to 25°C just before the application (total-weight 2800 g). Temperatures of the thermal conductor, PCM in the superficial layer, and the water bottle were monitored using thermistor probes (ThermoTrace, Dräger, Lübeck, Germany) placed on the upper surface of the subject covered with a small urethane insulator (1.4 cm diameter, 4 mm thick).

Figure 5.1: Diagrams demonstrating the construction and the position of the piglet on the PCM cooling mattress and the water bottles

(A) PCM cooling mattress



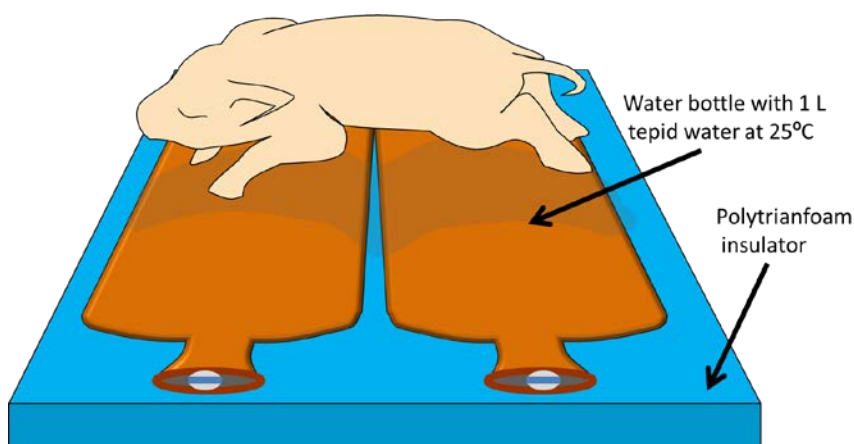
PCM mattress:

16 PCM packs with a specific melting point of 32°C were built into 2 layers of 4 × 2 PCM sheets.

The upper surface of the PCM mattress was covered by a plastic water bag to improve thermal conductance.

Abbreviation: PCM, phase changing material.

(B) Water bottles



Commercial water bottles:

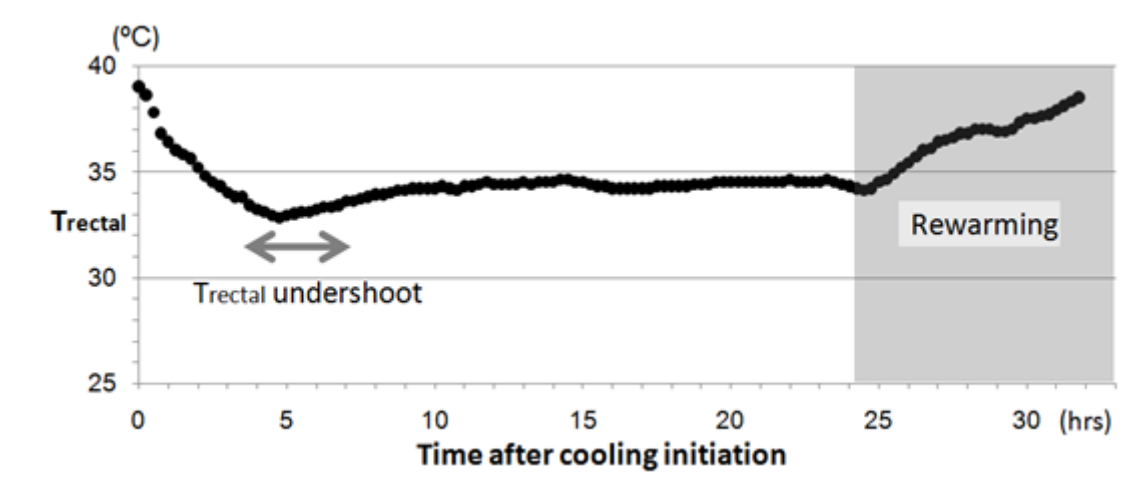
2 water bottles were filled with 1 liter each of tepid water at 25°C.

These cooling devices were placed underneath the piglet's head, trunk and limbs. An insulator mattress was used underneath the cooling devices so as to allow the heat exchange only with the ambient air (25°C) but not with the incubator surface.

5.1.6 Temperature control

Physiological data were recorded every 15 min. Two h following transient hypoxia-ischaemia and resuscitation, the piglet was placed on either the PCM mattress or the water bottles. We aimed to maintain T_{rectal} within the target range of 33-34°C during 2-26 h following resuscitation. To minimise transient overcooling, which was commonly observed in our pilot studies (Fig. 5.2), a cotton blanket was applied once T_{rectal} was recognised to be at or below 34°C with the regular (every 15 min) data recording. The T_{rectal} undershoot was adjusted using cotton blankets thereafter, however no more than one blanket was applied (or removed) every 15 min. If T_{rectal} deviated over this range, the cooling device was replaced. Twenty-six h after resuscitation, rewarming at $\sim 0.5^{\circ}\text{C/h}$ was commenced by reducing the exposed surface area of the piglet's skin to the cooling device. Intravenous phenobarbital was used when seizures were clinically detected. After rewarming to normothermia, the animal was humanely sacrificed (intravenous pentobarbitone overdose).

Figure 5.2: Pilot study showing transient temperature undershoot after the initiation of PCM mattress without blankets



When the PCM mattress was applied to a piglet without using blankets, the animal's T_{rectal} transiently dropped to 32.6°C; this was followed by a gradual recovery and establishment of a temperature equilibrium within the target T_{rectal} of 33-34°C at ~7 h after cooling initiation. In the main study, because we anticipated a similar period of excessive cooling, blankets were applied when the T_{rectal} reached the target range following the initiation of cooling.

Abbreviations: PCM, phase changing material. T_{rectal} , rectal temperature.

5.1.7 Data analysis

The results were compared using ANOVA or Fisher's exact test when appropriate. Results were adjusted for multiple comparisons using Bonferroni correction. For the comparison of the temperature stability within the target range, logistic regression with a generalised estimating equation was used, taking into account repeated measurements.

5.2 Results

Values are shown as mean (SD) unless described.

5.2.1 Summary

In this chapter, findings in temporal changes for T_{rectal} and its stability provided by low-tech cooling devices are presented with information in the clinical procedure required during cooling. Although the water bottles and the PCM mattress were both able to induce and maintain therapeutic hypothermia between 2-26 h after hypoxia-ischaemia, PCM cooling led to a longer period within target T_{rectal} and more stable cooling; water bottle cooling required frequent device renewal.

5.2.2 Physiological statement

One piglet cooled with PCM died 16 h after resuscitation due to respiratory failure. The data from this piglet were not used in the comparison during the maintenance phase and rewarming. All other piglets survived to the completion of rewarming. Physiological data before (6 PCM and 5 water bottles), during (6 PCM and 5 water bottles) and after (5 PCM and 5 water bottles) hypoxia-ischaemia were not significantly different between groups (Table 5.1). The number of animals developing seizures and requiring phenobarbital injection (3/6 for PCM and 2/5 for water bottles) were also similar between groups.

Table 5.1: Physiological variables

		Baseline*	Nadir of HI*	2 h*	14 h**	26 h**	End of experiment**
HR (min ⁻¹)	PCM	165 (29)	208 (24)	160 (16)	121 (18)	117 (29)	170 (8)
	WB	156 (19)	224 (23)	168 (31)	128 (22)	129 (18)	171 (20)
MABP (mmHg)	PCM	51.4 (15.5)	46.6 (20.0)	42.2 (5.1)	36.0 (4.5)	40.0 (5.8)	42.3 (7.9)
	WB	41.6 (5.9)	37.4 (9.6)	40.4 (4.3)	36.4 (4.7)	45.4 (6.8)	47.2 (9.9)
Arterial blood gas analysis							
pH	PCM	7.47 (0.06)	7.47 (0.08)	7.55 (0.11)	7.49 (0.09)	7.53 (0.07)	7.44 (0.10)
	WB	7.39 (0.05)	7.37 (0.08)	7.43 (0.06)	7.40 (0.13)	7.37 (0.17)	7.36 (0.10)
PaCO ₂ (kPa)	PCM	5.46 (0.96)	5.04 (0.82)	4.39 (1.05)	5.13 (1.22)	4.09 (0.67)	5.83 (1.49)
	WB	6.62 (0.92)	6.13 (1.56)	5.45 (0.49)	6.65 (1.51)	6.92 (1.67)	6.99 (0.76)
PaO ₂ (kPa)	PCM	11.20 (6.70)	2.61 (0.73)	11.68 (5.34)	9.20 (5.22)	8.22 (5.94)	10.42 (3.53)
	WB	12.24 (1.50)	3.08 (0.86)	12.10 (1.58)	12.26 (4.88)	13.85 (2.49)	11.67 (5.29)
BE (mmol/L)	PCM	5.3 (2.7)	3.6 (3.0)	5.9 (2.8)	5.6 (2.7)	2.8 (1.8)	4.6 (1.8)
	WB	4.4 (6.5)	0.7 (5.9)	2.9 (5.9)	5.7 (6.8)	3.8 (7.1)	3.0 (6.7)
Lactate (mmol/L)	PCM	2.67 (1.71)	3.68 (1.60)	2.17 (1.01)	1.35 (0.64)	1.46 (0.61)	1.21 (0.43)
	WB	2.66 (0.84)	6.45 (1.71)	2.44 (1.64)	0.97 (0.32)	1.04 (0.33)	1.23 (0.12)

Data shown as mean (standard deviation)

Number of subjects included: * 6 PCM and 5 WB. ** 5 PCM and 5 WB.

Abbreviations: HR, heart rate. MABP, mean arterial blood pressure. BE, base excess. PCM, phase changing material. WB, water bottle. HI, hypoxia-ischaemia.

5.2.3 Hypoxic-ischaemic insult and aEEG change with time

(6 PCM and 5 water bottles)

At the end of the insult the mean upper (and lower) margins of aEEG in PCM and water bottle groups were 38.4 (36.7) % and 28.6 (33.4) % of baseline; this gradually increased to 65.7 (42.7) % and 53.1 (51.8) % toward the end of the experiment (See Table 5.2). There was no intergroup difference in the aEEG margins throughout experiment. At the insult nadir (lower margin) and experiment end (upper margin), only water bottle cooled piglets had smaller aEEG margins compared with baseline (both $p < 0.05$).

Table 5.2: Electro-cortical activity with time

		Baseline	End of HI	2 h	14 h	26 h	End of experiment
PCM	upper	22.5 (7.9)	6.8 (6.6)	10.8 (10.0)	13.2 (9.0)	12.2 (7.9)	10.7 (5.9)
	lower	10.2 (4.1)	3.5 (3.6)	5.8 (4.5)	5.5 (2.8)	5.3 (2.8)	6.1 (2.5)
WB	upper	26.1 (1.9)	7.5 (8.7)	12.8 (12.9)	11.5 (8.2)	11.9 (7.4)	13.9* (4.1)
	lower	12.8 (1.2)	4.5* (3.7)	6.1 (6.3)	5.2 (3.5)	5.3 (3.3)	6.5 (2.2)

Data shown as mean (standard deviation)

* $p < 0.05$, comparison vs. baseline, ANOVA with Bonferroni correction

Abbreviations: PCM, phase changing material. WB, water bottle. HI, hypoxia-ischaemia.

5.2.4 Initiation of cooling

(6 PCM and 5 water bottles unless otherwise stated)

PCM cooled piglets achieved T_{rectal} 33-34°C at 1.9 (0.3) h after placing on the PCM mattress; water bottle cooled animals took 1.8 (0.5) h (this excluded one outlier which required water bottle replacement before reaching target range at 11.8 h (Table 5.3)). The T_{rectal} fell transiently below 33°C in 4 piglets, 2 from the PCM group (for 0.5 and 2.0 h) and 2 from the water bottle group (for 1.3 and 3.0 h), which resolved when the

temperatures of the PCM mattress and water bottles rose above 30°C following the application of blankets.

Table 5.3: Cooling profile with PCM and water bottles

	PCM	WB	p value
Induction phase	n = 6	n = 5	
Time to reach 34°C (h)	1.9 (0.3)	3.8 (4.5)	NS
Maintenance phase	n = 5	n = 5	
T _{rectal} (°C)	33.4 (0.2)	33.9 (0.5)	NS
Time within 33-34°C (h)	18.45 (2.06)	11.80 (5.23)	< 0.05 *
Requirements for Manipulation	n = 5	n = 5	
Average blanket number	1.5 (0.8)	0.6 (0.7)	NS
Change in blanket number	10.4 (4.0)	11.6 (8.6)	NS
Cooling device replaced	0 (0)	1.2 (1.1)	< 0.05 ¶

Data shown as mean (standard deviation)

* ANOVA

¶ Fisher's exact test

Abbreviations: PCM, phase changing material. WB, water bottle. NS, not significant.

5.2.5 Maintenance of cooling

(5 PCM and 5 water bottles)

Once the T_{rectal} target was achieved, there was a gradual increase in PCM temperature, which plateaued at around its melting point (Fig. 5.3 (A)). In PCM cooled piglets there was no requirement for PCM mattress renewal (Table 5.3). In the water bottle group, there was also a gradual increase in water bottle temperature which caused the gradual increase in T_{rectal} (Fig. 5.3 (B)). Four out of 5 water bottle cooled piglets required renewal of water bottles at 12.2 (4.4) h after induction of cooling (Table 5.3). The need for cooling device renewal differed between PCM and water bottle groups (p < 0.05).

During the cooling period the PCM group remained within the target T_{rectal} range for longer compared to the water bottle group ($p < 0.01$ when observations within the target range were compared and $p < 0.05$ when incorporating the correlation among repeated observations); there was no difference in the number of blankets applied and the number of blanket manipulations.

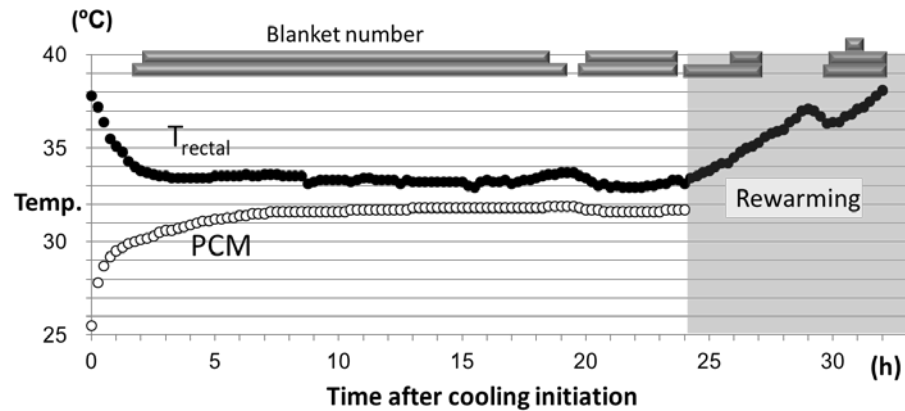
5.2.6 Rewarming

(5 PCM and 5 water bottles)

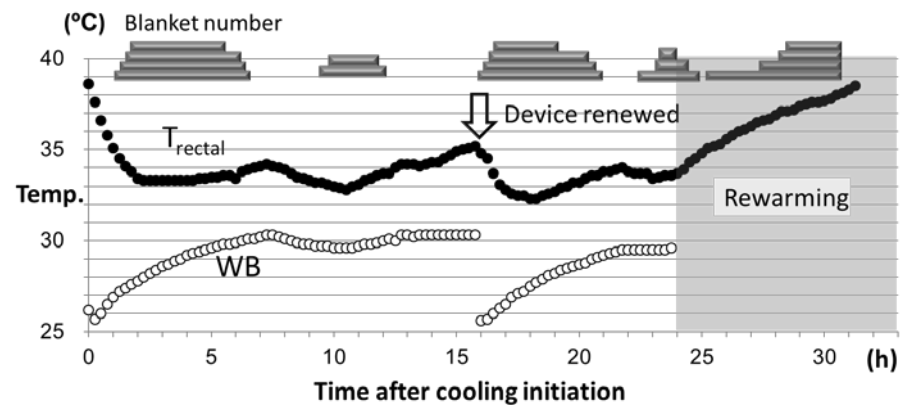
Gradual rewarming was achieved by reducing the exposed surface area of the piglet skin to the PCM mattress and water bottles. No adverse effects were apparent at the completion of rewarming (Fig. 5.3).

Figure 5.3: Changes in rectal and cooling device temperatures with time in a representative piglet from the group cooled with PCM and water bottles

(A) PCM



(B) Water bottles



The target T_{rectal} between 33-34°C was achieved ~2 h after the induction with PCM mattress and water bottles. In the piglet cooled with the PCM mattress, the device temperature remained at its melting point throughout the cooling period, leading to a more stable T_{rectal} (A). In the animal cooled with water bottles, there was a gradual increase in the T_{rectal} in parallel with the increase in the water bottle temperature; a new set of water bottles were required at 16 h after cooling initiation (B).

Both cooling devices required blankets to avoid excessive cooling (the use of blankets is shown as grey bars in the figures). Gradual rewarming was achieved by reducing the exposed surface area of the piglet skin to the PCM mattress and the water bottles.

Abbreviations: T_{rectal} , rectal temperature. PCM, phase changing material. WB, water bottle.

5.3 Brief Discussion

5.3.1 Summary

The current study confirmed the hypothesis that simple, low-tech cooling devices can induce and maintain therapeutic hypothermia effectively in a porcine model of neonatal encephalopathy. However, even with continuous monitoring of T_{rectal} , water bottle cooling resulted in considerable temperature drifts compared to PCM cooling. PCM mattress, with its heat-buffering function, appeared to be more suitable for the induction and maintenance of cooling before admission to tertiary cooling centres. The findings of this study are discussed in this chapter.

- Key Findings from this Study

This study demonstrates that simple, low-tech cooling devices, such as water bottles filled with tepid water at 25°C and a PCM mattress with a melting point of 32°C, were able to induce and maintain therapeutic hypothermia effectively in a porcine model of neonatal encephalopathy for at least 24 h. PCM cooling led to a longer period within target T_{rectal} and more stable cooling compared to water bottle cooling. However, both approaches required frequent fine tuning by adjusting the number of blankets insulating the piglet to maintain stable temperatures. Although PCM appeared to induce more stable cooling than water bottles, it is unknown whether it would remain effective for the 72 h recommended for therapeutic hypothermia and whether its composition may need modification for optimal treatment of human infants. Such clinical validation is urgently needed.

- What is already known and what our study adds

What is already known:

- Mild to moderate therapeutic hypothermia following perinatal hypoxia-ischaemia can reduce mortality and the incidence of moderate to severe neurological deficit at 18 months in an intensive care setting.
- This therapy does not yet reach low- and mid-resource settings where the incidence of neonatal encephalopathy is more common; the development and validation of effective, safe, low-maintenance and low-cost cooling methods is urgently required in the context of carefully conducted randomised controlled trials of cooling.

What this study adds:

- Two low-cost, low-tech cooling devices (water bottles and a mattress made from PCM with a melting point of 32°C) can induce and maintain mild to moderate hypothermia in a piglet model.
- Compared with water bottles, the PCM mattress provided more stable hypothermia without the need for device renewal for up to 24 h.

5.3.2 Induction and maintenance of hypothermia

With the exception of one animal in the water bottle group, hypothermia was induced within ~2 h with both cooling mattresses. Although this may appear to be slower than some clinical studies (8, 9, 145, 146), this does not account for the higher normal temperature for the piglet (38.5-39.0°C) than the human infant (~37°C); more rapid induction of cooling may be possible using pre-cooled or additional coolants. However our recent clinical study has highlighted that, even in equatorial Africa, many high risk infants in low-resource settings are hypothermic on admission to the baby unit (147) and so may require maintenance of, rather than induction of, hypothermia.

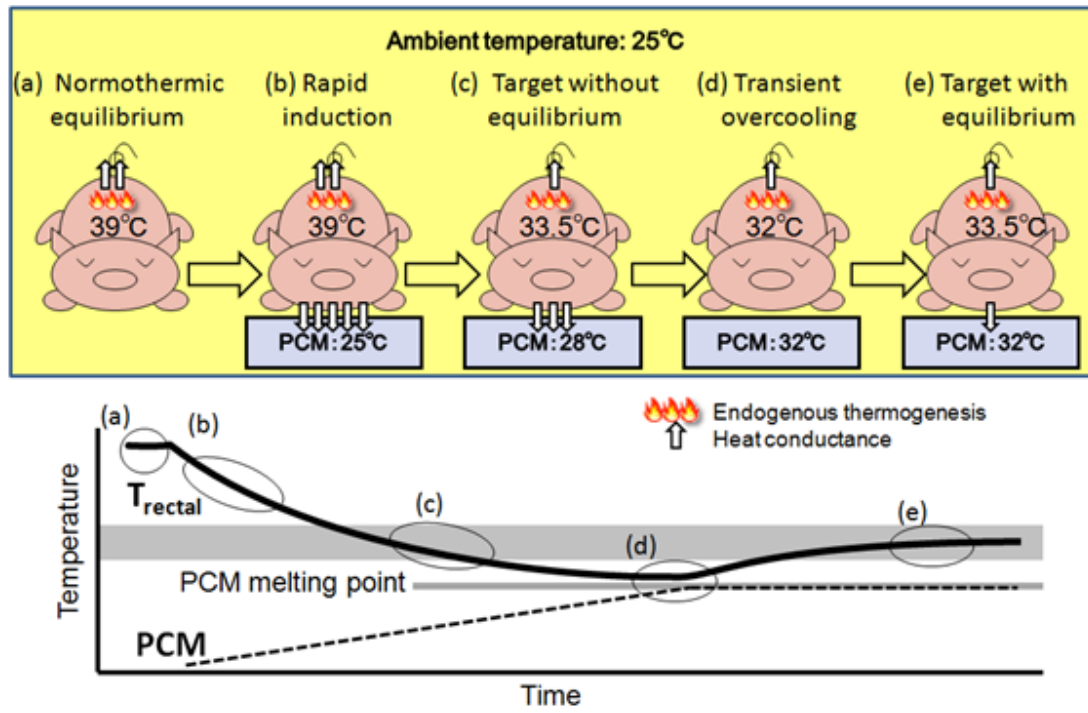
In human infants non-shivering thermogenesis contributes to maintain the body temperature utilising the relatively large stores of brown adipose tissue under the regulation of the sympathetic nervous system and an uncoupling protein (UCP1) (148-150). Caesarean section reduces postnatal non-shivering thermogenesis compared with vaginal delivery due to a lower postpartum surge in thyroid hormones and reduced sympathoadrenal activity (151, 152), while those born vaginally will activate heat production (152). In addition to the delivery mode, perinatal factors such as maternal analgesia, perinatal hypoxia-ischaemia, sleep status, seizures, ventilation and use of postnatal sedation may all influence the neonatal metabolic rate and heat production (153, 154). Fluctuations in T_{rectal} and occasional periods of excessive hypothermia have been observed even with high-tech electric cooling devices (6, 146, 155). Although no significant adverse effects have been seen with mild to moderate hypothermia used in the randomised controlled trials of cooling, it is possible that deeper hypothermia than that used in the trials may be associated with a higher risk of

adverse events, and it would be desirable that such temperature fluctuations are minimised. In this study, both low-tech cooling devices induced a T_{rectal} stability comparable to that seen in the clinical studies (146). The temperature plateau reached by the PCM mattress was more stable and may allow for less frequent temperature monitoring compared with water bottles and other cooling devices which do not have a temperature buffering effect.

With the PCM mattress any slight overcooling was addressed by adding cotton blankets (Fig. 5.3 (A)). A significant undershoot in the T_{rectal} from the target range may, however be difficult to manage with blankets alone due to the robust intrinsic heat buffering capacity of PCM. The use of PCM mattresses with multiple melting points may facilitate precise cooling in infants with differing levels of heat production.

Several important points were ascertained from a series of pilot studies not described here: (i) a polytrianfoam insulator is required underneath the cooling device to minimise heat exchange with the incubator surface; (ii) once the target T_{rectal} is attained, transient but prompt rewarming is essential to minimise T_{rectal} undershoot (Fig. 5.4); (iii) a larger coolant surface area is required for a longer cooling effect; (iv) a heat conductor is required between the animal and PCM mattress to prevent a localised phase change; (v) 32°C was more suitable than 28°C as the PCM melting point because the latter caused a prolonged period of overcooling. Although the current composition and amount of PCM was optimised for our experimental setting, further refinements in the amount and composition of PCM may be needed to provide optimal therapy for human infants.

Figure 5.4: Schematic diagram demonstrating the establishment of a temperature equilibrium following the induction of cooling with PCM



(a) Temperature equilibrium before the initiation of cooling: the animal's thermogenesis equilibrates with the heat release to the ambience. (b) Rapid induction of hypothermia with the PCM: the PCM mattress rapidly deprives heat from the animal due to a large temperature difference between the animal and the mattress, and also to the superior thermal conductance of the device compared with the ambient air. The heat buffering effect of PCM is not expected until the temperature of PCM reaches its melting temperature. (c) Target temperature without equilibrium: Although the target T_{rectal} is achieved, the PCM temperature still remains cooler than its melting point, and the animal's heat loss remains greater than its thermogenesis, leading to (d) transient excessive cooling. After the PCM temperature reaches its melting point, there remains only small heat exchange between the animal and PCM due to only small temperature difference between each other. This results in the equilibrium of heat exchange within target T_{rectal} (e).

Abbreviation: PCM, phase changing material.

5.3.3 Limitations of the study

The duration of cooling was shorter compared with clinical studies due to ethical and practical reasons. Our study was not designed to incorporate the variations in endogenous thermogenesis and environmental temperatures. A different ambient temperature, especially with diurnal temperature swing, may also affect the efficacy, stability and the life of coolant. Hypoxic-ischaemic insult given to the animal may be different from clinical perinatal asphyxia, as we used carotid occlusion simultaneously with systemic hypoxia. Indeed, there was only little change in arterial blood pH even at the nadir of the insult. However, the combination of these procedures is known to result in histopathological brain injury similar to human newborn infants following moderate to severe perinatal asphyxia (62, 156). In addition, the newborn piglet is smaller in size, has more mature skin, has a higher normal core temperature, and is more dependent on shivering thermogenesis compared with the human infant (150). These differences have to be carefully considered when interpreting the findings, and the effective cooling duration of the PCM-32°C mattress and optimal melting point for newborn infants need to be addressed.

5.3.4 Clinical Implications

Therapeutic hypothermia is a safe and promising treatment for birth asphyxia in the developed world. As many infants are born outside the regional perinatal centre, cooling during transport has become an increasingly common practice (6), because there is a consensus that, cooling should be started within 6 h of birth to be effective (13). Safe cooling methods and close monitoring of core temperature are vitally important during

transport; we suggest that water bottles at 25°C or PCM mattress (32°C) may be effective for transportation or early MRI.

The standardisation of such effective, safe, low-maintenance and low-cost cooling methods is urgently required for the possible benefit of therapeutic hypothermia to reach the areas of the world that have the highest prevalence of neonatal encephalopathy and its sequelae (13). We have shown that both water bottles and a mattress made from PCM-32°C may substitute for expensive cooling devices in a low-resource setting although continuous temperature monitoring and frequent modification of the insulation are essential. These low-tech devices are re-usable and much cheaper than the more sophisticated electronic devices used in the developed countries. Although the PCM mattress is currently more expensive (~£300-500 per mattress) compared with water bottles (~£10), the cost is likely to reduce significantly with expanded production. Therapeutic hypothermia with water bottles (water temperature 25°C) and PCM has been successfully achieved in pilot cooling studies in equatorial Africa and south-west India respectively, settings with minimal diurnal and seasonal swings in temperature (147, 157).

5.3.5 Conclusion

Therapeutic hypothermia can be induced and maintained using low-tech devices such as water bottles and a PCM mattress in a porcine model of neonatal encephalopathy. Although cooling with the PCM mattress provided more stable cooling compared with water bottles, further refinements in the size, composition, insulation and melting point of the PCM mattress may be required to optimise effective therapeutic hypothermia for the human newborn.

Chapter 6

Overall conclusion and implication of the findings from this thesis

6.1 Conclusion

Under whole body cooling, selective head cooling, and even normothermia, regional brain temperatures depend on the body weight of the subject presumably due to the difference in the surface to volume ratio of the head, and subsequent alteration in the efficacy of heat exchange at the scalp surface. In contrast to the observation in anaesthetised piglets, in human healthy newborn infants, brain temperatures were associated with cerebral perfusion and ambient temperature, but not with the body weight and cerebral metabolism. Cerebral perfusion showed closest positive association with superficial brain temperatures, suggesting the heat delivery to the superficial brain via blood flow. Temperature values and supportive treatments, which provide the optimal neuroprotective property to different brain regions, have to be investigated. Further identification of regulating factors for the regional brain temperature may help establish a cooling method to accomplish the precise temperature control within a given cerebral region.

Low-tech, low-cost heat buffering mattress made of PCMs provided more stable temperature control compared to non-heat-buffering water bottles, which may help facilitate safe cooling in low resource settings and cooling during transfer to tertiary centres when used with continuous body temperature monitoring. The relevance and utility of cotside monitoring techniques for cerebral perfusion and metabolism, and PCM cooling also need to be confirmed in the clinical cases.

6.2 Determinants of regional cerebral temperatures: variables recognised in this current thesis and other potential variables

In an anaesthetised newborn piglet model, where the cardio-pulmonary function is stably controlled, and the influence of motion and sympathetic stimulation to heat generation is minimised, the body size was the central determination factor of the cerebral temperature distribution. The dependence of the brain temperature on the body weight was present even at normothermic condition, suggesting that heat dissipation from the scalp surface to the cooler ambient air occurred at a greater extent in smaller subjects compared to peers. The influence of the body size on the brain temperature significantly increased under selective head cooling, where a substantial temperature gradient was created between the superficial brain and deep brain. In the clinical study which enrolled healthy newborn infants, we were not able to confirm the association between regional brain temperatures and the body weight, presumably because of the relatively limited variations in the body size and the brain temperature under normothermic, thermo neutral condition. Instead, a positive correlation was observed between cerebral perfusion and superficial brain temperatures, suggesting that the superficial brain may be warmed up by the blood flow, which is distributed to the brain surface and then penetrates the parenchyma towards the deep brain structure. Future clinical studies are required to obtain the surrogate temperature values for the regional brain tissue to distribute the optimal cooling level to the target brain regions.

6.3 Estimation of regional cerebral temperatures

Our current study suggested that the zero-heat flux tissue core thermometer can be a handy proxy for the cerebral tissue temperature. This technique may be applied under both normothermic condition and whole body cooling, where the scale of the intracerebral temperature gradient is not outstanding. Taken together with the previous reports which investigated the temperature gradient within the brain (55-57, 130, 158-161), the temperature measures obtained by the probe diameters of 15mm and 25mm were likely to reflect the temperatures of the cerebral cortex and subcortical white matter; for the estimation of the deep brain temperature, probes which can insulate a greater surface area would be required. In contrast, under selective head cooling, where a substantial temperature gradient is created within the brain tissue, the utility and accuracy of the zero-heat flux method has not been fully validated. In addition to the nasopharyngeal temperature, which was reported to reflect the deep brain temperature even under selective head cooling (57), our current investigation suggested that the scalp skin temperature measured using infrared thermometer may predict both the superficial and deep brain temperatures. Validation studies for these surrogate measures are required using non-invasive techniques such as magnetic resonance spectroscopic imaging for the 3-dimensional temperature maps.

6.4 Cerebral temperature control based on the understanding of its determinants

Currently in the clinical practice, the level of cooling is adjusted with the core body temperature as a proxy of the brain temperature (7-9). However, our current study highlighted that the brain temperature can widely vary between cerebral regions as well as between subjects, even when the core body temperature was successfully controlled to a similar target level. Especially when selective head cooling is used, changes in the cap temperature can cause considerable alteration of the temperature distribution to the cerebral tissue. Clinical studies have demonstrated that selective head cooling and whole body cooling are similarly safe and neuroprotective following perinatal asphyxia (47). In the light of this observation, the use of whole body cooling appears to have been the first choice in clinical practice (47, 141) because it is practical and simple, and leads to a stable core-body temperature. However, given that several clinical factors, which influence the beneficial effect of selective head cooling, have already been identified, further titration of cooling level according to the body size, encephalopathy grade, CBF, cerebral metabolism and other factors may significantly improve outcomes. In the future, at least for selective head cooling, protocols may have to be revised either to adjust to the reliable surrogate for superficial and deep brain temperatures, or to account for the body size and cerebral perfusion in setting the cap temperature e.g. to use relatively higher cap temperature for infants with small body size or poor cerebral perfusion to avoid excessive cooling in the superficial structure. Further understanding of regulatory factors of regional brain temperatures may help deliver a tailored cooling level to a given brain region, which may provide optimal protection at the site. The use of non-invasive cotside monitoring techniques of CBF and brain metabolism, like ours, may accelerate

the accumulation of clinical evidence required to refine neuroprotective treatments
although the accuracy of monitoring needs to be validated and improved.

6.5 Low-tech device to provide safe and stable pre-hospital cooling

Following hypoxia-ischaemia, despite resuscitation and apparent restoration of normal energy metabolism (the latent phase), neurotoxic cascades are activated (162, 163).

Both experimental and clinical evidence suggests that neuroprotective treatments should be commenced as early as possible, because the beneficial effect of neuroprotective treatments are less effective with increasing time from the injury especially following most severe hypoxia-ischaemia (20, 164-166). Currently in clinical practice, despite the best attempts of clinicians, a considerable number of encephalopathic newborn infants arrive at tertiary cooling centres close to or after 6 h of birth (the end of the apparent therapeutic window) (8, 9, 141, 166). The feasibility of initiating cooling during transport and before admission to tertiary centres has been investigated (89, 167-170). In some of the early studies, where non-servo-controlled cooling methods such as ice packs, fans or passive cooling were used without routine monitoring of core-body temperature, up to 34% of infants were cooled below the target temperature range. This emphasises the importance of continuous core temperature monitoring. In our study, even with the continuous T_{rectal} monitoring, cooling induced by water bottles resulted in substantial temperature drifts. In contrast, PCM mattress led to more stable body temperature profile over a period of several hours; the development of techniques which provide stable body temperature control of not only cooling but also smoothing the temperature drifts, may be highly beneficial for pre-hospital cooling.

Although PCMs are much more expensive compared to water bottles, they are re-usable and are still much cheaper than electronic devices used in the developed countries. Although further studies are needed to assess PCM's use in tropical setting

(i.e. warmer than the melting point of PCM), this cooling modality might be suitable for the use in low-resource settings (157).

6.6 Future works

Although our study provided clinically useful feedback in improving the safety and efficacy of therapeutic hypothermia in the newborn infant, further clinical studies are needed before changing the clinical protocol. To “deliver” the precise temperature values to given cerebral regions, more information has to be accumulated to allow the estimation of regional brain temperatures using clinical factors such as the cooling device temperature, ambient temperature, infant’s body size, cerebral perfusion, level of consciousness, and the metabolic state of the brain. Also, optimal temperature values for each cerebral region following specific injury type and depth have to be confirmed in preclinical and clinical studies using advanced neuroimaging techniques.

Simultaneously, cotside monitoring system for the regional cerebral temperature should be developed and validated, using temperature mapping techniques such as magnetic resonance spectroscopic temperature imaging (171). In addition, the establishment of non-invasive cotside technique to monitor the cerebral perfusion and metabolism may help adjust the level of cardiac support, cap temperature for selective head cooling, and sedation during cooling, which should also be addressed in the future experimental and clinical studies. Finally, our study suggested the benefit of using heat-buffering mattress made of PCMs under low-resource settings such as pre-hospital cooling; clinical studies need to confirm the benefit of initiating therapeutic hypothermia before admission with combined use of continuous core body temperature monitoring and PCM mattress.

6.7 Strength and limitation

We were able to prepare an ideal translational setting using the preclinical large animal model and the medium risk infants who were hospitalised at a tertiary neonatal intensive care unit. In the piglet model, comprehensive temperature monitoring was available using both indirect and direct brain tissue temperature monitoring. Our setting of the clinical study employed a wide range of non-invasive biomarkers to compensate the lack of invasive direct monitoring and to allow estimation of the temperature, perfusion and metabolism of the brain tissue by combining multiple factors.

Several major limitations of the current thesis have to be noted. In the first two studies, the study subject did not experience hypoxia-ischaemia, which may significantly alter the response in metabolism and perfusion to cooling. Subsequently, no infant in the clinical cohort was cared under therapeutic hypothermia. This resulted in a dataset of temperatures and other physiological variables within limited ranges of variations, leading to the difficulty in identifying important associations between variables.

Comprehensive data acquisition using a similar protocol to our clinical study is required in the future clinical study of therapeutic hypothermia in asphyxiated newborn infants.

6.8 Implication of the findings from this thesis

Spatial temperature distributions within the brain is various, with complex associations with clinical factors such as the body size, cerebral perfusion and oxygen metabolism. Therapeutic hypothermia, especially when selective head cooling is used, may have to account for the body size to deliver consistent cooling levels to the brain tissue.

Accounting for several central factors such as body weight and cerebral perfusion may significantly improve the precision of the temperature delivery to the regional brain tissue. Heat-buffering mattress made of PCMs may promote very early induction of therapeutic hypothermia before admission with minimum increase in the risk for excessive cooling. PCM mattresses may also be of use in low resource settings but this still needs to be assessed in the clinical studies.

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169. Fairchild K, Sokora D, Scott J, Zanelli S. Therapeutic hypothermia on neonatal transport: 4-year experience in a single NICU. *J Perinatol*. 2010;30(5):324-9. Epub 2009/10/23.
170. Hallberg B, Olson L, Bartocci M, Edqvist I, Blennow M. Passive induction of hypothermia during transport of asphyxiated infants: a risk of excessive cooling. *Acta Paediatr*. 2009;98(6):942-6. Epub 2009/06/02.
171. Karaszewski B, Wardlaw JM, Marshall I, Cvorovic V, Wartolowska K, Haga K, et al. Early brain temperature elevation and anaerobic metabolism in human acute ischaemic stroke. *Brain*. 2009;132(Pt 4):955-64. Epub 2009/04/07.

Supplementary materials and appendix

Supplementary Materials

List of Abstracts and Presentations at Meetings from the analysed data

6-8/10/2007 The 3rd Congress of Asian Society for Pediatric Research (Tokyo, Japan)

Low-tech, Low-cost cooling device for global infant population with birth asphyxia

Iwata S, Iwata O, Olson L, Kapetanakis A, Kato T, Evans S, Setterwall F,
Matsuishi T, Lagercrantz H, Wyatt JS, Robertson NJ

6-8/10/2007 The 3rd Congress of Asian Society for Pediatric Research (Tokyo, Japan)

Body-size determines regional cerebral temperature at normothermia, whole body cooling
and selective head cooling.

Iwata O, Iwata S, Thornton J, Bainbridge A, Matsuishi T, Wyatt JS, Cady EB,
Robertson NJ

24-26/11/2007 The 52nd Annual Meeting of the Japan Society for Premature and Newborn
Medicine (Takamatsu, Japan)

Low-tech low-cost modality to provide safe and stable whole-body cooling using
phase-changing materials

Iwata S, Iwata O, Kato T, Kapetanakis A, Evans S, Olson L, Setterwall F, Kanda H,
Maeno Y, Matsuishi T, Lagercrantz H, Wyatt JS, Cady EB, Robertson NJ

24-26/11/2007 The 52nd Annual Meeting of the Japan Society for Premature and Newborn
Medicine (Takamatsu, Japan)

Unexpected tight relationship between regional cerebral temperature and body-weight in the
newborn piglet

Iwata S, Iwata O, Kanda H, Maeno Y, Matsuishi T, Kato T, Wyatt JS, Cady EB,
Robertson NJ

3-6/05/2008 The 4th Congress of Asian Society for Pediatric Research (Honolulu, Hawaii, USA)

Low-tech, Low-cost cooling device for global infant population with birth asphyxia

Iwata S, Iwata O, Olson L, Kapetanakis A, Kato T, Evans S, Araki Y, Kakuma T,
Setterwall F, Matsuishi T, Lagercrantz H, Robertson NJ

3-6/05/2008 The 4th Congress of Asian Society for Pediatric Research (Honolulu, Hawaii, USA)

Body-size determines regional cerebral temperature at normothermia, whole body cooling and selective head cooling.

Iwata O, Iwata S, Thornton J, Bainbridge A, Matsuishi T, Wyatt JS, Cady EB, Robertson NJ

25-27/04/2008 The 111th Annual Meeting of the Japan Pediatric Society (Tokyo, Japan)

Determinants of regional brain temperature in the newborn infant: Are small body-weight infants at increased risks of excessive cooling?

Iwata S, Iwata O, Kato T, Wyatt JS, Cady EB, Kanda H, Maeno Y, Matsuishi T, Robertson NJ

25-27/04/2008 The 111th Annual Meeting of the Japan Pediatric Society (Tokyo, Japan)

Development of cooling mattress using low-tech heat buffering system: Is stable whole body cooling provided safely to asphyxiated newborn infants without electronic devices?

Iwata S, Iwata O, Kato T, Kapetanakis A, Evans S, Olson L, Setterwall F, Kanda H, Maeno Y, Matsuishi T, Lagercrantz H, Wyatt JS, Cady EB, Robertson NJ

20-22/05/2010 The 52nd Annual Meeting of the Japanese Society of Child Neurology (Fukuoka, Japan)

Determination factors of regional cerebral temperatures in the newborn infant

Iwata S, Iwata O, Oya T, Matsuishi T

20-22/04/2012 The 115th Annual Meeting of the Japan Pediatric Society (Fukuoka, Japan)

Determinants of regional cerebral temperature in newborn infants.

Iwata S, Unno M, Saitsu H, Tanaka S, Okada J, Hirose A, Kanda H, Maeno Y, Matsuishi T, Iwata O.

List of publications derived from this work

1. Superficial brain is cooler in small piglets: neonatal hypothermia implications.
Iwata S, Iwata O, Thornton JS, Shanmugalingam S, Bainbridge A, Peebles D, Wyatt JS, Cady EB, Robertson NJ.
Ann Neurol. 2006 Nov;60(5):578-85.
2. Therapeutic hypothermia can be induced and maintained using either commercial water bottles or a "phase changing material" mattress in a newborn piglet model.
Iwata S, Iwata O, Olson L, Kapetanakis A, Kato T, Evans S, Araki Y, Kakuma T, Matsuishi T, Setterwall F, Lagercrantz H, Robertson NJ.
Arch Dis Child. 2009 May;94(5):387-91.

List of publications related to this work

1. Brain temperature in newborn piglets under selective head cooling with minimal systemic hypothermia.
Iwata O, Iwata S, Tamura M, Nakamura T, Sugiura M, Ogiso Y.
Pediatr Int. 2003 Apr;45(2):163-8.
2. Early head cooling in newborn piglets is neuroprotective even in the absence of profound systemic hypothermia.
Iwata O, Iwata S, Tamura M, Nakamura T, Sugiura M, Ogiso Y, Takashima S.
Pediatr Int. 2003 Oct;45(5):522-9.
3. Depth of delayed cooling alters neuroprotection pattern after hypoxia-ischemia.
Iwata O, Thornton JS, Sellwood MW, Iwata S, Sakata Y, Noone MA, O'Brien FE, Bainbridge A, De Vita E, Raivich G, Peebles D, Scaravilli F, Cady EB, Ordidge R, Wyatt JS, Robertson NJ.
Ann Neurol. 2005 Jul;58(1):75-87.
4. Delayed whole-body cooling to 33 or 35 degrees C and the development of impaired energy generation consequential to transient cerebral hypoxia-ischemia in the newborn piglet.
O'Brien FE, Iwata O, Thornton JS, De Vita E, Sellwood MW, Iwata S, Sakata YS, Charman S, Ordidge R, Cady EB, Wyatt JS, Robertson NJ.
Pediatrics. 2006 May;117(5):1549-59.
5. "Therapeutic time window" duration decreases with increasing severity of cerebral hypoxia-ischaemia under normothermia and delayed hypothermia in newborn piglets.
Iwata O, Iwata S, Thornton JS, De Vita E, Bainbridge A, Herbert L, Scaravilli F, Peebles D, Wyatt JS, Cady EB, Robertson NJ.
Brain Res. 2007 Jun 18;1154:173-80.
6. Abnormal white matter appearance on term FLAIR predicts neuro-developmental outcome at 6 years old following preterm birth.
Iwata S, Iwata O, Bainbridge A, Nakamura T, Kihara H, Hizume E, Sugiura M, Tamura M, Matsuishi T.
Int J Dev Neurosci. 2007 Dec;25(8):523-30.

7. Supra- and sub-baseline phosphocreatine recovery in developing brain after transient hypoxia-ischaemia: relation to baseline energetics, insult severity and outcome.
Iwata O, Iwata S, Bainbridge A, De Vita E, Matsuishi T, Cady EB, Robertson NJ.
Brain. 2008 Aug;131(Pt 8):2220-6.
8. Subtle white matter injury is common in term-born infants with a wide range of risks.
Iwata S, Bainbridge A, Nakamura T, Tamura M, Takashima S, Matsuishi T, Iwata O.
Int J Dev Neurosci. 2010 Nov;28(7):573-80.
9. Determinants of outcomes following acute child encephalopathy and encephalitis: pivotal effect of early and delayed cooling.
Kawano G, Iwata O, Iwata S, Kawano K, Obu K, Kuki I, Rinka H, Shiomi M, Yamanouchi H, Kakuma T, Takashima S, Matsuishi T; Research Network for Acute Encephalopathy in Childhood.
Arch Dis Child. 2011 Oct;96(10):936-41.
10. Filling the evidence gap: how can we improve the outcome of neonatal encephalopathy in the next 10 years?
Iwata O, Iwata S.
Brain Dev. 2011 Mar;33(3):221-8.
11. Hypothermia for neonatal encephalopathy: Nationwide Survey of Clinical Practice in Japan as of August 2010.
Iwata O, Nabetani M, Takenouchi T, Iwaibara T, Iwata S, Tamura M; Working Group on Therapeutic Hypothermia for Neonatal Encephalopathy, Ministry of Health, Labor and Welfare, Japan; Japan Society for Perinatal and Neonatal Medicine.
Acta Paediatr. 2012 May;101(5):e197-202.
12. Qualitative brain MRI at term and cognitive outcomes at 9 years after very preterm birth.
Iwata S, Nakamura T, Hizume E, Kihara H, Takashima S, Matsuishi T, Iwata O.
Pediatrics. 2012 May;129(5):e1138-47.

Appendix 1:

Superficial brain is cooler in small piglets: neonatal hypothermia implications.

Iwata S, Iwata O, Thornton JS, Shanmugalingam S, Bainbridge A, Peebles D, Wyatt JS, Cady EB, Robertson NJ.

Ann Neurol. 2006 Nov;60(5):578-85.

Appendix 2:

Therapeutic hypothermia can be induced and maintained using either commercial water bottles or a "phase changing material" mattress in a newborn piglet model.

Iwata S, Iwata O, Olson L, Kapetanakis A, Kato T, Evans S, Araki Y, Kakuma T, Matsuishi T, Setterwall F, Lagercrantz H, Robertson NJ.

Arch Dis Child. 2009 May;94(5):387-91.

Appendix 3:
Article associated with the current thesis

Methyl-isobutyl amiloride reduces brain Lac/NAA, cell death and microglial activation in a perinatal asphyxia model.

Robertson NJ, Kato T, Bainbridge A, Chandrasekaran M, Iwata O, Kapetanakis A, Faulkner S, Cheong J, Iwata S, Hristova M, Cady E, Raivich G.

J Neurochem. 2013 Mar;124(5):645-57.