Core temperature and brown adipose tissue activity during therapeutic whole body cooling in human neonates

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THESIS SUMMARY

Brain injury caused by the combination of inadequate blood flow and oxygen delivery to the brain before and during birth is called neonatal hypoxic ischaemic encephalopathy (HIE). Whole body hypothermia has been shown by randomised controlled trials to reduce brain injury due to hypoxic-ischaemic insults, and is an accepted treatment for HIE. However, HIE still results in considerable morbidity with 40% or more of cooled neonates nevertheless dying or suffering moderate or severe long-term impairment.

The aim of whole body hypothermia is to lower brain temperature to 33-34°C within 6 hours of birth and continue this for 72 hours before rewarming to normothermia. However, direct measurement of brain temperature is not generally possible in the human neonate during whole body hypothermia and there is a reliance on proxy measures of systemic core temperature, the most common of which is rectal temperature (T_{rec}).

The inability of hypothermia to prevent all brain injury following HIE is likely to relate to a complex interplay of many factors, including the timing of injury and application of cooling, and inadequate knowledge of the ideal depth and duration of cooling. However, hypothermia is also energetically costly where thermoregulation is activated to defend a core temperature set-point, and induces a hormonal stress response which may be harmful. An important concept is that induced hypothermia is quite different to anapyrexia, which is the adaptive response to hypoxia highly conserved in evolution where the core set point is lowered thus reducing oxygen consumption and stress responses.

Brown adipose tissue (BAT) thermogenesis is the metabolic mechanism used by neonates to defend core temperature during exposure to cold, and has an essential role in warming the brain in animal models. The activity of BAT during hypothermia treatment for HIE has not been examined in the human neonate.

Furthermore, the premise that T_{rec} reflects brain temperature is weakly supported with a paucity of human neonatal data. Extrapolation from animal data and adult human data may be invalid due to thermoregulatory differences, in particular BAT thermogenesis. T_{rec} as a monitoring site has not been adequately validated by comparison with temperature at other core sites during whole body hypothermia.

In this thesis, temperature control during whole body hypothermia in human neonates is studied, with an emphasis on BAT activity and temperature gradients between core body sites.

The following hypotheses are tested: (i) thermogenesis in BAT is active during therapeutic hypothermia for HIE; (ii) BAT activity influences temperature in the lower oesophagus (T_{oes}) more than T_{rec}; (iii) T_{rec} does not accurately reflect T_{oes}; (iv) BAT activity is associated with severity of brain injury.

Manually controlled hypothermia using T_{rec} as the target temperature site was studied because this is the standard practice in South Australian tertiary neonatal intensive care units. Furthermore, servo-controlled cooling blankets (by cooling the skin of the back) preclude the assessment of changes in inter-scapular skin temperature (T_{scap}) that may be associated with BAT thermogenesis. A series of experiments measured core temperature (T_{rec} and T_{oes}) and T_{scap} using standard temperature probes, and exposed surface temperatures using infrared imaging, both in healthy neonates, and in critically ill normothermic and hypothermic neonates nursed supine with the back in contact with an insulating mattress of an open radiant warmer.

These studies conclude that rectum is an inappropriate site to monitor and regulate core temperature during manually controlled hypothermia. T_{rec} underestimates T_{oes}, appears to be influenced by leg skin temperature, and demonstrates a long lag time to change after a change in environmental temperature that promotes temperature fluctuations in more rapidly responding sites such as T_{oes}. Evidence is presented that supports the presence of BAT thermogenesis in many neonates during whole body hypothermia, and that thermogenesis is more closely aligned to T_{oes} than T_{rec}. If T_{oes} is considered more reflective of central venous and aortic blood temperature, then T_{rec} monitoring will result in warmer than expected blood perfusing deep brain. T_{oes} is therefore more likely to provide a stable and relevant measure of brain temperature and should be used in clinical practice.

The data presented in this thesis also suggest that neonates with HIE can be divided into those that demonstrate anapyrexia and those that defend core temperature via activation of BAT. Those neonates with anapyrexia have a greater likelihood of MRI visible brain injury. The data do not suggest that activation of BAT results in harm to the brain. However conclusions are limited by small study numbers and a lack of clinical follow-up. The influence of BAT thermogenesis on the recovery of the brain during hypothermia requires further study and consideration should also be given to the study of therapeutic interventions that turn off BAT as a means of inducing therapeutic hypothermia.

DECLARATION OF ORIGINALITY OF THIS REPORT

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Anthony Carlisle Date:

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LIST OF COMMON ABBREVIATIONS

BAT	Brown adipose tissue
HIE	Hypoxic ischaemic encephalopathy
IR	Infra-red
NICU	Neonatal intensive care unit
NST	Non-shivering thermogenesis
RCT	Randomised controlled trial
T_{amb}	Local environmental temperature
T_{belly}	Exposed abdominal skin temperature
T_{core}	Core (systemic) body temperature
T_{oes}	Temperature in the lower oesophagus
T_{rec}	Rectal temperature
T_{scap}	Inter-scapular skin temperature
ZHF	Zero heat flux

CHAPTER ONE

1 INTRODUCTION AND THESIS OBJECTIVES

1.1 INTRODUCTION

Brain injury caused by the combination of inadequate blood flow and oxygen delivery to the brain before and during birth is called hypoxic ischaemic encephalopathy (HIE).[1] While brain injury occurs acutely (cellular necrosis), cell death is known to continue for some time afterwards.[2] Animal studies have shown that numerous brain cells are programmed to die in a process called apoptosis, sometime after a HIE insult. The lowering of brain temperature helps to inhibit this secondary cell death effect.[3] Based on animal experiments, several authors speculated that lowering brain temperature for 72 hours in human neonates shortly after suffering a HIE insult could inhibit apoptosis and improve neurological outcome.[2, 4-11]

Cerebral cooling via whole body or selective head cooling benefits human neonates with HIE

Several pilot studies of rescue hypothermia were undertaken on human HIE neonates that showed a degree of neuronal rescue that led to several randomised controlled trials (RCTs).[2, 6, 12-14]

These RCTs deliberately lowered body temperature in HIE human neonates and provided evidence that cooling reduced death or disability.[2, 4-11] Jacobs et al in 2013 published results of a Cochrane review of eleven therapeutic hypothermia RCTs for HIE neonates.[15] A subgroup of eight quality RCTs collectively studied 1344 neonates where 666 neonates underwent no therapeutic cooling and 678 neonates received cooling.[15] Jacobs et al concluded that there was evidence to show that induced hypothermia helped improve both survival and development at 18 to 24 months of age.[15]

Outcomes from these eight RCTs showed that death or major disability occurred in 409 uncooled neonates (61%) and only 312 (46%) with cooled neonates. While these results show benefit from therapeutic hypothermia, it is clear that significant morbidity continues from HIE despite cooling.

Cooling studies used body temperature as an estimate of brain temperature

The aim of therapeutic hypothermia treatment is to sufficiently lower brain temperature for a period of 48 to 72 hours before gradual rewarming to a normal temperature.[1, 6] However brain temperature was not measured as part of any of these trials. Instead, 'core' body temperature was used as an estimate of brain temperature with different core sites used including rectum, lower oesophagus and nasopharynx, along with different cooling methods.[4, 7-11] In these eight RCTs, rectum was used in 945 (70%) of neonates, lower oesophagus was used in 205 (15.5%) and nasopharynx was used in 194 (14.5%) of neonates.

Methods of achieving cerebral cooling varied between RCTs

Jacobs et al stated that the quality of the neurodevelopmental outcome assessment was considered to be high in six RCT studies that followed survivors to at least 18 months of age.[15] These studies can be divided into five subgroups based on the following cooling methodologies: (i) selective head cooling where rectal temperature was used to target the level of body cooling;[2, 7] (ii) selective head cooling where nasopharyngeal temperature was used to target the level of cooling;[11] (iii) total body cooling where lower oesophageal temperature (T_{oes}) was used to servo-control a cooling blanket system;[9] (iv) total body cooling where rectal temperature (T_{rec}) was targeted using manual control with a cooling blanket system;[4, 6, 10] (v) manual method of total body cooling using refrigerated cool packs for additional cooling where T_{rec} was monitored by staff to achieve a target range.[8]

As brain cooling is important, do systemic temperatures reflect brain temperature?

Animal studies showed that brain cooling for approximately 72 hours was the basis for hypothermia treatment.[16-22] Therefore brain temperature should be the parameter that is monitored during hypothermia treatment in human neonates because using body temperature from a core site in a neonate may not reflect brain temperature.

The assumption that core temperature measurement sites reflect brain temperature was derived from animal studies that involved inducing HIE in piglets followed by a period of mild therapeutic hypothermia.[16-22] Shankaran et al measured brain temperature in piglets at 2 cm depth along with T_{oes} and reported that T_{oes} was a valid marker of deep brain temperature with a mean (±SD) brain to oesophageal temperature difference of -0.1 (±0.3)°C.[13]

Human adult and animal studies suggested proxies for brain temperature. Are they applicable to neonates requiring brain cooling?

Adult human studies have compared brain temperature to various 'core' body sites and showed that brain temperature was higher than other core body sites.[23-34] However the methods used in these studies varied and measurements may not have been made during stable environmental conditions.[29, 33, 35-37] Assumptions were made that a steady offset temperature exists and could be used to derive brain temperature from a particular core measurement site.[34]

Thermoregulation in the human neonate may be different to animals as well as human adults and children, because neonates lack an adequate ability to generate heat from shivering in response to cold exposure.[38] Instead, neonates are reported to activate brown adipose tissues (BAT) to generate heat when they are cold which has been termed non-shivering thermogenesis (NST).[38-40] Several authors reported that piglets do not have BAT and instead use huddling and shivering to generate heat.[41, 42] Considering that piglets, human children and adults use shivering to generate heat, thermoregulatory mechanisms used in human neonates appear markedly different and may exhibit different thermal responses at various body sites. Therefore, brain temperature proxies that are used in animals, human children and adults may not be applicable in neonates.

To date, no study has compared direct brain temperature measurement in neonates to another body site. As a result, there is no evidence that a suitable proxy exists for brain temperature measurement in human newborns.

1.2 THESIS OBJECTIVES

This thesis explores how two core body temperatures in the newborn human neonate may relate to brain temperature. How do rectal and lower oesophageal temperatures behave and compare in neonates? Are body core sites likely to reflect brain temperature? What are the factors that influence core body temperatures? Do a neonates' adaptive responses to hypothermia alter rectal and lower oesophageal temperatures? Answers to these questions will allow a greater understanding of therapeutic hypothermia and its application to the treatment of neonatal HIE.

The literature review in Chapter 2 discusses current concepts of therapeutic hypothermia following birth asphyxia in human neonates. The review includes what is known about how body core sites relate to brain temperature as well as clinical aspects of thermoregulation in the newborn human neonate and how they respond to cold stress. The literature review concludes with a synthesis of what is known in relation to brain temperature and thermoregulatory responses during hypothermia and presents a rationale for the thesis that includes hypotheses to be tested.

Chapters 3 and 4 then review biomedical engineering aspects of the measurement of temperature and oxygen consumption that are fundamental to subsequent experimental designs. These two chapters also detail methods and equipment used to sense and record temperatures and to measure oxygen consumption.

The first hypothesis tested is that brown adipose tissue (BAT) thermogenesis is active during whole body cooling. A defence of core temperature via activation of BAT as a normal thermoregulatory response may influence the effectiveness of whole body cooling in lowering brain temperature. In Chapters 5 and 6 studies in normothermic babies detail the development of a method for monitoring putative BAT activity using inter-scapular skin temperature and comparing this to lower oesophageal temperature. The hypothesis is tested in Chapter 8 in an observational study of neonates with HIE receiving whole body cooling.

BAT activity may have a greater influence on central core temperature (reflected by oesophageal temperature) than rectal core due to the anatomical distribution of BAT. The second hypothesis is therefore that BAT activity influences T_{oes} more than T_{rec}, and this is examined in Chapter 8.

Thirdly, if BAT activity has a significant influence on oesophageal temperature then rectal temperature is not likely to reflect oesophageal temperature during hypothermia. This hypothesis is tested in Chapters 7 and 8.

The relationship between BAT activity and neuroprotection from hypothermia is addressed in this thesis by the fourth hypothesis that BAT activity is associated with brain injury. This can be alternatively stated as BAT activity counteracts the neuroprotective properties of whole body cooling. This hypothesis is tested in Chapter 9 in a study of MRI brain abnormalities after HIE.

In the concluding Chapter (Chapter 10), the limitations of the data, and implications of the results to clinical practice are discussed, and future research directions are proposed.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 RATIONALE FOR THERAPEUTIC COOLING, MEASUREMENT OF CORE AND BODY TEMPERATURES ALONG WITH ADAPTIVE RESPONSE OF NEONATES TO COLD STRESS

HIE following perinatal asphyxia contributes significantly to neonatal mortality and morbidity including long-term neurodevelopmental sequelae in up to 25%-60% of survivors.[43] HIE is estimated to occur in 2.5 out of every 1000 term births in high-income countries and can be ten times higher in less affluent countries.[44, 45] Worldwide, approximately a quarter of all neonatal deaths are attributed to perinatal asphyxia, which is a leading cause of HIE.[46] The HIE neonates analysed in these studies can be divided into three major categories: (i) neonates with mild encephalopathy who do not have an increased risk of motor or cognitive deficits; (ii) neonates with moderate encephalopathy who are more likely to suffer death or survive with severe impairments with other significant deficits such as memory impairment, visual motor or visual perceptive dysfunction, increased hyperactivity and delayed school readiness;[47, 48] (iii) neonates with persistent severe encephalopathy who have a high risk of death, increased risk of cerebral palsy and mental retardation.[1]

Gunn et al noted that after the primary phase of brain damage during asphyxia, cerebral metabolism may initially recover in a latent phase but then deteriorate in a secondary phase of brain injury 6 to 15 hours later.[2] Animal studies have shown that undertaking mild to moderate hypothermia within 30 minutes after a controlled HIE event is neuro-protective.[3, 16, 17, 19, 49] While establishing therapeutic brain cooling shortly after cerebral ischemia showed potential for neuronal rescue, this short length of time (30 min) before treatment may not be possible to achieve with human newborns in clinical practice. Gunn et al were aware of this and presented evidence in foetal sheep that prolonged cerebral cooling, starting 5.5 hours after cerebral ischemia was associated with significant neuronal rescue.[20]

Several authors speculated that lowering brain temperature for 48 to 72

hours in HIE human neonates, could produce a similar outcome.[2, 4-11] This led to five published pilot studies that investigated hypothermic therapy in HIE human neonates.[2, 6, 12-14]

2.1.1 Pilot studies of cerebral cooling in human newborns

Five pilot studies were conducted on HIE human neonates to investigate the practicability and safety of hypothermia therapy as a potential for neuronal rescue. Although the methods used in each study were different their conclusions were similar. Further details are explored with these pilot studies.

2.1.1.1 Pilot study 1

Gunn, A.J., Gluckman, P.D., and Gunn, T.R., *Selective head cooling in newborn infants after perinatal asphyxia: a safety study*. Pediatrics, 1998. 102(4 Pt 1): p. 885-92

Gunn et al experimented with selective head cooling on HIE foetal lambs and found that selective head cooling offered potential for neuronal rescue.[18, 20] Gunn et al then carried out selective head cooling on human HIE neonates for 72 hours and chose selective head cooling as a way to avoid potential side effects associated with systemic hypothermia.[2] They randomly assigned 22 HIE neonates to one of three groups; no cooling (n=10), selective head cooling with minimal systemic hypothermia (n=6) or selective head cooling with mild systemic hypothermia (n=6). The two hypothermia groups commenced cooling within 2 to 5 hours after birth. Head cooling was achieved by circulating water at 10°C (to avoid skin damage) through a coil of clear silicone tubing wrapped around the head. All neonates were warmed by overhead radiant heaters to maintain a target rectal temperature (T_{rec}). The T_{rec} targets for two hypothermia groups were a minimal systemic cooling target T_{rec} of 36.3°C, and a mild systemic cooling target T_{rec} of 35.7°C. The non-cooling (or normothermia) group had a target T_{rec} of 37°C.[2]

The authors commented that while selective head cooling was used, exposed skin was kept warm from an overhead radiant warmer to reduce non shivering thermogenesis (NST).[2] If NST is not reduced, then it would warm the brain and increase oxygen consumption and be counterproductive. Gunn et al 1998 mentioned that intra-cerebral temperatures were not measured

and that nasopharyngeal temperature is a common alternative used in some infant and adult studies. Nasopharyngeal temperatures are known to correlate well with jugular venous blood temperature with intubated adults[50] and so the authors added nasopharyngeal temperature to their observations. Nasopharyngeal temperature was reported cooler than T_{rec} by 1.2°C during cooling in the mild systemic cooled group.[2] The authors discounted nasopharyngeal temperature readings due to concern that ventilatory gas temperature may affect nasopharyngeal temperature.[2]

Gunn et al 1998 concluded that selective head cooling combined with mild systemic hypothermia in term neonates after perinatal asphyxia was a practical method of quickly reducing cerebral temperature with an increased gradient between the surface of the scalp and core temperature.[2] While this is likely to leave predominantly cool superficial brain tissue, the authors decided that selective head cooling would avoid potential adverse effects associated with deeper systemic hypothermia. Further studies of selective head cooling were recommended to establish efficacy before hypothermia was considered for clinical practice.[2]

Achieving a stable T_{rec} in this study appeared difficult based on graphical results which showed considerable variability in the mean and standard deviation (SD) of T_{rec} with each of the 3 groups of neonates.[2]

2.1.1.2 Pilot study 2

Azzopardi, D.V., et al., *Moderate hypothermia to treat perinatal asphyxial encephalopathy*. N Engl J Med, 2009. 361(14): p. 1349-58

Azzopardi et al in 2000 investigated the feasibility of quickly selecting potential HIE neonates with a poor neurological prognosis using amplitude integrated electro-encephalographic monitoring (aEEG) and beginning hypothermic therapy within 6 hours of birth (n =16).[12] Instead of using selective head cooling, the authors chose whole body cooling as cooling machines were available and they were uncertain whether head cooling lowered deep brain temperature effectively. A target core temperature was set between 33-34°C (T_{rec}), but for a shorter cooling duration (48 hours) compared to the study of Gunn et al (72 hours).[12] A form of active cooling

blew cool air over the neonate and the cool air temperature was manually adjusted to keep T_{rec} within the target range.[12]

Although the authors reported a mean T_{rec} of 33.2 ±0.06°C during cooling, the temperature range of T_{rec} of 10 neonates, shown graphically in the paper was between 32 and 34.5°C for most of the 24 hours, which reflects substantial T_{rec} variability. The authors stated that "direct measurement of brain temperature was too invasive for routine clinical use" and chose T_{rec} as a less invasive surrogate for brain temperature. The authors also cited Schwab et al 1998 and Mellergard et al 1992, stating that "studies in adults found close correlation between core temperature and direct measurements of brain temperature during mild hypothermia".[12]

However, the references quoted do not support the statement of Azzopardi et al in this paper. Schwab et al 1998 measured bladder temperature ($T_{bladder}$) not T_{rec}, to indicate core temperature in neuro critical care adults receiving moderate total body cooling to lower intra cranial pressure after severe acute stroke.[12] Schwab et al stated that "the brain temperatures of all 25 patients were consistently higher than body-core temperatures, confirming previous data that showed a significant gradient between body-core and brain temperatures in neurotrauma patients".[51] Although Schwab et al did not indicate how often temperature measurements were made, they did measure intra-parenchymatous brain temperature and reported a mean (range) temperature difference between brain and bladder of 0.3 (-0.3 to 1.0)°C during cooling. While mean brain temperature was only 0.3°C above $T_{bladder}$, a -0.3 to 1.0°C temperature range is large and does not indicate that bladder temperature is necessarily a good indicator of brain temperature.[51] Furthermore, Maxton et al compared $T_{bladder}$ and T_{rec} to pulmonary artery blood temperature, a 'gold standard' core temperature site, in cooled children after cardiac surgery and reported that $T_{bladder}$ provided a suitable indication of core temperature measurement (T_{core}) and that T_{rec} was not a suitable measure for T_{core}.[52]

Mellergard et al compared T_{rec} to ventricular brain temperature in neurosurgical intensive care adult patients where they tried to lower brain

temperature and measure the effectiveness of different brain cooling methods.[53] The authors developed a novel method of continuous brain temperature monitoring in the brain ventricle in conjunction with intra-cerebral pressure monitoring. Although Mellergard et al measured brain temperature and T_{rec}, no results were presented other than graphs from some typical subjects. The graphs showed approximately hourly intervals of T_{rec}, ventricular and sometimes epidural brain temperature that graphically showed considerable variability over time and between each site.[53] However, the majority of patients were febrile and as a result the data are not applicable to the clinical context of hypothermia in neonates.

In the pilot study of Azzopardi et al, as with Gunn et al, nasopharyngeal temperature was also observed. However, nasopharyngeal temperature was not used to direct cooling as they were concerned that airway gas temperature could potentially influence nasopharyngeal temperature. The authors also measured tympanic temperature but decided not to use it as they reported that tympanic temperatures were consistently lower than T_{rec} (-0.49°C) and they were concerned that some of the cooling air used to cool the body could influence tympanic temperature.[12]

Azzopardi et al found that amplitude integrated electro-encephalography (aEEG) criteria were a useful enrolment tool to predict a high probability of death or morbidity in a short enough time to commence hypothermia within 5 to 6 hours.[12] Azzopardi et al 2000 concluded that there were no significant complications due to whole body hypothermia and they recommended proceeding with a randomised controlled trial (RCT) of hypothermia therapy.[12]

2.1.1.3 Pilot study 3

Shankaran, S., et al., *Whole-body hypothermia for neonatal encephalopathy: animal observations as a basis for a randomized, controlled pilot study in term infants*. Pediatrics, 2002. 110(2 Pt 1): p. 377-85

Prior to human studies, Shankaran et al compared direct measurement of brain temperature at 1 cm and 2 cm depth to T_{oes} in anaesthetised miniature swine placed on a servo-controlled cooling blanket during total body cooling

experiments.[13] The authors reported a small temperature difference of 0.1 ±0.3°C from T_{oes} to 2 cm brain depth and suggested that T_{oes} was a good marker of deep brain temperature.[13] The authors found that adding an adult cooling blanket in parallel to the infant cooling blanket reduced the magnitude of the water temperature swings in the infant cooling blanket. Adding the extra water volume of an adult cooling blanket to their system demonstrated their keenness to reduce the thermal instability of their cooling equipment. It appears that using the lower oesophagus as a core site for servo-control may have also helped improve thermal stability.

When studying HIE human neonates reported in the same paper, Shankaran et al 2002 randomly assigned 19 neonates to either whole body hypothermia (n=10) or normothermia (n=9).[13] Neonates in the hypothermia group were placed supine on a cooling blanket that was servo-controlled and based on T_{oes} with a set point temperature of 34.5°C for 72 hours.[13] The purpose of their pilot study was to determine the feasibility of their cooling method as well as to note potential serious adverse events.

Shankaran et al 2002 concluded that modest systemic hypothermia seemed a promising therapy for HIE neonates. They demonstrated the feasibility and safety of initiating whole-body hypothermia within six hours of birth to a constant T_{oes} of 34.5°C, using a commercial servo-controlled cooling system.[13]

While conducting experiments with animals seems a reasonable precursor to introducing a new therapy for human neonates, the authors did not caution readers that there could be differences in thermoregulatory mechanisms between miniature swine and human neonates. The absence of BAT and NST in piglets may be important, as a central physiological role of BAT is to heat the brain.[54] Furthermore, the general anaesthesia used in the piglets abolishes thermoregulatory defence of shivering.[55] Thus the results of the piglet studies of Shankaran et al should be interpreted with caution.

2.1.1.4 Pilot study 4

Debillon, T., et al., *Whole-body cooling after perinatal asphyxia: a pilot study in term neonates*. Dev Med Child Neurol, 2003. 45(1): p. 17-23

Debillon et al studied the practicability and safety of whole body cooling for 72 hours using two different methods in 25 moderate to severe HIE neonates.[14] In the first method, neonates were placed on a cooling blanket that was controlled to a set water temperature and was manually adjusted to keep T_{rec} within a target range of 33 to 34°C (n=18). In the second method neonates had rubber gloves that were filled with 4°C water and placed alongside their body to keep T_{rec} within 33 to 34°C (n=7).[14] Debillon et al concluded that whole body cooling was feasible and safe, with no life threatening effects were seen, and they recommended proceeding with a larger RCT.[14]

The authors pointed out that 60% of neonates had excessive instability in T_{rec} during cooling regardless of cooling method.[14] Although the authors did not offer an explanation for this, a possibility is that T_{rec}, as an indicator of core body temperature, has a slow response time and that manual methods to achieve a target T_{rec} between 33 to 34°C makes it difficult to accomplish T_{rec} stability.

While the authors found no statistical significance between the two methods of cooling, the processes used to cool neonates were very different. For example, neonates placed on the cooling mattress had the circulating water temperature never lower (or warmer) than 1°C from T_{rec}, that is likely to produce a desired small thermal gradient through the body. However, the rubber gloves placed next to the axillas in the second neonate group (and if necessary, on their trunk) were filled with 4°C water which is approximately 30°C lower than their core temperature. This is likely to have a different effect on neonates such as inducing more rapid swings in core temperature. The clinical significance of such different cooling methods on neuro-protection is uncertain.

2.1.1.5 Pilot study 5

Eicher, D.J., et al., *Moderate hypothermia in neonatal encephalopathy: efficacy outcomes*. Pediatr Neurol, 2005. 32(1): p. 11-7

Eicher et al published two concurrent papers on their pilot study where one study reported safety aspects and the other study reported on efficacy outcomes.[6, 56] Their study randomised 65 HIE neonates to either normothermia (n =33) or whole body hypothermia (n =32). Their procedure applied ice to the head and body for approximately 2 hours, then a T_{rec} of 33 ±0.5°C was maintained by a servo-controlled cooling blanket (Blanektrol II) based on T_{rec} for a cooling period of 48 hours.[56]

Among these five pilot studies, Eicher et al used the lowest systemic target T_{rec} (33°C) and the authors tabled clinical side effects introduced by systemic hypothermia. Mortality and developmental outcomes were compared to normothermic neonates after 12 months of age.[56] Their safety study concluded that side effects from cooling were manageable with minor interventions and regarded therapeutic hypothermia as a promising therapy and suggested reporting on the adverse effects of hypothermia in future trials.[56] Furthermore, their efficacy outcomes study concluded that the incidence of death and severe motor scores at 12 months in this pilot trial was reduced, indicating that hypothermia may be helpful even in severe neonatal hypoxic-ischaemic injury.[6]

Eicher et al mentioned that 22% of neonates had a T_{rec} greater than 39°C after cooling and during cooling the mean (range) variability in T_{rec} 25 to 48 hr after enrolment was 1.6 (0.6 to 2.7)°C.[6] This considerable variability in T_{rec} during cooling and substantial overshoot during rewarming could mean that the servo-controlled cooling machine was struggling to keep T_{rec} stable. Zichella et al's abstract [3833.344] titled 'Elevated Blanket Temperatures during Whole Body Cooling with Servo-Controlled Blanketrol III, PAS conference, Denver, 2011, reported ongoing large temperature oscillations with the Blanketrol III cooling machine when servo controlled for 72 hours on T_{rec} such that blanket temperature was >40°C for 23% of the time. Shankaran et al also reported large temperature oscillations in the Blanketrol II blanket temperature used for cooling. These oscillations suggest that the

Blanketrol II and III servo control was very sensitive (underdamped) causing a form of hunting to occur.

2.1.2 Summary of pilot studies

A total of 88 HIE neonates from five pilot studies spanning seven years (from 1998 to 2005), were given hypothermia treatment for 48 hours (in two trials) to 72 hours (in three trials). Each trial confirmed the safety and practicability of therapeutic hypothermia in human neonates with HIE. Four of the five studies used whole-body hypothermia as a method to cool the brain in contrast to one study that utilised selective head cooling. Four of the five studies used T_{rec} as an indicator of core temperature and all found temperature instability with T_{rec}. Shankaran et al compared intra-cerebral temperature (at 2 cm depth) to T_{oes} in piglets and suggested that T_{oes} was a good marker of deep brain temperature.[13]

Azzopardi et al's study was the first to use aEEG to help rapidly define the severity of HIE and the first pilot study to use total body cooling.[12] T_{rec} was used to target the depth of total body cooling where the assumption is that deep brain temperature is similar to the systemic temperature of blood perfusing deep brain and that T_{rec} is a good indicator of whole body temperature. Azzopardi et al cited two previous published papers from Schwab et al[51] and Mellergard et al[53] where Azzopardi et al interpreted that T_{rec} was a surrogate for T_{brain}.[12] However, Schwab et al's article used $T_{bladder}$, not T_{rec} to guide the depth of total body cooling and both cited articles did not suggest that T_{rec} was a surrogate for brain temperature. In this regard, Azzopardi's misinterpretation may have misled subsequent total body cooling studies.

Shankaran et al's[13] animal research observed that T_{oes} was a good marker of deep brain temperature during cooling and the authors used this site of core temperature measurement with neonates in their pilot study.[13] Three years after Shankaran et al's study, Eicher et al in 2005 did not cite Shankaran's pilot study. This is notable given that both studies were conducted in the United States.[56] It appears as though Eicher et al 2005 overlooked

Shankaran's study, as Eicher et al continued to use T_{rec} as an indicator of core and brain temperature which resulted in some instability of T_{rec}.

Although these pilot studies used different methods to achieve cerebral cooling, they all reported that hypothermia therapy was safe and practicable and paved the way for larger randomised controlled trials to compare the efficacy of hypothermia therapy.

2.1.3 Randomised controlled trials of therapeutic hypothermia

The small pilot studies of hypothermia treatment for HIE neonates discussed above showed potential for improved outcomes which led to several larger randomised controlled trials (RCTs). In this section, the six large hypothermia RCTs that are the basis of current clinical practice are summarised and discussed. Their methodological deficiencies in thermoregulatory management are critically examined.

2.1.3.1 CoolCap trial

Gluckman, P.D., et al., *Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial*. Lancet, 2005. 365(9460): p. 663-70

Introduction

Based on the earlier pilot study of Gunn et al,[2] Gluckman et al were the first to report outcomes from a large therapeutic hypothermia RCT (n =234) where they used selective head cooling of neonates with moderate to severe HIE. The study was termed the 'Cool Cap Trial' and they investigated whether neonates randomly assigned to 72 hours of selective head cooling coupled with mild systemic hypothermia (n =116), started within 6 hours of birth, improved neurodevelopmental outcome at 18 months of age, compared to HIE neonates randomly assigned to a control normothermia group (n=118).[7]

HIE neonates ≥ 36 weeks gestation were eligible for enrolment and Gluckman et al used clinical inclusion criteria as well as amplitude integrated electro-encephalo-graphic (aEEG) recordings to improve the specificity of

selection, to control for: (i) severity of encephalopathy (moderate or severe) and (ii) to allow subgroup analysis to test a hypothesis that hypothermia is not protective in neonates with the most severe aEEG abnormalities.[7]

Methods

Control group (non-cooled) treatment methodology

HIE neonates randomly assigned to the control group were given conventional intensive care and were placed under an overhead radiant heater that was servo-controlled to the neonate's exposed abdominal skin temperature with manual adjustment to maintain rectal temperature (T_{rec}) between 36·8 to 37·2°C.[7]

Hypothermia treatment methodology

Cooled HIE neonates had a commercial cooling cap (Olympic Medical Cool Care System, USA) fitted over their head for 72 hours which circulated cold water from a cooling machine through inner tubes of the cap. Initial water temperature was set between 8-12°C and these neonates were nursed under an overhead radiant heater, servo-controlled to the neonate's abdominal skin temperature and manually adjusted to maintain a target T_{rec} of 34.5 ±0.5°C. Manual adjustments were also made to the cooling cap water temperature to help keep T_{rec} within these limits. Temperature recording frequency was not mentioned. At the end of the 72 hr cooling period, the neonates were slowly rewarmed at no more than 0.5°C per hour until T_{rec} was within normal temperature range (i.e., 36·5 to 37·5°C).[7]

Gluckman et al described two concurrent manual adjustment methods to keep the one monitored core T_{rec} between 34 to 35°C. One method adjusted the radiant heater energy output and the other method adjusted the temperature of the circulating water through the cap.[7] How these two thermal control methods were balanced was not described in the study. Therefore some neonates could have received high radiant heating along with cooler circulating water through the cap to achieve the same target T_{rec}, compared to neonates receiving low radiant heating along with less cool water

circulating through the cap.

The level of systemic cooling used in this study (T_{rec} = 34.5°C)[7] was 1.2°C lower than used in Gunn et al's 1998 pilot study (T_{rec} = 35.7°C).[2] Having a lower systemic temperature may have been chosen to lower deep brain temperature in tissues such as basal ganglia which may have improved neuro-protection.[57]

Results

The authors' subgroup analysis with severe HIE neonates (based on aEEG readings shortly after birth) showed no statistically significant difference in the primary outcome of death or severe disability which affected 55% of cooled neonates, and 66% of control neonates (OR 0.61, 95% CI 0.34 – 1.09, p =0.1). However, in the moderate HIE neonate subgroup, hypothermia treatment was associated with an improvement in the primary outcome, with a greater than 50% reduction in severe neuromotor disability in survivors and improved continuous BSID-II (neurodevelopmental assessment by a developmental psychologist) scores at 18 months of age.[7]

Discussion

Gluckman et al's CoolCap trial showed that selective head cooling of moderate or severe HIE neonates, along with mild systemic cooling started soon after birth (< 6h), was clinically feasible.[7] Wyatt et al's secondary analysis extended their initial findings and showed that hypothermia can reduce rates of death or disability at 18 months of age after moderate neonatal encephalopathy.[58] Wyatt et al also showed that "outcomes after hypothermic treatment are affected greatly not only by the severity of encephalopathy but also by birth size",[58] such that higher birth weight neonates were associated with more adverse outcomes.

As direct measurement of brain temperature is not feasible, Gluckman et al used T_{rec} as a measure of systemic temperature and assumed that the surface of the brain was cooled close to the temperature of the circulating water in the CoolCap device.

Using two concurrent forms of manual temperature adjustment (radiant heater energy and circulating water temperature) to control T_{rec}, may have resulted in variation in the depth of systemic cooling and the amount of selective head cooling each neonate received. Furthermore, Gluckman et al did not report the insertion distance of the rectal temperature probe, as rectal probe insertion depth could be an important factor that may influence core temperature accuracy.[59]

In both study arms, temperature control was suboptimal. During cooling, Gluckman et al defined adverse temperature control as T_{rec} being less than 33.5°C for more than an hour. Here, 32% of the cooled neonates were reported to have difficulties in T_{rec} temperature control.[7] Therefore the high frequency of T_{rec} below target range indicates that T_{rec} monitoring and/or the concurrent manual methods of temperature control were not well suited for temperature stability during cooling.

Furthermore, in the control group, 31% of neonates had a T_{rec} >38°C at some point during the 76 hour monitoring period. All neonates had exposed abdominal skin servo-controlled temperature with manual intervention to achieve a target T_{rec}. Servo temperature control of the required body site (T_{rec}) was not used and may have contributed to pyrexia. Intermittent monitoring of rectal temperature could also contribute to instability, although the frequency of temperature measurement was not specified by the authors.

Finally, Gluckman et al mentioned that they included nasopharyngeal temperatures in their study as an indicator of core temperature.[7] However their results section was relatively short and they did not report nasopharyngeal temperature results. Nasopharyngeal temperature observations may have offered some insight. For example, comparing nasopharyngeal temperature to T_{rec} may have shown differences in response rates to changes in environmental temperature.

In contrast, Wyatt et al in 2007 carried out secondary analysis of the CoolCap study and noted that control (non-cooled) neonates that had pyrexia (T_{rec} ≥ 38°C) were associated with adverse outcomes.[58] Of the 31% non-cooled patients who had a T_{rec} of 38°C or more at some time during the 76 hour

monitoring period, 82% had unfavourable outcomes.[58] These results suggest that either elevated brain temperature after birth asphyxia is deleterious or that elevated temperature is a marker for severe brain injury.[60]

Wyatt et al were not able to explain why spontaneous pyrexia occurred but stated that heat production could have been related to intense seizures or induction of inflammatory cytokines.[58] Another possibility may relate to damaged neurons in deep brain regulating thermogenesis, resulting in BAT thermogenesis.

Wyatt et al ended with a recommendation that pyrexia should be rigorously prevented.[58] This implies that better thermal management is required during normothermia, such as more frequent recording of T_{rec} to avoid thermal overshoot.

2.1.3.2 NICHD trial

Shankaran, S., et al., *Whole-body hypothermia for neonatal encephalopathy: animal observations as a basis for a randomized, controlled pilot study in term infants*. Pediatrics, 2002. 110(2 Pt 1): p. 377-85

Introduction

Based on an earlier pilot study of Shankaran et al,[13] a second large cooling RCT was sponsored by the National Institute of Child Health and Human Development (NICHD) Neonatal Research Network and published in 2005.[9] Neonates were clinically assessed for encephalopathy and those with moderate or severe HIE and at least 36 weeks gestational age were randomly assigned to either a whole body hypothermia group (n =102) with treatment beginning within 6 hours of birth, or to a control group where normothermia was maintained (n =106).[9]

Shankaran et al pointed out the tendency for hypoxic–ischaemic injury to affect deep-brain structures such as basal ganglia in the human neonate. As a result they chose whole-body cooling instead of selective head cooling to achieve a consistent reduction in deep brain temperature.[9] The level of systemic cooling was directed by lower T_{oes} targeted at 33.5°C for 72 hours.[9] Shankaran et al commented that T_{oes} was used as a marker for brain

temperature based on earlier research with piglets that were cooled on a servo-controlled cooling blanket.[13] Compared to Shankaran et al's earlier pilot study,[13] the depth of systemic cooling was 1°C lower to a T_{oes} of 33.5°C,[9] instead of 34.5°C. Deeper cooling was likely chosen in the hope of better outcomes and the absence of any serious adverse events occurring during the pilot study.

The CoolCap trial used aEEG as an aid in the determination of the severity of encephalopathy and the primary outcome was a combined end point of death or severe disability.[7] The NICHD trial differed in two ways: (i) neonates were clinically assessed for encephalopathy; (ii) the primary outcome was a combined end point of death or moderate or severe disability.[9] The primary outcomes are therefore not directly comparable between the two trials.

Methods

Control group (non-cooled) treatment methodology

Neonates randomly assigned to the control group were given routine intensive care and placed on a mattress of an overhead radiant warmer that was servo-controlled to exposed abdominal skin temperature. Neonates had T_{oes} recorded four hourly but this was not used to control systemic temperature.[9]

Hypothermia treatment methodology

Neonates in the hypothermia group were placed on an infant size cooling blanket (Blanketrol II, Cincinnati Sub-Zero, USA) that was servo-controlled to T_{oes} targeted to 33.5°C and cooling commenced within 6 hours of birth.[9] A second cooling blanket (adult size) was also attached in parallel to the cooling system that circulated water simultaneously through both blankets to help minimise the magnitude of the water temperature swing and hence the variability in T_{oes}.[9] This was used in the earlier pilot study of Shankaran et al.[13]

Although the servo-controlled cooling machine was continuously measuring T_{oes}, T_{oes} was only documented every 15 minutes for the first 4 hours, every

hour for the next 8 hours and every 4 hours during the remaining 60 hours of cooling period of cooling. After 72 hours of hypothermia, the set point of the automatic control on the cooling system was increased by 0.5°C per hour to reach normothermia at which time exposed abdominal skin temperature was used to servo-control an overhead radiant warmer as per the non-cooled neonate method.[9]

Results

Shankaran et al reported that although adverse events were similar in the two groups during the 72 hours of cooling, death or moderate or severe disability occurred in 44% in the hypothermia group and 62% in the control group (OR 0.72, 95% CI 0.54 – 0.95, p <0.01).[9] There were no statistically significant differences between the two study groups in mortality. However, with the control group there was more neurodevelopmental issues and motor or neuro-sensory impairment.[9] Shankaran et al's cooled neonate outcomes also differed to Gluckman et al's by demonstrating a trend towards better outcomes with severe HIE neonates.

Discussion

The NICHD trial was the first published neonatal RCT to use: (i) whole body cooling; (ii) T_{oes} to direct depth of cooling; (iii) a servo-controlled cooling blanket based on a core temperature site.

Both the NICHD trial[9] and the earlier pilot study of Shankaran et al[13] used T_{oes} to servo-control a cooling blanket system to achieve systemic hypothermia. However there was no mention in either study of the oesophageal probe insertion distance which is reported to influence temperature readings.[61]

An adult size cooling blanket was added in parallel to the cooled neonate's cooling blanket, to reduce the magnitude of swings in circulating water temperature. This occurred in the earlier pilot study of Shankaran et al 2002.[9, 13] The reason why the equipment had large swings in circulating water temperature were not stated but may be due to either the servo-control algorithm used in the cooling device being underdamped (Blanketrol II), or

time lag with T_{oes} in response to changes in circulating water temperature, or a combination.

Mean ± standard deviation (SD) of T_{oes} were graphically depicted in Shankaran's article showing that T_{oes} had less variability with the cooled neonates than the control neonates.[9] The 25th and 75th percentiles of the cooled group for T_{oes} were reported as 33.2°C and 33.5°C, and in the control group, 36.9°C and 37.5°C.[9] T_{oes} in the control group also exceeded 38°C on at least one measurement in 39% of neonates. Likely contributions to this greater variability during normothermia were differences in thermal management (blanket versus radiant heating) and the site of thermal monitoring (oesophagus versus skin).

In summary, this study showed benefits to systemic hypothermia where T_{oes} was servo-controlled. Subsequent publications of long term follow-up data on the study confirmed that hypothermia benefits were sustained to the age of 6 to 7 years.[62] An assumption was made by Shankaran et al that T_{oes} correlated with deep brain temperature, however the basis for this came from animal data and may not directly translate to the human neonate undergoing hypothermia. The use of T_{oes} and servo-control is in contrast to other RCTs of systemic hypothermia that have used T_{rec} and manual control of temperature during hypothermia.

2.1.3.3 TOBY Study Group

Azzopardi, D.V., et al., *Moderate hypothermia to treat perinatal asphyxial encephalopathy*. N Engl J Med, 2009. 361(14): p. 1349-58

Introduction

Azzopardi et al published the Total Body Hypothermia for Neonatal Encephalopathy Trial (TOBY). This was a multicentre RCT that compared 72 hours of total-body cooling that commenced within 6 hours of birth, with routine intensive care, but without cooling, among neonates of at least 36 weeks gestational age.[4] There were 325 moderate to severe HIE neonates recruited and these were randomly assigned to either a non-cooled group

(n =162) or to a whole-body hypothermia group (n =163).[4]

Neonates were selected on the basis of clinical criteria and the presence of abnormalities on amplitude-integrated electroencephalography (aEEG).[4] This was similar to the earlier pilot studies of Azzopardi et al,[12] and the CoolCap trial,[7] but different to the NICHD trial.[9] The primary outcome of Azzopardi et al's study was combined death and severe neurodevelopmental disability.

Methods

Control group (non-cooled) treatment methodology

Neonates randomly assigned to the non-cooled group were placed under a radiant heater or in an incubator that was servo-controlled according to exposed abdominal skin temperature with manual adjustment to target rectal temperature (T_{rec}) at 37.0°C.[4]

Hypothermia treatment methodology

Neonates assigned to the cooled group were treated in closed incubators with the heater power turned off. The neonates were placed on a cooling blanket (Tecotherm TS 200, Germany) in which the circulating fluid temperature was manually adjusted to keep T_{rec} within 33 to 34°C. Cooling concluded after 72 hours of treatment or earlier if clinical circumstances dictated, where T_{rec} was allowed to rise by no more than 0.5°C per hour to reach 37°C. T_{rec} was recorded hourly in all neonates throughout the 72 hour intervention period.[4]

Results

Azzopardi et al reported that there was a statistical difference between the two groups in the composite primary outcome of the study, with combined death and severe neurodevelopmental disability (RR 0.86, 95% CI 0.68 – 1.07, p =0.17).[4] However, neonates in the cooled group had an increased rate of survival without neurologic abnormality. Among survivors (RR 1.57, 95% CI 1.16 – 2.12, p =0.003) cooling resulted in reduced risks of cerebral palsy (RR 0.67, 95% CI 0.47 – 0.96, p =0.03). The cooled group also showed improved scores on the Mental Developmental Index and Psychomotor

Developmental Index of the Bayley Scales of Infant Development II and the Gross Motor Function Classification System.[4]

Discussion

Azzopardi et al cited the NICHD RCT and quoted that the NICHD trial used "slightly different cooling regimens",[4] acknowledging that T_{oes} control was different to T_{rec} control, and that servo-controlled cooling was different to manually adjusted cooling. However, Azzopardi et al did not discuss the potential clinical significance of these differences.

The trial of Azzopardi et al was the first large RCT to report the insertion distance into the body of the core temperature probe. In this case the rectal probe was at least 2 cm within the rectum.[4] Therefore rectal probe insertion depth could be an important factor as insertion distance is reported to influence temperature readings.[59]

In the TOBY trial, both the control group and cooled group used T_{rec} to target systemic temperature. However the control group were not servo-controlled to T_{rec} but rather, servo-controlled according to exposed abdominal skin temperature with manual adjustment that targeted a T_{rec} of 37.0°C.[4] Servo-controlling a body temperature site that is not the target site could lead to thermal instability in the targeted body temperature site (T_{rec}) and is therefore a limitation of the study. The reason for this limitation was that servo-controlling a core temperature site was not an available option for at that time with infant radiant warmers.

Azzopardi et al included a graphic of mean rectal temperatures (and standard deviation) during the study period for both the control (non-cooled) and cooled groups that showed temperature variation in both groups that was most marked in the control group.[4] Among the neonates who were not cooled, during the treatment period the temperature rose above 38°C on one occasion in 9% of subjects and on more than one occasion in 14% of subjects.[4]

Hypothermia was maintained by placing the neonate on a cooling blanket in

which the circulating fluid temperature was manually adjusted to target T_{rec} 33-34°C. Any lag time in T_{rec} to changes in cooling mattress temperature or delay with manual intervention is likely to produce thermal instability in T_{rec}. Servo-control would minimise fluctuation in temperature but this technology was not available at the time of their study.

As with the study of Shankaran et al,[9] the greater variation in T_{rec} in the control group may reflect servo-control, based on exposed abdominal skin temperature rather than T_{rec}.

In a nested sub-study, Rutherford et al demonstrated that cooled neonates had fewer MRI abnormalities than controls (OR 0.41, 95% CI 0.18 – 0.91, p = 0.03), further supporting the clinically demonstrated benefit of hypothermia in reducing brain tissue injury.[63]

2.1.3.4 China Study Group

Zhou, W.H., et al., *Selective head cooling with mild systemic hypothermia after neonatal hypoxic-ischemic encephalopathy: a multicenter randomized controlled trial in China*. J Pediatr, 2010. 157(3): p. 367-72, 372 e1-3

Introduction

In China, Zhou et al investigated the efficacy and safety of selective head cooling along with mild systemic hypothermia in neonates ≥37 weeks gestational age with mild, moderate or severe HIE. They were randomly assigned to either selective head cooling (n =100) or non-cooling (control, n =94) groups in a multicentre RCT.[11]

This trial was similar to the CoolCap RCT in that neonates in the cooling group received 72 hours of selective head cooling along with mild systemic cooling that commenced within 6 hours after birth.[7] The methodology of Zhou et al differed to that of the CoolCap study by requiring a gestational age ≥37 weeks for eligibility, and by not using aEEG to help assess the level of encephalopathy. Neurodevelopmental outcome was assessed at 18 months of age and the primary outcome was a combined end point of death or severe disability.[11]

Methods

Non-cooled methodology (control group)

Neonates randomly assigned to the control group were given conventional intensive care and were placed under an overhead radiant heater that was servo-controlled to the neonate's exposed abdominal skin temperature with manual adjustment to a T_{rec} between 36 to 37·5°C.[11]

Hypothermia treatment methodology

The authors used a locally made cooling system (Henyang Radio Manufacturer, Hunan, China). The design used a cooling cap placed over the neonate's head that circulated cold water through the cap and servo-controlled to a nasopharyngeal target temperature of 34 ±0.2°C. These neonates were placed on a mattress of an open radiant warmer which was servo-controlled to the neonate's abdominal skin temperature and manually adjusted to maintain a target T_{rec} of 34.5 to 35°C. At the end of the 72 hour cooling period, spontaneous rewarming occurred.[11]

Results

Although Zhou et al analysed all HIE neonates, including those with mild HIE (21% in the selective head cooling group and 19% in control group), their results showed that neonates with mild HIE in both groups had no risk of death or major disability, supporting the exclusion of this subgroup in the other published RCTs. Zhou et al also reported that the combined outcome of death or severe disability was 31% of neonates in the cooled group and 49% in the non-cooled group (RR 0.47, 95% CI 0.26 – 0.84, p =0.01).[11]

The authors also showed a positive benefit to cooling in the secondary outcomes, a composite of death or severe disability in neonates with moderate, and moderate to severe HIE, but not with severe HIE. Neonates with moderate to severe HIE were less likely to survive with severe disability if cooled (RR 0.36, 95% CI 0.15 – 0.87, p =0.02).[11]

Discussion

The unique features of this trial are that neonates were: (i) of a higher

gestational age (≥37 weeks); (ii) servo-controlled to a nasopharyngeal target temperature, to guide the depth of selective head cooling; (iii) spontaneously rewarmed after cooling.

The study of Zhou et al is limited by the lack of information on the frequency of recorded temperatures. The majority of their temperatures were only graphically shown with 6 hourly increments. Furthermore, the authors did not mention rectal probe insertion distance or how they positioned a temperature probe in the nasopharynx. Consequently the stability of core temperature is difficult to ascertain.

Nasopharyngeal and rectal temperatures were recorded for all neonates for 96 hours and simultaneously plotted in a graphic, showing the relative difference between nasopharyngeal and T_{rec} for both cooled and non-cooled neonates.[11] Zhou et al showed that T_{rec} was consistently warmer by approximately 1°C in the non-cooled group.[11] They provided no evidence to validate nasopharyngeal temperature as a stable measurement site not influenced by the ambient temperature of inspired air.[64]

Zhou et al did not report any temperature mean or standard deviation (SD) results in nasopharynx or rectum.[11] However, they reported that approximately 5% of neonates in the control and cooled groups after rewarming had, a temperature >38°C at least once to an age of 96 hours.[11]

2.1.3.5 neo.nEURO.network

Simbruner, G., et al., *Systemic hypothermia after neonatal encephalopathy: outcomes of neo.nEURO.network RCT*. Pediatrics, 2010. 126(4): p. e771-8

Introduction

In the study of Simbruner et al (the neo.nEURO.network trial), 111 term neonates (≥36 weeks of gestation) with electroencephalography (EEG) criteria and clinical evidence of moderate to severe HIE were evaluated.[10] Neonates were randomly assigned to either a control group with a target rectal temperature (T_{rec}) of 37°C (n =58), or to a hypothermia group with a target T_{rec} of 33.5°C using a cooling blanket for 72 hours, with slow rewarming (n =53). Neurodevelopmental outcomes were assessed at the age

of 18 to 21 months where the primary outcome was death or severe disability.

The neo.nEURO.network study methodology was based on the protocol of the CoolCap trial authored by Gluckman et al.[7] However, it differed by using total body cooling and rigorous co-treatment with morphine for both cooled and non-cooled neonates.[10]

Methods

Control group (non-cooled) treatment methodology

Neonates randomly assigned to the control group were given conventional intensive care and nursed naked on a mattress of an open radiant warmer that was manually adjusted to achieve a target T_{rec} of 37°C.[10] Whether exposed abdominal skin temperature had been utilised was not discussed.

Hypothermia treatment methodology

Neonates assigned to the hypothermia group were placed on a cooling blanket for 72 hours with manual adjustment of the cooling machine (Tecotherm TS Med 200, Germany) to achieve a T_{rec} in the range of 33 to 34°C. The T_{rec} target was intended to be achieved within 60 minutes after the start of cooling. Rewarming was achieved by setting the circulating fluid temperature 2°C higher in a stepwise manner, that allowed T_{rec} to increase 0.5°C per hour until normothermia was reached.[10]

Results

Simbruner et al reported that 51% of neonates in the hypothermia group died or had severe disability and 83% of neonates in the normothermia group died or had severe disability (adjusted OR 0.21, 95% CI 0.09 – 0.54, p =0.001).[10] Although there were fewer clinical seizures in the hypothermia group, the rates of adverse events during the intervention were similar in the two groups.[10]

Discussion

Simbruner et al reported that no previous RCT had used morphine as a co-treatment with hypothermia and suggested that this contributed to the neuro-protective efficacy of hypothermia.[10] Simbruner et al suggested that opioids may blunt stress, metabolic and hormonal responses thereby reducing neurological injury.[10] However, the extent to which morphine was used on a regular basis in the other RCTs is unclear.

Simbruner et al reported that the insertion depth of the rectal temperature sensor was 2 cm from the anus.[10] While this insertion depth is the same as used in TOBY study, Mead et al reported that insertion depth of a rectal probe in adult humans has a large influence on the temperature reading.[59] Therefore the results from the neo.nEURO.network study and the TOBY study may not be comparable to other studies (ICE RCT) that targeted T_{rec} using at least 5 cm insertion depth.[8]

Although Simbruner et al did not include any temperature plots, they reported that 87% of cooled neonates had episodes below 33°C (mean =5 hours) and 61% above 34°C (mean =2.5 hours).[10] This suggests that the majority of cooled neonates had episodes outside the target temperature range. In the control group 76% of non-cooled neonates had episodes below 36.5°C (mean =14.9 hours) and 39% above 37.5°C (mean =6.8 hours).[10] In addition 14% of neonates in the control (normothermia) group experienced at least one episode of T_{rec} >38°C during the intervention period and the highest T_{rec} was 39.3°C.[10]

A contributor to this instability may have been the method of manually adjusting the temperature of the cooling blanket fluid to achieve the target T_{rec}.[65] Manual adjustment may have created instability through delays in recognition of T_{rec} approaching the limits of the target temperature range.

2.1.3.6 ICE trial

Jacobs, S.E., et al., *Whole-body hypothermia for term and near-term newborns with hypoxic-ischemic encephalopathy: a randomized controlled trial.* Arch Pediatr Adolesc Med, 2011. 165(8): p. 692-700

Introduction

Jacobs et al authored a large RCT known as the Infant Cooling Evaluation (ICE) trial. The ICE trial was a multi-centre RCT for 221 term and near-term neonates ≥ 35 weeks gestational age with moderate or severe HIE born in hospitals with or without a neonatal intensive care unit (NICU). The study aim was to determine the effectiveness and safety of moderate whole-body hypothermia targeting T_{rec} to 33.5 ±0.5°C for 72 hours, and commenced within 6 hours of birth (n =110).[8] This was compared to a control group of HIE neonates nursed at normal body temperature (n =111).[8]

Neonates born in a facility that did not have a NICU (outborn neonates) were retrieved to a participating facility that had one. Exposure to ambient temperature and refrigerated gel packs were used to achieve systemic hypothermia. Hypothermia was commenced at the birth hospital by dedicated neonatal retrieval teams and continued during transport to participating NICUs.[8] Cooling blankets were not used.

Clinical criteria were used to identify neonates at risk of brain injury after hypoxia-ischemia to determine the severity of encephalopathy and the primary composite outcome was mortality or major sensorineural disability at 2 years of age.[8] The ICE trial planned to recruit 300 neonates but stopped at 221 due to a loss of equipoise following publication of other RCTs that showed evidence for the efficacy of therapeutic hypothermia.[8] Ethical concerns were raised about randomly assigning further HIE neonates to non-cooled groups who might benefit from hypothermia therapy.[8]

Methods

Control group (non-cooled) treatment methodology

Neonates randomly assigned to the non-cooling group were nursed under a radiant warmer and had T_{rec} manually targeted to 37.0°C.[8] Although likely,

there was no mention about using exposed abdominal skin temperature to control the radiant warmer.

Hypothermia treatment methodology

Cooled neonates had whole-body hypothermia to a T_{rec} target of 33.5°C for 72 hours. Neonates were placed naked on a mattress under a radiant warmer or in a transport incubator with the heater initially turned off.[8] Neonates were exposed to ambient temperature to lose heat and if greater cooling was required, refrigerated gel packs were applied across the chest and/or under the head and shoulders using an algorithm based on T_{rec}. The radiant warmer heater output, or transport incubator temperature, was manually increased if T_{rec} was below 33.5°C. After 72 hours, neonates were slowly rewarmed over 8 to 12 hours.[8]

Results

Jacobs et al reported that 51.4% of neonates in the hypothermia group died or had a major sensorineural disability at 2 years of age. They also reported that 66.3% of neonates in the normothermia group died or had a major sensorineural disability at 2 years of age (RR 0.7, 95% CI 0.62 – 0.98, p = 0.03).[8] Mortality rates were decreased by hypothermia (RR 0.65, 95% CI 0.93 – 0.97, p =0.04), while the survival rate, free of any sensorineural disability, also increased (RR 1.75, 95% CI 1.13 – 2.70, p =0.01).[8]

Jacobs et al demonstrated that whole-body hypothermia commenced at the birth hospital within 6 hours of birth (without cooling machines), was achievable and appeared to be safe in term and near-term HIE neonates in non-intensive care, intensive care, and transport settings.

Discussion

The ICE trial differed from the previous hypothermia trials because cooling blankets were not used and rectal probe insertion distance was at least 5 cm. Furthermore, the method of hypothermia management was not only used in

the non-intensive care setting (awaiting retrieval) but also during retrieval and in the tertiary intensive care setting.[8]

Jacobs et al included a graphic of mean (±SD) rectal temperatures during the study period for both the control (non-cooled) and cooled groups. This showed that the mean (±SD) of T_{rec} for the non-cooled group was 36.9°C (±0.3°C), and for the cooled group 33.8°C (±0.4°C).[8] This graphic suggested little variability in T_{rec}. However, the authors pointed out that refrigerated gel packs were used to lower T_{rec} in 84.5% of cooled neonates during the first 6-hour initiation phase while 78.2% of cooled neonates required refrigerated gel packs in the maintenance phase (between 6 and 72 hours). This caused undershoot in T_{rec} as more than half (58%) of cooled neonates had a T_{rec} reading that was below 33°C while 56.4% of neonates had a T_{rec} reading in the range 29.8 to 32.9°C.[8] This latter T_{rec} range can be classified as severe hypothermia.[66] This may be a clinical concern especially during retrieval.

With control (non-cooled) neonates, 14.4% had a temperature of 38.0°C or higher at some stage which Jacobs et al associated with a trend toward increased mortality[8]. This suggests instability in T_{rec} but is not discernible from the presented temperature plots and resultant mean (±SD) temperatures.

With cooled neonates, Jacobs et al offered no explanation for the thermal undershoot except to report that undershoot in T_{rec} below 33°C, mostly occurred during the first 6 hour initiation phase of hypothermia.[8] This is further evidence of instability in T_{rec}. It is likely that the undershoot observed in T_{rec} is due to a lag time in T_{rec} coupled with the time taken for the attending nurse to observe T_{rec} and manually intervene.

As with the TOBY study, a sub-study of the ICE trial examined MRI abnormalities on cooled and control neonates.[67] Both studies showed reduced brain injury in cooled neonates, supporting the neuro protective benefits of hypothermia.

2.1.4 Summary of Randomised Controlled Trials

The aforementioned RCTs have been the subject of a Cochrane meta-analysis.[15] In this meta-analysis, Jacobs et al concluded that cooling results in a reduction in combined death or major neurodevelopmental disability to 18 months of age (RR 0.75, 95% CI 0.68 to 0.83, NNT 7, 95% CI 5 to 10), a reduction in mortality (RR 0.75, 95% CI 0.64 to 0.88, NNT 11, 95% CI 8 to 25), and a reduction in neurodevelopmental disability in survivors (RR 0.77, 95% CI 0.63 to 0.94, NNT 8, 95% CI 5 to 14).[15] The positive effects of cooling holds true for selective head cooling, whole body cooling and for both moderate and severe HIE neonates.[15] Based on this evidence, hypothermia therapy is now a standard of care at tertiary medical centres. However, there was considerable variability between the methods and technologies used in the RCTs to achieve hypothermia and monitor the depth of cooling (Table 2.1).

As discussed, there was variability between the individual RCTs in neurological outcomes. Furthermore, cooling is not a panacea because HIE continues to have considerable morbidity. Cotton and Shankaran commented that 40% or more of cooled neonates are still dying or suffering moderate or severe long-term impairment and recommended work to discover additional neuro-protective strategies.[1]

The two large RCTs that utilised selective head cooling used different methods to determine the depth of selective head cooling even though both used the same method to guide the depth of coupled mild systemic hypothermia. Although selective head cooling avoids potential problems associated with moderate total body hypothermia, the two competing manual adjustment methods used in the CoolCap trial to guide cooling treatment seemed counterproductive. The cooling method reported by Zhou et al seems to have used a logical approach by servo-controlling to nasopharyngeal temperature to guide the depth of selective head cooling, independent from concurrent mild systemic hypothermia.

RCT	Authors	Trial Name	Servo Cooling	Hypothermia measurement site	Servo-control exp. abd. skin temp. (control group)	Pyrexia in control group
1	Gluckman et al[7]	Cool Cap	No	Rectum	Yes, man adj T_{rec} 37°C	31%
2	Shankaran et al[9]	NICHD	Yes	Oesophagus	Yes, T_{oes} observed	39%
3	Azzopardi et al[4]	TOBY	No	Rectum	Yes, man adj T_{rec} 37°C	14%
4	Zhou et al[11]	China Study Group	Yes	Nasopharynx	Yes, man adj T_{rec} 37°C	6%
5	Simbruner et al[10]	neo.nEURO.network	No	Rectum	Unknown man adj T_{rec} 37°C	14%
6	Jacobs et al[8]	ICE	No	Rectum	Unknown man adj T_{rec} 37°C	14%

Table 2.1 Temperature measurement methodology used in six RCTs

(Pyrexia is defined as having a core temperature above 38°C).

The other four large RCTs that used whole body hypothermia to determine the depth of systemic cooling assumed that deep brain was likely be cooled to a similar temperature. Three of these trials used cooling blanket systems to lower systemic temperature but only one (NICHD) used servo-control to T_{oes}. The TOBY and neo.nEURO.network trials only used manual control of the circulating fluid temperature to target systemic temperature (T_{rec}). Rectum was used as a target site in 3 of the whole body cooling RCTs while oesophagus was used in 1, and the depth of rectal probe insertion also differed.

The inherent delay with manual adjustments along with some lag time in T_{rec} are both contributing factors of temperature instability and undershoot.[65] Reducing manual intervention time such as using servo-control and a body site that is faster in responding to changes in environmental conditions, will improve temperature control and stability.

As two trials identified worse outcomes among neonates who experienced core body temperatures >38°C, taking steps to avoid hyperthermia among HIE neonates will result in less morbidity.[8, 58]

Rescue hypothermia treatment was trialled in the RCTs as a potential neuro-protective strategy through lowering brain temperature. However brain temperature was not monitored. Instead, different body sites were chosen as a substitute for brain temperature and the studies failed to respect the impact this could have on the results.[68] In contrast, core temperature is known to vary throughout the adult human body under normal thermal states,[69, 70] and core temperature differences in the body are accentuated by hypothermia.[71] Furthermore, different core sites have different lag times before changing in response to warming or cooling the skin, with rectum being particularly slow.[52, 65, 72] Rapidly responding, but non-monitored sites, such as brain may show considerable change while a slowly responding site such as rectum is the target monitoring site.

2.2 CONCLUSION OF CLINICAL DATA REVIEW AND COOLING RCTs

The question arising from this review of clinical data from pilot studies and RCTs of rescue hypothermia is whether the continued morbidity observed with HIE neonates is related to the manner in which an indication of brain temperature is measured and controlled.

To begin to answer this question it is important to understand what determines brain temperature. This is the subject of the next section of this literature review.

2.3 WHAT DETERMINES BRAIN TEMPERATURE?

The human adult brain is a highly vascular organ and accounts for approximately 20% of the body's total oxygen consumption at rest.[73] Brain neurons require substantially more energy to function than other cells.[73] While the power consumption of a single central neuron is about 0.5–4.0 nano-Watts, it is 300–2500 times more than the average body cell (1.6 pico-Watts).[73] In addition to neurons, the brain contains metabolically active glial and endothelial cells, with numbers that greatly exceed those of neural cells.[73]

Brain temperature is considered to be an extremely important variable in both normal brain functioning and brain development.[54, 73] Brain temperature is part of the body's autonomic thermoregulatory system,[68] and is considered to be an open thermodynamic system, where brain temperature is determined by the temperature of the incoming arterial blood perfusing the brain, the amount of heat generated by cerebral metabolism and the amount of heat that is lost.[30, 68, 73-79]

The temperature of incoming arterial blood to the brain is related to core body temperature which is typically maintained within ±1°C from normal temperature (37°C) by an effective thermoregulatory system.[68] A slight variation in core temperature of approximately ±0.2°C from normal, quickly triggers a thermoregulatory response that is closely controlled to avoid thermal imbalance.[68, 80] In humans, core temperature is more tightly regulated than other important physiological parameters such as blood pressure and heart rate.[80] Temperature is sensed by the transient receptor potential (TRP) family of ion channels. The subtype TRP-V3 senses heat whereas cold is sensed by TRP-M8.[80] Thermal information from the skin surface, peripheral tissues, vital organs and the central nervous system (CNS) are integrated at various levels and finally arrive at the hypothalamus that is considered the dominant centre of thermoregulatory control.[80]

Heat is an essential feature of brain metabolism with most of the energy used for brain functioning eventually released as heat.[81] In the brain, heat is mostly produced by mitochondrial oxidative chemical reactions.[79] Energy required for brain activity is generated from the net chemical reaction of oxygen and glucose, where approximately 1/3 of this energy is immediately dissipated into heat, and the remaining 2/3 is used to synthesise ATP.[79] The final ATP hydrolysis releases part of the energy back to the system also as heat.[81]

The metabolic activity of brain is reflected by the cerebral metabolic rate of oxygen ($CMR0_2$). The neonatal brain has a lower $CMR0_2$ under basal conditions as measured by measured by near infrared spectroscopy and MRI methods, when compared to adult brain $CMRO_2$.[82-85] This reflects the lower cerebral cortex synaptic activity in the neonate.[83, 86] $CMRO_2$ increases with

gestational age and postnatal age in preterm infants.[83, 85] Glucose utilisation by brain (an indirect measure of metabolic rate) increases to a peak at approximately 4 years of age before a slow decline to adult values.[86]

Although $CMRO_2$ is increased by seizure activity in animal,[87] $CMRO_2$ in the human neonate has been shown to be reduced with HIE.[82] A greater reduction in glucose metabolism as assessed by PET is also associated with increasing severity of HIE.[88]

The structure and arrangement of the brain contained within the skull creates a thermal gradient even within the normothermic brain, such that deep brain is typically warmer than the brain surface and warmer than pulmonary artery blood.[23, 25, 27-34, 53, 89-93] In neonates, a brain temperature gradient has been measured from the brain surface to a depth of 4 cm where the temperature gradient was approximately 0.1°C per cm over the deepest 3 cm, with a largest gradient from the brain surface to 1 cm depth (0.8°C).[92] Radiant heat loss through the scalp of the human neonate is greater than in the adult due to a larger surface area to volume ratio.[94]

The second law of thermodynamics specifies that heat can only flow down a temperature gradient (from high to low temperature). Heat loss within deep brain is a combination of conduction to more superficial regions, and convection via cerebrospinal fluid (CSF) circulation and cerebral blood flow.[68]

However, most of the heat that leaves the brain is through cerebral blood flow,[73, 74, 78, 79] and therefore venous blood leaving the brain is considered to be at a higher temperature than the incoming arterial blood.[81] Consequently, a lower/higher than normal cerebral blood flow would increase/decrease relative brain temperature respectively.[92]

Cerebral blood flow studies using a Xenon 133 clearance method in human neonates with HIE not treated with hypothermia have demonstrated increased cerebral blood flow on the first day of life, and impaired autoregulation in response to changes in blood pressure and arterial CO_2 levels.[95] The degree of hyperaemia correlates with the severity of the insult.[95, 96] These findings are supported by studies using near infrared spectroscopsy.[96]

During whole body hypothermia, MRI studies of arterial perfusion in neonates with HIE show that early hypoperfusion of brain is followed by regional hyperperfusion, with hyperperfused brain regions correlating with subsequent MRI identified injury.[97] Cerebral blood flow increases over the first 48 hours of life with whole body hypothermia.[98] Lower cerebral blood flow has been found in the first 2 days of life in babies with more severe encephalopathy.[98] De Vis et al showed similar global cerebral blood flow in babies with HIE on day 2-7 compared to healthy control preterm babies at term corrected age using MRI.[82] Hochwald et al have shown that superior vena cava (SVC) flow (a proxy for cerebral blood flow) is increased on day 3 of life during whole body hypothermia for HIE.[99]

Changes in global and regional blood flow during HIE appear to reflect impaired autoregulation and re-perfusion injury. How these changes influence brain temperature is unknown, and will depend on the extent of brain heat production via metabolism. As global $CMRO_2$ is lowered during HIE, lower CBF is less likely to result in heating of the brain when compared to conditions where $CMRO_2$ is normal or raised. However, regional hot spots in brain may occur due to difficulty in transferring heat due to local oedema and vascular blockage and focal increases in neuronal activity.[100] The data of Wintermark et al however do not support focal brain overheating as a mechanism of injury, as regional hyperaemia should enable greater heat transfer from brain.[97] Mechanisms that prevent dissipation of heat in jugular venous blood would be expected to increase temperature. In this respect, the jugular venous system in the neck is encased by BAT which if active will rapidly heat brain by reducing convective heat loss. While blood flow through the scalp tends to thermally isolate the brain from the environment,[68] superficial brain exchanges heat with superficial vessels and by thermal conduction through the skull.[79] However if the scalp does not lose heat (head covering) or is warmed by IR radiation, then both superficial and deep brain temperature can increase.[101]

The amount of heat loss from the body and the amount of heat generated are controlled through thermoregulatory mechanisms via active central command to keep the brain at its desired temperature. Although brain temperature is

the endpoint of thermoregulation, the mechanisms employed to achieve this are located extra-cranially. Therefore brain temperature is normally above, yet linked to core body temperature. But what is known about temperature within the brain?

2.4 INTRACRANIAL TEMPERATURE

As a thermal gradient normally exists within the brain, it is challenging to determine the most appropriate site in the brain to measure temperature. While the premise of rescue hypothermia is to cool brain tissue, it is not clear which parts within the brain require the most cooling as cells in the cortex and deep gray nuclei as suggested by Volpe et al,[102] are highly metabolically active and may be more susceptible to damage. Furthermore, glial cells offer support to brain neurons and may also benefit from cooling.[103] Therefore it may be advantageous to cool the whole brain which can be done with whole body hypothermia.[13] Another method of cerebral cooling, known as selective head cooling, tends to cool the outer cortex more than deep brain.[104] Due to the paucity of data from intracerebral temperature measurements within human neonates, animal experiments have compared temperature differences within the brain during normothermia as well as cerebral cooling. These important studies offer valuable insight.

2.4.1 Temperature difference: deep brain and superficial brain in animals

Temperature differences (site offset) between deep and superficial brain during normothermia and cerebral cooling from the literature are shown in Table 2.2. This table outlines methods used, limitations and brief comments of the most important studies to date. The most frequently used model in experimental neonatology was the piglet,[3, 13, 16, 19, 21, 22, 42, 57, 101, 104] as newborn pigs were thought to have comparable physiology and brain maturation to human neonates.[101] Gunn et al also studied brain and body temperatures in foetal sheep,[18] and this study is included in Table 2.2.

Animal (Author)	Brain Reference Site	Other Brain Site	Method	Site Offset from ref (°C)	Limitations/Comments
Piglet. (Liu et al, 2011)[101]	Parenchyma, 22 mm below surface via burr hole	Parenchyma, 3 mm below surface via burr hole	Normothermia (T_{rec}), Radiant heater on, no hat, Gen anaesthesia. 10 sec measurements. 0.1°C sensor accuracy.	0.0°C (same)	N =6, small sample size. Anaesthesia: N_2O (66%) and Isoflurane (1-2%). Without heated humidification. Room temperature unknown. With radiant heater on and with no hat suggests warm scalp, therefore minimal heat loss from superficial brain.
As above	as above	as above	Normothermia (T_{rec}), Radiant heater off, no hat. (same remaining methods)	-1°C	As above, but with radiant heater off and with no hat. Suggests cooler scalp, therefore superficial brain heat loss via skull and scalp.
As above	as above	as above	Heater/Cooler blanket in contact with scalp after rewarming. (same remaining methods)	+1°C (warmer)	As above, and with radiant heater off. Heater/Cooler blanket up to 40°C touching scalp suggests scalp warming, and superficial brain warming through skull.
As above	Parenchyma, 3 mm below surface via burr hole (no hat)	Parenchyma, 3 mm (same as ref site). But with a hat	Normothermia, with hat, no radiant heating or, Hypothermia with hat, servo cooling blanket (T_{rec}) (same remaining methods)	+0.78°C (normothermia) +1.2°C (hypothermia)	As above. Heater/Cooler blanket maintaining normothermia (T_{rec}). The authors suggested that the cotton hat avoided heat loss. Therefore scalp warming lead to retention of heat in superficial brain when wearing a cotton hat.
As above	Parenchyma, 22 mm below surface via burr hole	Parenchyma, 22 mm (same as ref site). But with a hat	as above	+0.53°C (normothermia) +0.7°C (hypothermia)	As above. A more pronounced effect occurred with superficial brain temperature above. Even so, a warmer superficial brain lead to an increased deep brain temperature when wearing a cotton hat.

Table 2.2 Temperature between deep and superficial brain in animals detailing methods and limitations

Animal (Author)	Brain Reference Site	Other Brain Site	Method	Site Offset from ref (°C)	Limitations/Comments
HIE Piglet. (Tooley et al, 2005)[105]	Parenchyma, 20 mm below surface via burr hole	Subdura, just below surface via burr hole	Normothermia (T_{rec}), radiant heater on, IR shield above head. Gen anaes. 1 min meas. 0.1°C sensor accuracy	-0.8°C	N =10. Without heated humidification. Room temp unknown. Baseline conditions. IR shield above head. Deep brain temp was 39.3°C, Data suggest some heat loss from brain surface through skull and scalp. Similar result to Liu et al 2011.
As above	as above	as above	SHC (24 hours) with radiant heater on, Mild whole body cooling. (same remaining methods)	-3.7°C	As above except N=8. IR shield above head. Deep brain temp was 30.4°C, suggesting some contribution from cooler systemic blood perfusing deep brain and some contribution from heat loss from deep brain to superficial brain through skull and scalp to cool cap (16°C).
Piglet. (Iwata et al in 2003)[104]	Parenchyma, 20 mm below surface via burr hole	Parenchyma, 5 mm below surface via burr hole	SHC only. 10°C cap water Radiant heater on. Gen anaes. 5 min meas. 0.1°C sensor accuracy.	-3.4°C	N =7. Without heated humidification. Room temp 20°C. During thermal steady state (<0.1°C for 20 min). Deep brain temp was 35.8°C, suggesting some heat loss from deep brain to superficial brain through skull and scalp to cool cap.
As above	Parenchyma, 20 mm below surface via burr hole	Parenchyma, 20 mm below surface via burr hole. (same as ref site)	Mild whole body cooling (cooling blanket) + SHC (same remaining methods)	Deep brain reduced by 1.1°C	N =3. Caution, small sample size. Mild WHC plus SHC with 10°C water through cool cap. Deep brain temp reduced a further 1.1°C, suggesting a direct influence from systemic blood temperature perfusing deep brain.

Table 2.2 continued

Animal (Author)	Brain Reference Site	Other Brain Site	Method	Site Offset from ref (°C)	Limitations/Comments
Piglet. (Shankaran et al 2002)[13]	Parenchyma, 20 mm below surface via sealed burr hole	Parenchyma, 10 mm below surface via sealed burr hole	Whole body cooling (WBC) using heater/cooler blanket servo T_{oes} to 33.5°C. Gen anaes. Meas rate unknown. 0.2°C sensor accuracy.	-0.3°C	Caution as N =3. Without heated humidification. Room temp unknown. Reportedly after thermal stabilisation, however, a graphic shows ongoing oscillation of ±0.9°C in deep brain temp therefore not thermal steady state. Required a second adult size blanket (Blanketrol) to reduce magnitude of water temp oscillation. Deep brain warmer than mid brain.
as above	as above	Epidural, below sealed burr hole	as above	-1°C	Brain surface shows greater heat loss than mid brain.
HIE foetal sheep. (Gunn et al 1997)[18]	Parenchyma, 25 mm below surface via sealed burr hole	Parenchyma, 10 mm below surface via sealed burr hole.	SHC to 32°C (extradural) In utero, T_{oes} 38.4°C. No anaes. 1 min meas ave. Sensor accuracy unknown	-0.8°C	N =1. Caution, very small sample size and performed in utero (where brown adipose tissue is inhibited). Reportedly stable temperatures, accuracy unknown. Toes core temp. Deep brain temp was 37.7°C i.e. 5.7°C warmer than brain surface. Some deep brain cooling.

Table 2.2 continued

Discussion of temperature difference between deep and superficial brain in neonatal piglets and foetal sheep

The above studies of Liu et al, Tooley et al, Iwata et al, Shankaran et al and Gunn et al had small sample sizes. Furthermore, these studies used general anaesthesia during experiments. Although anaesthesia is known to blunt thermoregulatory responses,[55, 106] anaesthesia is not used during neonatal therapeutic hypothermia. Therefore caution is required with interpreting their results when considering the human neonate.

Liu et al, Tooley et al, Iwata et al, Shankaran et al and Gunn et al showed that with a cool scalp, superficial brain was cooler than deep brain, demonstrating that a thermal gradient normally exits within the animal brain. However, Liu et al showed that extra scalp warming occurred under three conditions: (i) IR absorption from radiant heater; (ii) the scalp contacting a warm heater/cooler blanket; (iii) covering the head with a cotton hat.

Liu et al demonstrated that a warm scalp reduced heat loss from superficial brain while a very warm scalp (warmer than deep brain) warmed superficial brain above that of deep brain.[104] This clearly shows that heat flow between deep brain, superficial brain, skull and scalp is bidirectional and based on a thermal gradient.

Conclusion of temperature comparison between deep and superficial brain in animals

Liu et al, Tooley et al, Iwata et al, Shankaran et al and Gunn et al showed that a thermal gradient normally exists between deep brain, superficial and the scalp. When deep brain was warmer than superficial brain, heat flowed away from deep brain. However, when the scalp was warmer than deep brain, heat flowed from the scalp to superficial brain to deep brain, resulting with an increased deep brain temperature.

Despite the paucity of data, the above observations of intracerebral temperatures with neonatal animals when used with caution are likely to be translatable to intracerebral temperatures of the human newborn.

To gain further insight, studies of intracerebral temperatures in human adults is reviewed next.

2.4.2 Temperature difference: deep brain and superficial brain in humans

Studies showing temperature differences within the brain in human adults are shown in Table 2.3. Where similar regions within the brain were reported, they have been grouped sequentially. One study showing temperature differences within the brain in two human neonates is shown in Table 2.4. The tables detail where brain temperature was measured, temperature difference between the sites (site offset), methodology, study limitations and summary comments.

Author	Deep Brain Reference Site	Other Brain Site	Method	Site Offset from ref (°C)	Limitations/Comments
Schwab et al 1997[28]	Parenchyma Next to frontal horn of lateral ventricle in normal hemisphere	Parenchyma. Next to frontal horn of lateral ventricle in infarcted hemisphere	Adults with severe ischaemic stroke in Neuro-ICU. 15 min recordings, sensors within 0.1°C. Room temperature was 18 to 20°C	+0.6°C at 1hr -0.9°C > 6hr (large variability)	Mean age was 45yrs. N =7, small sample size. Infarcted hemisphere was at first warmer than normal hemisphere. May be due to local hypo-perfusion (less heat loss from blood circulation). This temp then reduced linearly for 7 hrs after onset of symptoms, possibly due to less functional brain tissue. After 4hrs, this temp was lower than the normal hemisphere and remained steady and low from 7hrs onwards. This suggests that results measured in infarcted tissue are likely to be unreliable due to temporal variation.
Stone et al 1995[36]	Parenchyma, 4 cm insertion depth after rewarming (normothermia)	Parenchyma, 3 cm insertion depth after rewarming (normothermia)	Anaesthetised & ventilated adults during cerebral aneurysm surgery. 1 min recordings, sensors within 0.1°C	-0.1°C	Mean age is unknown. Caution is required as N =4. While no evidence of thermal steady state, oesophageal, tympanic, and operating room temps remained constant during experiments. Results show little difference between 3 and 4 cm depth in parenchyma even though during open craniotomy where brain surface was exposed to room temperature (18 to 21°C).
Fountas et al 2004[107]	Parenchyma, most compromised side approx. 5 cm insertion depth during normothermia	Parenchyma, most compromised side approx. 3 cm insertion depth during normothermia	Traumatic brain injured (TBI) adults in Neuro ICU with sedation requiring ICP mon via burr hole. For stability, meas after 90 min with no change in cerebral perfusion or ICP. Sensor accuracy unknown.	+0.05°C (warmer)	Mean age was 41 yrs. N =61, good sample size. Although the authors took care to measure temp 90 min after positioning probe, there was no evidence of thermal steady state This site was slightly warmer than deeper tissue. This may be due to compromised blood flow coupled with compromised local tissue metabolism. Caution is required with measurements in compromised tissue.

Table 2.3 Temperature between deep and superficial brain in human adults detailing methods and limitations

Author	Brain Reference Site	Other Brain Site	Method	Site Offset from ref (°C)	Limitations/Comments
Stone et al 1995[36] (as above)	Parenchyma, 4 cm insertion depth after rewarming (normothermia)	Parenchyma, 2 cm insertion depth after rewarming (normothermia)	Anaesthetised & ventilated adults during cerebral aneurysm surgery. 1 min recordings, sensors within 0.1°C	-0.9°C	Mean age is unknown. Caution is required as N =4. While no evidence of thermal steady state, oesophageal, tympanic, and operating room temps remained constant during experiments. Results show a lower tissue temperature at this depth during open craniotomy. The brain surface was exposed to room temperature (18 to 21°C) and seems to have influenced tissue temperature at 2 cm beneath the brain surface.
Fountas et al 2004[107] (as above)	Parenchyma, most compromised side approx. 5 cm insertion depth during normothermia	Parenchyma, most compromised side approx. 2 cm insertion depth during normothermia	Sedated TBI adults in Neuro ICU requiring ICP monitoring via burr hole. For stability, meas after 90 min with no change in cerebral perfusion or ICP. Sensor accuracy unknown.	+0.04°C (warmer)	Mean age was 41 yrs. N =61, good sample size. Although the authors took care to measure temp 90 min after positioning probe, there was no evidence of thermal steady state This site was unexpectedly slightly warmer than deeper tissue, which may be due to compromised blood flow coupled with compromised local tissue metabolism. Caution is required with measurements in compromised tissue.
Stone et al 1995[36] (as above)	Parenchyma, 4 cm insertion depth after rewarming (normothermia)	Parenchyma, 1 cm insertion depth after rewarming (normothermia)	Anaesthetised & ventilated adults during cerebral aneurysm surgery. 1 min recordings, sensors within 0.1°C	-3.5°C (large difference)	Mean age is unknown. Caution is required as N =4. While no evidence of thermal steady state, oesophageal, tympanic, and operating room temps remained constant during experiments. Results show a large reduction in parenchymal temperature during open craniotomy, as the brain surface was exposed to room temperature (18 to 21°C). Suggests that the skull and scalp provide a level of thermal insulation for the outer cortex (1 cm beneath the brain surface).

Table 2.3 continued

Author	Brain Reference Site	Other Brain Site	Method	Site Offset from ref (°C)	Limitations/Comments
Fountas et al 2004[107] (as above)	Parenchyma, most compromised side approx. 5 cm insertion depth during normothermia	Parenchyma, most compromised side approx. 1 cm insertion depth during normothermia	Sedated TBI adults in Neuro ICU requiring ICP monitoring via burr hole. For stability, measured after 90 min with no change in cerebral perfusion or ICP. Sensor accuracy unknown.	-0.31°C	Mean age was 41 yrs. N =61, good sample size. Although the authors took care to measure temp 90 min after positioning probe, there was no evidence of thermal steady state. The results show the temperature suddenly reduced at this depth (approaching brain surface), suggesting some heat loss from the outer cortex through the skull and scalp to the environment. Room temperature unknown. Caution is required with measurements in compromised tissue.
Schwab et al 1997[28] (as above)	Parenchyma, next to frontal horn of lateral ventricle in infarcted hemisphere	Epidural, near the base of the skull	Adults with severe ischaemic stroke in Neuro-ICU. 15 min recordings, sensors within 0.1°C.	-1°C	Mean age was 45yrs. N =15, reasonable sample size. Graphically a steady temp offset over 3.5 hrs. Room temp was 18 to 20°C. Caution is required as temperature reference was measured in infarcted tissue.
Mellergard 1994[25]	Lateral Ventricle Frontal horn of right hemisphere.	Epidural 1 to 2 cm lateral to a covered burr hole	Sedated adults in Neuro ICU requiring ICP monitoring. Measurement frequency unknown. Bandage covered head. Sensors within 0.1°C	-0.55°C	Mean age is unknown. N =10, slightly small sample size. Results show that deeper brain is warmer. Compared to Schwab et al above, head bandages may have reduced heat loss. Therefore epidural temperature may have been slightly warmer. Thermal steady state is unknown.

Table 2.3 continued

Author	Brain Reference Site	Other Brain Site	Method	Site Offset from ref (°C)	Limitations/Comments
Simbruner et al 1994[92]	Centre of 4th ventricle near brain core (approx. 4 cm below cortex surface).	3 cm from core (approx. 1 cm below cortex surface).	Thermo-neutral neonates in NICU having ventricular drainage. Incubator temperature not stated. Sedation unknown. Sensors within 0.1°C	-0.3 to -0.4°C	Caution is required with results as N=2. These neonates had severe post-haemorrhagic hydrocephalus and were treated by external ventricular drainage. Measurement frequency unknown. No therapy during measurements. Reportedly during thermal steady state, but no evidence. Results clearly demonstrate that deep brain is warmer than the outer cortex, suggesting some heat was lost through the skull to the environment.

Table 2.4 Temperature between deep and superficial brain in human neonates detailing methods and limitations

Discussion of temperatures between deep brain and superficial brain in humans

Brain temperature is a generic term and could relate to temperature in one of the brain ventricles, within brain tissue (parenchyma) or on the brain surface (epidural). However, all authors in Tables 2.2, 2.3 and 2.4 showed a thermal gradient from deep brain to brain surface when the scalp was not heated. Fountas et al concurrently measured temperatures in the lateral ventricle of one hemisphere and in the lateral ventricle of the opposite hemisphere.[107] However they did not report their findings if there was a temperature differential, only a statement that there exists no statistically significant differences between intra-ventricular and intra-parenchymal temperature, as they slowly withdrew one of the probes from one of the ventricles.[107] All authors in Tables 2.3 and 2.4 demonstrated a thermal gradient within the brain such that the outer superficial brain was cooler than deeper brain.[25, 28, 36, 92, 107] Mellergard et al and Schwab et al measured brain surface temperature and showed a pronounced reduction in temperature compared to deeper brain.[25, 28]

Within the brain, CSF flows through the lateral ventricles[108] and has a thermal conductivity similar to water.[109] Therefore CSF flow would help equilibrate temperature within the immediate area of the lateral ventricles,[107] including the adjacent basal ganglia. Consequently the preferred site for global cerebral temperature measurement is within the lateral ventricle with the least abnormality.

While Simbruner et al was the only study that directly measured temperature within the brain of human neonates,[92] data from adult human studies suggest that lateral ventricular temperature is the most stable and representative of global cerebral temperature. Clinically then, this is the most logical site to measure brain temperature because it is warmer and the thermal conductivity of CSF flow from the immediate area equilibrates temperature within the relatively large ventricle.

2.5 CONCLUSION OF INTRACRANIAL TEMPERATURE MEASUREMENT

Brain temperature is a dynamic process modulated by thermal inputs, heat production and thermal outputs. The temperature of arterial blood feeding the brain is a thermal input. This is the net result of heat absorption and heat loss from the body as well as heat generation within the body.

The amount of heat generated from cerebral metabolism adds to the temperature of arterial blood feeding the brain and the rate of cerebral blood flow through regions of the brain also influences local temperature. The temperature differential between deep brain and the scalp surface influences the amount of heat loss through the skull and scalp and results in a thermal gradient from deep brain to the brain surface. Heat loss from the brain also depends on a thermal gradient between the venous blood leaving the brain and the temperature of blood in the heart. Each of these factors either independently or in combination play a role in determining brain temperature.

There are four common areas within the brain where temperature can be measured: (i) deep brain (parenchyma); (ii) deep brain (lateral ventricle); (iii) shallow brain (parenchyma); (iv) brain surface (epidural). The data suggest that lateral ventricular temperature in the least damaged hemisphere is the most stable and representative of global cerebral temperature. Therefore, this is the most logical site to measure brain temperature clinically.

The next section reviews comparison between brain and body temperature. However, only studies referencing deep brain have been included in this review.

2.6 BODY TEMPERATURE REFLECTING BRAIN TEMPERATURE

During neonatal therapeutic hypothermia, direct measurement of brain temperature is not clinically feasible due to the following issues cited with brain temperature monitoring in adult intensive care: difficulty with access, risk of brain damage, risk of infection and special clinical care management.[110-113] Therefore during therapeutic hypothermia, a representative measure of brain temperature is required to direct the depth of cerebral cooling. The following section discusses various body sites used to

measure temperature with a specific focus on how they relate to brain temperature and where in the brain the temperature was measured.

Temperature comparisons between brain and less invasive body sites have occurred during normothermia, selective head cooling and whole body cooling. As superficial brain is substantially influenced by scalp temperature, then selective head cooling studies that reported deep brain temperature have been included in the following tables. Likewise, studies that involved whole body cooling and reported deep brain temperature have also been included.

2.6.1 Animal studies of body temperature reflecting brain temperature

Historically, neonatal research into therapeutic hypothermia that compared deep brain temperature to a body site commenced with trials involving neonatal piglets and foetal sheep. These studies are reviewed in Table 2.5.

Animal (Author)	Deep Brain Reference Site	Other Body Site	Method	Site Offset from ref (°C)	Limitations/Comments
Piglet. (Liu et al, 2011)[101]	Parenchyma, 22 mm below surface via burr hole	Rectum 6 cm insertion	Normothermia (T_{rec}) Radiant heater on, no hat Gen anaesthesia. 1 min measurements. 0.1°C sensor accuracy.	-0.8°C	N =6, small sample size. Anaesthesia: N_2O (66%) and Isoflurane (1-2%), without heated humidification. Room temperature and steady state unknown. Radiant heater on and no hat suggests: warm scalp, therefore low or no heat loss from superficial brain; radiant heat may warm exposed skin and influence i.e. warm T_{rec}. No comparative results during cooling.
HIE Piglet. (Tooley et al, 2005)[105]	Parenchyma, 20 mm below surface via burr hole	Rectum 6 cm insertion	Normothermia (T_{rec}), radiant heater on, IR shield above head. Gen anaes. 1 min meas. 0.1°C sensor accuracy	-0.2°C (close)	N =10. Without heated humidification. Room temp unknown. Baseline conditions. IR shield above head. Deep brain temp was 39.3°C. Data suggest warming of exposed skin which may warm T_{rec}. Similar result to Liu et al 2011 above.
As above	as above	as above	Selective head cooling (SHC), cool cap 16°C with radiant heater on, Mild whole body cooling. (same remaining methods)	+4.4°C (warmer)	As above except N=8. IR shield above head. Deep brain temp was 30.4°C, suggesting that blood temperature perfusing deep brain may be slightly below deep brain temp. Therefore T_{rec} does not represent systemic or deep brain temperature.
Piglet. (Iwata et al in 2003)[104]	Parenchyma, 20 mm below surface via burr hole	Rectum 6 cm insertion	SHC 10°C cap water Radiant heater on. Gen anaes. 5 min meas. 0.1°C sensor accuracy.	+2.7°C to +3.2°C (warmer)	N =6. Without heated humidification and room temp 20°C. During thermal steady state (<0.1°C for 20 min). Deep brain temp was 35.8°C. Suggests that blood temperature perfusing deep brain may be slightly below deep brain temp. Therefore T_{rec} does not represent systemic or deep brain temperature

Table 2.5 Temperature between deep brain and a core body site in animals detailing methods and limitations

Animal. Author	Deep Brain Reference Site	Other Body Site	Methods	Site Offset from ref (°C)	Limitations/Comments
Piglet. (Thoresen et al 1996)[17]	Parenchyma, 20 mm below surface via sealed burr hole	Rectum 6 cm insertion	SHC cap water temp 10°C + radiant heating. Positioned prone. Gen anaes. 1 min meas ave. Sensor accuracy 0.1°C.	+5.3°C	N =4. Caution, small sample size. Reportedly stable conditions. Rectum was warmer than deep brain due to selective head cooling. T_{rec} did not reflect deep brain temperature during SHC.
As above	as above	as above	Normothermia (after rewarming) + radiant heating. (Same remaining methods)	+4.3°C	As above. Discrepancy between deep brain and rectal temperature was greater during SHC. T_{rec} did not reflect deep brain temperature after rewarming from SHC, suggesting lag time with T_{rec}.
As above	as above	as above	as above	+4.5°C	As above. Meas recommenced 1hr after a hypoxic insult (HI). Deep brain was 0.2°C cooler after HI during SHC. This may reflect a transient period of early post-HI hyper-perfusion. T_{rec} did not reflect deep brain temperature after SHC.
As above	as above	as above	SHC cap water temp 10°C + radiant heating. (Same remaining methods)	+3°C	As above. SHC recommenced 1hr after a hypoxic insult (HI). Deep brain was 2.3°C warmer after HI during SHC. This may reflect a transient period of early post-HI hypo-perfusion. T_{rec} did not reflect deep brain temperature after SHC.
HIE Piglet. (Shankaran et al 2002)[13]	Parenchyma, 20 mm below surface via sealed burr hole	Oesophagus (lower)	Whole body cooling (WBC) using heater/cooler blanket servo T_{oes} to 33.5°C. Gen anaes. Meas rate unknown. 0.2°C sensor accuracy.	-0.1°C (close)	Caution as N =3. Without heated humidification. Room temp unknown. A graphic shows ongoing oscillation of ±0.9°C in deep brain temp therefore not thermal steady state. Required a second adult size blanket to reduce magnitude of water temp oscillation. Authors reported that T_{oes} was 'a good marker of deep brain temperature' during WBC.
HIE foetal sheep. (Gunn et al 1997)[18]	Parenchyma, 25 mm below surface via sealed burr hole	Oesophagus (lower)	SHC to 32°C (extradural) In utero, T_{oes} 38.4°C. No anaes. 1 min meas ave. Sensor accuracy unknown.	+0.7°C	N =1. Caution, very small sample size and performed in utero Brown adipose tissue inhibited. Reported stable temps, accuracy unknown. T_{oes} core temp. Oes was warmer than deep brain due to selective head cooling. T_{oes} shows some indication of deep brain temperature during SHC.

Table 2.5 continued

Discussion of animal studies of body temperature reflecting brain temperature

While animal studies offer important information, caution is required with interpreting their results. Extrapolation to the human neonate may not be valid because the physiology of thermoregulation of piglets is different to that of humans.[68, 75] Unlike humans, piglets have a carotid rete to help cool their brain with nasal airflow.[21, 114, 115] The carotid rete is a form of heat exchanger where venous blood from the nose and face cools arterial blood flowing to the brain. Therefore the depth of brain cooling achieved with experimental cooling methods with piglets may be different to human neonates. Greater cooling of facial venous return in piglets can exaggerate the extent of brain cooling.

Another limitation with piglet experiments was that the subjects had general anaesthesia and intubation to ventilate the lungs. However, unlike ventilated human neonates, heated humidification was not used and this may have reduced the temperature of the blood exiting the lungs and the arterial blood temperature perfusing the brain.

Unlike the other studies, Gunn et al studied selective head cooling with un-anaesthetised near term foetal lambs.[18, 20] Lambs have BAT to generate heat when cold and so their thermoregulatory mechanisms might be a closer animal model to human neonates than piglets. However BAT activation is inhibited by adenosine and prostaglandin E2 which are produced in the placenta of the sheep.[116]

When cool, human neonates lack an ability to shiver intensely to generate heat and instead use BAT to heat blood returning to the central circulation.[38] However piglets do not have BAT,[41, 117] and rely on shivering to augment body temperature during hypothermia which may be abolished or blunted by general anaesthesia.[55, 106] Therefore comparison of T_{brain} to other T_{core} sites documented in animal studies may be misleading for neonatal rescue hypothermia.[21]

Although experiments that compared T_{rec} to deep brain temperature before cooling showed similarity, during selective head cooling and after rewarming there were large discrepancies. Experiments that compared T_{oes} to deep brain temperature during whole body cooling and after rewarming showed similar values. This suggests that lower T_{oes} may better reflect deep brain temperature in HIE animals during whole body cooling.

2.6.2 Conclusion of animal studies that compared deep brain temperature to a core body site

The studies in Table 2.5 showed that T_{rec} was similar to deep brain temperature before cooling despite the results showing that during selective head cooling, T_{rec} did not reflect deep brain temperature. On the other hand, T_{oes} showed similarity to deep brain temperature during whole body cooling. The data suggest that T_{oes} may approximate deep brain temperature during whole body cooling and that T_{rec} did not approximate brain temperature during selective head cooling.

For the majority of animal experiments, the scalp was cooler than superficial brain. This supports the general concept that one path of brain heat loss occurs when heat flows from the warmer deep brain to superficial brain and the other through the skull and scalp to the environment. Heat loss through the scalp is likely to be applicable to human neonates because wearing a hat may trap heat over the scalp. Furthermore, if a heating/cooling blanket is in contact with the scalp, then scalp temperature is likely to be influenced as well. These assertions are supported by Liu et al's animal studies that showed that deep and superficial brain increased in temperature when a hat was worn or when a heating blanket contacted the scalp.[101]

2.6.3 Human studies of body temperature reflecting brain temperature

The limitations observed with animal studies have directed this review to include human studies that compared brain temperature to other core body sites as potential measures of brain temperature. While there are many studies that have compared two core body sites other than brain, this thesis limits the review to comparisons between deep brain temperature and a core

body site. As there are limited publications on this topic with neonates and children, studies on adults have been included.

The following tables from adults (Table 2.6) and children (Table 2.7) show temperature measurements from either intra-ventricular or deep parenchyma as a global indication of deep brain temperature. Superficial or extradural temperatures are not included. Comparisons were made to another core body site showing the methods, temperature offset and limitations. Data comparing T_{oes} to an indirect measure of global brain temperature in the human neonate (from averaging four forehead ZHF sensors) are shown in Table 2.8.

Human Adults	Brain Reference Site	Body Site	Method	Mean Offset from ref (°C)	Limitations/Comments
Schwab, Spranger et al. 1997[28]	Parenchyma Next to frontal horn of the lateral ventricle, in infarcted white matter	Jugular Bulb	Adult stroke patients in Neuro-ICU. 15 min recordings, sensors within 0.1°C.	-1°C (cooler)	Mean age was 45yrs. N =15. Graphically, some variation (±0.5°C), therefore not ideal steady state. Reference in infarcted brain, therefore unreliable results.
Verlooy, Heytens et al 1995[27]	Right lateral brain ventricle (frontal horn)	Internal Jugular Vein (not bulb)	Sedated brain injured adults in Neuro ICU. 15 min recordings, sensors within 0.1°C.	-0.3°C	Mean age was 37yrs. Caution required as N =6. Graphically a steady offset during IV Paracetamol (1g). Graphically followed T_{brain} closer than $T_{bladder}$.
Rumana et al 1998[31]	Parenchyma. 1 to 2 cm deep. 3 cm lateral to the midline & 1 cm anterior of coronal suture	As above	Sedated head injured adults in Neuro ICU. 60 min between results. Sensors within 0.1°C.	-1.2°C	Mean age was 31yrs. N =14. Graphically approximately 1°C regular variation, therefore not ideal steady state. Large offset. Authors state 'jugular vein temperature will not substitute for brain temp'.
Stone et al 1995[36]	Parenchyma. 3 cm insertion depth.	Pulmonary Artery (PA)	During deep hypothermia. Anaes + vent adults during neurosurg + CPB. 1 min recordings, sensors within 0.1°C.	+0.4°C (warmer)	Mean age =unknown. N =27. PA warmer than brain, may be due to the CPB unit returning warm, oxygenated blood back to the circulation. Authors recommend PA plus two other sites for brain temp proxy.
As above	As above	As above	At end of rewarming. (same remaining methods)	+0.9°C	As above. At end of rewarming PA shows similarity to brain temp. Authors recommend PA plus two other sites for brain temp proxy.

Table 2.6 Temperature between deep brain and a core body site in human adults detailing methods and limitations

Human Adults	Brain Reference Site	Body Site	Method	Mean Offset from ref (°C)	Limitations/Comments
Stone et al 1995[36] (as above)	Parenchyma 3 cm insertion depth.	Lower Oesophagus (Oes)	During deep hypothermia. Anaes + vent adults during neurosurg + CPB. 1 min recordings, sensors within 0.1°C.	+0.6°C	As above. T_{oes} warmer than brain, possibly due to the CPB unit returning warm, oxygenated blood back to the circulation.
As above	As above	As above	At end of rewarming. (same remaining above methods)	-0.1°C	Mean age unknown. N =27. The most steady period was after rewarming. T_{oes} very close to brain temperature. Authors recommend this site + PA + NP (all 3) for brain temp proxy.
Whitby and Dunkin 1971[37]	Parenchyma 4 cm below the surface of the cortex.	Lower oesophagus. 24 cm below corniculate cartilage	Anaesthetised and ventilated adults during normothermic surgery (crainiotomy). Rate of measurement and sensor accuracy is unknown.	-0.25°C	Mean age is unknown. N =70. Thermal SS is unknown. T_{oes} similar to brain temperature. Authors commented that T_{oes} will give a sufficiently accurate estimation of brain temperature for most clinical purposes.
As above	As above	Nasopharynx (NP)	As above	-0.35°C	Mean age is unknown. N =40. Thermal SS is unknown. Authors commented that T_{NP} gave a less accurate and less reliable estimation of brain temperature than T_{oes}.

Table 2.6 continued

Human Adults	Brain Reference Site	Body Site	Method	Mean Offset from ref (°C)	Limitations/Comments
Stone et al 1995[36] (as above)	Parenchyma 3 cm insertion depth. (At end of rewarming)	Nasopharynx (NP)	During deep hypothermia. Anaes + vent adults during neurosurg + CPB. 1 min recordings, sensors within 0.1°C.	+0.6°C	Mean age =unknown. N =27. This site is warmer than brain
As above	As above	As above	At end of rewarming. (same remaining above methods)	0.0°C (same)	Mean age =unknown. N =27. Warmer than brain due to some lag time. The most steady period was after rewarming. Authors recommend this site + PA + Oes for brain temp proxy.
As above	As above	Tympanum	During deep hypothermia. (same remaining above methods)	+1.7°C	Mean age =unknown. N =27. This site is much warmer than brain.
As above	As above	As above	At end of rewarming. (same remaining above methods)	-0.3°C	Mean age =unknown. N =27. Measurements were reported during cooling and rewarming. Graphic showed large temperature differences due to lag time. The most steady period was after rewarming. Cooler than brain due to some lag time.

Table 2.6 continued

Human Adults	Brain Reference Site	Body Site	Method	Mean Offset from ref (°C)	Limitations/Comments
Stone et al 1995[36] (as above)	Parenchyma 3 cm insertion depth. (At end of rewarming)	Urinary Bladder	During deep hypothermia. Anaes + vent adults during neurosurg + CPB. 1 min meas, sensors within 0.1°C	+3.5°C	Mean age =unknown. N =27. Much warmer than brain, may be due lag time during cooling. Authors did not recommend bladder temperature as a proxy for brain temperature.
As above	As above	As above	At end of rewarming. (same remaining above methods)	-1.3°C	Mean age =unknown. N =27. Much cooler than brain, may be due lag time during rewarming. Authors do not recommend this site for proxy brain temperature indication.
Verlooy, Heytens et al 1995[27] (as above)	Right lateral brain ventricle (frontal horn)	As above	Sedated brain injured adults in Neuro ICU. 15 min recordings, sensors within 0.1°C.	-0.5°C	Mean age was 37yrs. Caution required as N =6. Graphically a steady offset during IV Paracetamol (1g). Authors commented that $T_{bladder}$ was more reliable than T_{rec} if brain temperature was not available.
Schwab, Spranger et al. 1997[28] (as above)	Parenchyma Next to frontal horn of the lateral ventricle, in infarcted white matter	As above	Adult stroke patients in Neuro-ICU. 15 min recordings, sensors within 0.1°C	-1.5°C	Mean age was 45yrs. N =15. Graphically a steady yet large offset. Good method except that reference in infarcted brain, therefore results may be unreliable.
As above	Brain ventricle in infarcted hemisphere	As above	As above	-1.9°C	Caution required as N =5. Large offset. No graphic, therefore steady state not known. Reference in ventricle of infarcted hemisphere, therefore results may be unreliable.

Table 2.6 continued

Human Adults	Brain Reference Site	Body Site	Method	Mean Offset from ref (°C)	Limitations/Comments
Mellergard 1994[25]	Right lateral brain ventricle (frontal horn)	Rectum. 3 to 4 cm insertion depth	Normothermic sedated adults in Neuro ICU. Measurement frequency is unknown. Sensors within 0.1°C	-0.33°C	Mean age is unknown. N =27. Small offset during normothermia. Thermal steady state unknown.
Stone et al 1995[36] (as above)	Parenchyma 3 cm insertion depth.	Rectum Insertion depth unknown	During deep hypothermia. Anaes + vent adults during neurosurg + CPB. 1 min meas, sensors within 0.1°C	+7.3°C	Mean age is unknown. N =27. Very large offset. T_{rec} appeared slow to respond during induction of deep hypothermia.
As above	As above	As above	At end of rewarming. (same remaining above methods)	-3.3°C	Mean age is unknown. N =27. Very large offset (T_{rec} was much cooler), probably due to long lag time after rewarming. Approaching steady state, as the most steady period was after rewarming.
Soukup et al 2002[34]	Parenchyma At a depth of 2 to 2.5 cm. During spontaneous hypothermia	As above	Sedated TBI adults in Neuro ICU. 30 minute temp ave from 3s sample rate. Sensor accuracy unknown.	Normothermic 0.0°C Spontaneous hypothermic +0.8°C (warmer)	Mean age unknown. Caution required as N was unstated. Some measurements were pre surgery and some were post-surgery, therefore unreliable. Authors do not recommend T_{rec} for therapeutic hypothermia due to large variability.
Fountas et al 2004[107]	Lateral Ventricle	As above	Sedated adults in Neuro ICU. During stable clinical conditions. Measurement rate unknown. Sensor accuracy unknown.	-0.19°C	Mean age was 41 yrs. N=61. Good sample size. Although measurements occurred during stable clinical conditions, a graphic only showed results every 90 min. for 7.5 hrs. Thermal steady state (SS) not known.

Table 2.6 continued

Human Children	Brain Reference Site	Body Site	Method	Mean Offset from ref (°C)	Limitations/Comments
Whitby and Dunkin 1971[37]	Parenchyma 4 cm below the surface of the cortex.	Lower oesophagus	Anaesthetised and ventilated children during normothermic surgery (crainiotomy). Rate of measurement and sensor accuracy unknown.	-0.2°C	Caution required as N =5. A single reading was measured from each child. Thermal steady state unknown. Mean age unknown.

Table 2.7 Temperature between deep brain and the lower oesophagus in children

Human Neonates	Brain Reference Site	Body Site	Method	Mean Offset from ref (°C)	Limitations/Comments
Simbruner et al. 1994[92]	Average reading of 4 heat flux forehead sensors. Note: Approximate of core brain temperature.	Lower Oesophagus	Healthy neonates naked in NICU incubator air at 31.1°C (thermo-neutral). Sedation unknown. Sensors within 0.1°C	-0.72°C	Caution required as N=7. GA=38.3 wks, BW=2929g, PNA=4.1 days. No therapy during measurements. Frequency of measurement unknown but during thermal SS. Results during normal thermogenesis.
As above	As above	As above	HIE neonates (same remaining methods)	-0.16°C	As above. Results suggest reduced brain metabolism decreasing brain temperature, or increased body thermogenesis triggered by damaged brain.

Table 2.8 Temperature between deep brain and the lower oesophagus in neonates

2.6.3.1 Discussion of body site suitability as a measure of global brain temperature in adults

While many body sites may approximate deep brain temperature during normothermia and in thermal steady state, comparisons made during therapeutic hypothermia are the most relevant and showed the greatest discrepancy. Caution is required in interpreting results from Schwab et al as brain temperature was measured in infarcted tissue which probably compromised perfusion and altered local brain temperature. Caution is also required if the measurements were not made during thermal steady state, as different response times at each site would lead to temperature variability. Several core body sites were compared to deep brain temperature and are discussed below.

Jugular Vein/Bulb

Brain metabolism adds heat to cerebral perfusion and the venous blood returns via the internal jugular vein. Verlooy et al showed that the internal jugular vein was 0.3°C cooler that deep brain.[27] However, cool venous return from the scalp and face also enters the internal jugular vein just below the jugular bulb.[118] Therefore measuring temperature in the jugular bulb seems a logical site to approximate deep brain temperature. However, this is an invasive method and difficult to apply to the neonate.

Pulmonary artery (PA)

PA blood temperature is often regarded as the 'gold standard' of systemic body temperature.[119] During thermal steady state, PA temperature is likely to reflect a temperature slightly less than deep brain temperature and can be used as a measure of deep brain temperature. However as this is an invasive method, it is not commonly used in NICU.

Lower Oesophagus

T_{oes} in children and neonates showed similarity with brain temperature.[37, 92] T_{oes} is reported to reflect aortic blood temperature.[74, 120] As a large proportion of aortic blood flow perfuses deep brain, T_{oes} is likely to reflect a temperature that is slightly below deep brain temperature and is a possible measure for

deep brain temperature.[36] This method is only minimally invasive and access is possible through the nose or mouth. Therefore it ought to be considered for further investigation.

Nasopharynx and Tympanum

The posterior wall of the nasopharynx and tympanum and are in close physical proximity with superficial brain. As indicated in Table 2.2, deep and superficial brain were influenced by scalp temperature and Zhu et al mathematically determined that the reduction in brain temperature from deep brain approaching the brain surface was exponential.[121] Therefore a temperature measurement site in superficial brain or brain surface is likely to be influenced by environmental conditions. However, there are limitations as nasopharyngeal temperatures are only suitable with intubated subjects and some HIE neonates who require cooling may not be intubated.

During thermal steady state, achieving reliable thermal contact with a temperature probe in the nasopharynx or next to the tympanum presents a challenge. While Stone et al commented that nasopharyngeal temperature usually approximates brain temperature and placing a temperature sensor in the nasopharynx is possible, Stone et al pointed out that temperatures vary between different probe positions,[36] making this site unsuitable for reliability.

Stone et al commented that tympanic temperature was not recommended as a measure of brain temperature during cooling due to the very large discrepancy between deep brain temperature during hypothermia (Table 2.6).[36] To monitor tympanic temperature for 72 hours, a temperature probe needs to constantly rest gently against the tympanum while the external ear canal is thermally insulated from environmental temperature. This suggests that probe positioning is critical especially when the subject's head moves and there is also a risk of damage to the tympanum from the temperature probe. Furthermore, considering that the middle ear is separated from the brain by a layer of skull bone, thermal approximation of brain temperature is likely to reflect brain surface temperature at best, not deep brain temperature.

Bladder

Stone et al showed that bladder temperature varied greatly in adults and the authors did not recommend using the bladder as a measure of brain temperature during cooling.[36] Although bladder temperature is a convenient measure of systemic temperature due to high kidney perfusion, it requires a steady or frequent urine flow to reflect systemic temperature as the bladder itself is not highly perfused with arterial blood. Therefore measuring bladder temperature in cooled HIE neonates may not be reliable.

Rectum

Table 2.6 showed that several authors compared rectal temperature to deep brain temperature.[25, 27, 31, 34, 36, 107] The majority of authors showed a pronounced variability in the temperature differences and some authors recommended against using rectal temperature as an indicator of brain temperature particularly during cooling.[27, 34, 36]

Rectal temperature has other limitations for monitoring that are relevant to NICU. In 1948, Bazzet et al inserted plastic covered thermocouples into arteries and veins in the limbs of human adults, simultaneously measuring rectal temperature (T_{rec}) at 15 cm insertion distance.[69] The authors determined that there was no uniform body temperature and concluded that rectal, brachial artery and common iliac blood temperatures differ from one another and vary with different degrees of lag time.

In 1949, Mead and Bonmarito measured rectal temperature at different insertion depths in human adults.[59] The authors constructed a multi sensor probe that measured temperature at 3, 4, 5, 6 and 8 inches insertion depth. They reported simultaneous temperature variations of 0.05 to 0.83°C at different depths and the variations were greatest in individuals whose body temperatures were decreasing. Mead and Bonmarito concluded that cooled blood from the surface of the body passing through veins in the pelvic wall adjacent to the terminal portion of the rectal probe were responsible for the deviations observed.[59]

Although these authors studied temperature in adults over 60 years ago, their

observations have not been heeded by neonatal intensive care clinical practice, as rectal temperature monitoring remains widespread.

In 2004, Maxton et al[52] compared pulmonary artery, bladder, nasopharyngeal, axillary, tympanic and rectal temperature in neonates (mean age 18 days) for approximately 6 hours postoperatively after cardiac surgery. Although Maxton et al supported pulmonary artery temperature monitoring, they suggested cessation of rectal temperature monitoring after cardiac surgery due to significant lag time during the initial postoperative period.[52]

Tabbutt et al in 2006 investigated 100 infants (mean age 128 days) undergoing cardiac surgery with cardiopulmonary bypass.[72] The authors compared right sided intra-atrial blood temperature and T_{rec}.[72] They concluded that T_{rec} underestimated central temperature significantly in the first 80 hours after cardiac surgery with cardio pulmonary bypass.[72] The authors highlighted the clinical importance of measuring central temperature accurately as a strategy to avoid neurological harm from pyrexia.

Hoque et al reported core temperature undershoot during four different methods of inducing therapeutic hypothermia in HIE neonates using T_{rec} to target the depth of cooling.[65] This may have arisen from T_{rec} responding slowly.

2.7 CONCLUSION OF BODY TEMPERATURE REFLECTING BRAIN TEMPERATURE

The majority of studies reported that during the initiation of cooling and with rewarming, rectal temperature was unreliable as a measure of brain temperature. This relates to the slower response time of rectum compared to other core sites after a step change in environmental temperature. During therapeutic hypothermia frequent changes in environmental temperatures are the norm suggesting that rectum may be a poor choice of monitoring site. These observations may cause larger temperature swings in the brain as the brain is a faster responding site than rectum. In addition, the propensity for cold blood from the legs to lower rectal temperature in comparison to other core body sites may have greater implications in the neonate during

hypothermia. This arises because there is more intense limb vasoconstriction in response to cold that is notable at this age.

Considering the above limitations, monitoring temperature in the lower oesophagus seems a suitable, minimally invasive option during neonatal therapeutic hypothermia. For this reason the behaviour of T_{oes} is examined in subsequent Chapters.

From this review, the paucity of data concerning the validity of alternative measures of brain temperature in the newly born human neonate is apparent. The direct extrapolation of animal, human adult and child data to the neonate may not be valid because of differences in the thermoregulatory responses. Therefore it is important to understand how the neonate regulates body and brain temperature which is the focus of the next section of this review.

2.8 NEONATAL THERMOREGULATION. HOW DO NEWLY BORN HUMAN NEONATES REGULATE THEIR TEMPERATURE IN RESPONSE TO COLD?

2.8.1 Adaptive responses to cold

Like other mammalian species, human neonates try to maintain a stable core temperature.[38] Thermoregulation is the ability to balance heat production and heat loss in order to maintain core body temperature within a 'normal' range.[122] Temperature is tightly controlled by physiological responses to within several tenths of a degree Celsius.[80] However, a circadian temperature variation exists where the temperature set point varies by approximately 1°C in adult humans.[123]

The homoeothermic response to environmental temperature begins with the sensation of temperature.[38] Temperature is sensed by skin surface, body organs and the brain and spinal cord and this information is integrated by the hypothalamus and other central control centres.[80] Both warm and cold temperature sensing are integrated as the cold skin sensory response is inhibited by a hyperthermia core.[38] Shivering due to mild hypothermia is also inhibited by skin warming.[124]

Adaptive responses to cold are behavioural and autonomic.[80] Behavioural responses include flexion of limbs to minimise exposed skin surface area, agitation and crying to seek maternal attention. Autonomic responses are mediated via the sympathetic nervous system and include vasoconstriction, shivering and BAT thermogenesis.[38]

Vasoconstriction is the first autonomic response to cold and involves the closing of arterio-venous connections and arterioles in peripheral limbs that reduces the flow of warm blood to the skin and retains heat in the core of the body.[80] Peripheral tissue temperature is not tightly regulated and thus serves as a buffer between the well-protected core and the environment.[106] Vasoconstriction is metabolically efficient as it doesn't require additional energy consumption, in contrast to shivering or BAT thermogenesis.[80] In neonates, acrocyanosis is a common clinical observation suggesting that vasoconstriction is more intense than in adults.

With mild cold exposure, behavioural responses and skin vasoconstriction can maintain core body temperature without metabolic cost. The range of environmental temperatures over which these mechanisms can maintain normothermia is called the neutral thermal zone. However, below a critical environmental temperature, heat has to be generated at a metabolic cost. The neutral thermal zone for neonates has been defined by Hey and Katz with studies of oxygen consumption that reveal the zone varies depending on gestation and body weight.[125]

Shivering is the next physiological response to cold in adults and children with an activation threshold approximately 1°C lower than for vasoconstriction.[126] While shivering is occasionally noted in human neonates, it is not commonly observed. Non-shivering thermogenesis is the main autonomic adaptive response to cold in the human neonate.[39] Non-shivering thermogenesis is considered to occur in BAT.[39]

2.8.2 Brown adipose tissue and non-shivering thermogenesis

BAT can be characterised as a sympathetic effector organ that provides a metabolic source of non-shivering thermogenesis.[38] The anatomical presence

and foetal development of BAT in the human neonate is well defined by post-mortem dissection.[127] BAT in the human foetus is deposited after 28 weeks gestation, and principally found around the scapulae, kidneys, adrenals, neck and axillae.[122]

The visible appearance of BAT from autopsy studies is due to the high density of blood vessels within the tissue.[38] BAT differs morphologically and metabolically from ordinary white adipose tissue because BAT contains numerous mitochondria, fat vacuoles, an abundant sympathetic innervation and blood supply.[116]

There are considerable data from animal models that document the importance of BAT thermogenesis in defence of body temperature in neonatal mammals which have been the subject of comprehensive reviews.[39] However, there are less data available from the human neonate.

BAT Thermogenesis and uncoupling protein

Thermogenesis in BAT is mediated via noradrenalin (NA) released from sympathetic nerves which richly innervate BAT. NA stimulates beta 3 receptors which act to increase intracellular cAMP resulting in lipolysis and free fatty acid release.[39] Free fatty acids are the substrate for beta oxidation within the mitochondria and generation of NADH which is then transferred to the electron transport chain.[39] Heat is produced by uncoupling the proton gradient generated by the electron transport chain from adenosine triphosphate (ATP) synthesis. This is achieved by uncoupling protein 1 which is richly expressed in BAT.[128] These reactions generate heat in the term neonate which is conducted into nearby blood vessels that carry the heat into the core circulation.[38]

Evidence for BAT activity in the human neonate

Although anatomically well defined, the functional role of BAT in temperature homeostasis in the human neonate is not as well characterised as in other mammalian species.[39] Current evidence that BAT is important for thermogenesis in human neonates is derived from three sources: autopsy

data, metabolic studies and surface skin temperature measurements after cold exposure.

Depletion of BAT lipid has been noted in neonates who die after exposure to cold, in contrast to preservation in neonates who die in a warm environment or following inutero malnutrition.[129] Dawkins and Scopes showed increased oxygen consumption without shivering, and increased plasma glycerol levels following mild cold exposure.[130] Karlberg et al described increased oxygen consumption and a fall in respiratory quotient during NA infusion.[131] In the rabbit, both cold and NA produce similar metabolic changes to those seen in the human neonate, as well as an increase in the temperature of BAT.[132]

In animal models the functional role of BAT has been clarified via direct measurement of BAT temperature. However, there are no data regarding direct measurement of BAT temperature in human neonates, as such experiments have ethical constraints. Data regarding indirect measurement of BAT temperature in human neonates are limited and difficult to interpret.

Several authors have previously described warmer skin surface temperatures over areas of presumed BAT deposits in human neonates following exposure to cold.[133-135] Silverman et al studied 16 stable neonates born at 31-40 weeks gestation and of postnatal ages varying from 1-78 days, exposed to an ambient incubator temperature of 25-32°C for 1-4 hours when naked.[135] These authors observed that the temperature of the neck nape skin fell less than other skin sites, and that the colon-nape temperature gradient was relatively smaller in comparison to other skin sites.[135]

Grausz studied 9 stable neonates born at gestations of 30-40 weeks at between 1 and approximately 28 days of age during 25-60 minutes exposure to an environmental temperature of 25-30°C.[133] Inter-scapular surface skin temperature increased slightly in most cases in conjunction with a fall in rectal temperature such that inter-scapular skin temperature was higher than rectum.[133]

Rylander et al observed the nape and inter-scapular areas to be the warmest on the back in most of 43 healthy full term neonates studied at 1-10 days of

life after 30 minutes of exposure to 21-23°C.[134] In a further study of 58 healthy term gestation neonates between 0 and 6 months of age exposed to temperatures of 18-23°C for 30 minutes, Rylander et al noted that the warm nape was mainly seen in neonates more than 72 hours of age.[134]

While these temperature data are consistent with BAT thermogenesis in the human neonate, two important methodological problems limit interpretation of the data. First, the use of rectal core as a reference for inter-scapular skin temperature, and secondly, the conduct of the studies in non-steady state thermal conditions.

T_{rec} is not an appropriate core reference for inter-scapular skin during mild cooling. For example Bazzett et al showed that T_{rec} was influenced by the temperature of venous return from the legs and buttocks when skin was cooled and therefore does not accurately reflect the temperature of more central core sites.[69] An increased difference between T_{rec} and inter-scapular temperature with cooling may therefore reflect rectal cooling rather than heating of inter-scapular skin. In non-steady state, differences in regional skin temperature may reflect regional differences in vasoconstriction or heat transmission from adjacent structures.

Although BAT temperature measurement data are incomplete, autopsy, metabolic studies and animal observations indicate that BAT is likely to be the important thermogenic organ in the human neonate.[136]

Control of BAT activity

The mechanisms of control of BAT thermogenesis have received considerable attention in animal models. Peripheral and central temperature sensing is integrated within the preoptic nucleus of the hypothalamus as the central point of control by warm-sensitive GABAergic neurons.[137] Thermoregulatory output from the preoptic nucleus is tonically inhibitory, mediated by GABA, with projections to the dorso-medial hypothalamus.[137] Cold stimulus dampens inhibitory output from the preoptic nucleus.[137] Neurons then project via the rostral raphe pallidus to the spinal cord, sympathetic ganglia and then to BAT adipocytes.[137]

While BAT activity is influenced by peripheral and central thermal information, other sensory input also appears to be important. The response of BAT to cold stress in the neonate may be affected by hypoxic-ischaemic brain injury in central control regions. Hypoxaemia in rabbits results in a transient block to the normally observed increase in oxygen consumption noted following exposure to cold which is known to be due to BAT activity.[138] Neonates who have suffered asphyxia have been found to drop core temperature more rapidly than non-asphyxiated neonates, suggesting impaired temperature regulation.[139] However, there have been no studies in neonates as to how hypoxia or neurological injury specifically affects BAT activity.

Recruitment of BAT and adaptation to prolonged cold exposure

BAT is capable of recruitment and involution under differing environmental conditions.[39] While mild cold exposure in rat pups (an altricial species) is required over the first days of life to recruit BAT, it's development is inhibited by warm ambient temperature.[140] Conversely in the guinea pig (a precocial species), well developed BAT at birth involutes in the absence of cold exposure.[141] BAT recruitment has been demonstrated in adult humans as part of cold acclimation using 18 fluorodeoxyglucose positron-emission tomography (PET).[142] Chronic nor-adrenergic stimulation of BAT via sympathetic nervous system activation is considered the basis for recruitment of BAT in response to cold.[39]

However, there are few data on the effects of prolonged cold exposure, as occurs with therapeutic hypothermia, on BAT recruitment in human neonates. Hu et al have recently shown a possible reduction in supraclavicular BAT deposits post therapeutic hypothermia in HIE neonates compared to healthy control neonates using MRI imaging.[143] However, the observations in the adult human and animal studies just discussed, suggest that BAT recruitment is the more likely response in the neonate during therapeutic hypothermia. Hu et al showed no differences in the quantity of other BAT deposits in HIE neonates which raises a question as to the significance of this finding.[143]

Role of BAT in brain warming

In addition to maintaining core body temperature, activation of BAT appears to

be important in warming the brain. Animal investigations of BAT activation conducted simultaneous and direct measurement of brain, core and BAT temperatures, and showed an increase in BAT temperature ahead of the brain together with a larger temperature increase in BAT.[54, 144] The increase in brain temperature from BAT activation in animals is considered to facilitate planning and action as well as possible contribution to emotional hyperthermia.[144]

BAT is anatomically located near to the jugular venous return from the brain, and it's activation will immediately reduce convective heat loss from brain. In addition, the venous drainage of BAT is directed centrally to heat central venous blood and will have a consequent rapid effect on aortic blood temperature that perfuses deep brain. The concept of BAT as an organ that heats the brain suggests that activation of BAT during therapeutic hypothermia could contribute to neurological injury by counteracting efforts to cool the body.

Consequences of thermoregulatory defences during therapeutic hypothermia

The autonomic response to cold involves increased circulating levels of noradrenalin.[145] Shivering and non-shivering thermogenesis are metabolically stressful, resulting in increased oxygen consumption and carbon dioxide production, and consumption of glucose and fatty acids.[80] Noradrenalin causes peripheral vasoconstriction, an increase in peripheral vascular resistance, increased blood pressure and increased cardiac afterload.[80] Heart rate may fall either as a primary response to cold or as a reflex in response to raised blood pressure. While bradycardia is more common with therapeutic hypothermia, randomised controlled trials have not shown a greater risk of serious hypotension or need for inotropic support.[15]

Stress has effects on the brain that may be clinically important in the human neonate. In the rat, paralysis without anaesthesia increases brain metabolic rate and blood flow as a consequence of release of adrenaline from the adrenal gland.[146] Increased glucocorticoid levels arising from activation of the hypothalamic-pituitary adrenal axis, impairs cerebral glucose uptake and increases sensitivity of neurones to excite-toxic damage.[147] In a piglet model of global hypoxic brain injury, 24 hours of mild hypothermia was not neuro-

protective when administered without anaesthesia.[148] In contrast, data in the same model have shown that hypothermia with anaesthesia is protective of brain following hypoxia.[16]

The implications of these findings for the newborn during therapeutic hypothermia are uncertain. However, cold stress, shivering and BAT thermogenesis may attenuate the beneficial effects of hypothermia on the brain following hypoxic-ischaemic injury.

2.9 SUMMARY AND RATIONALE FOR THESIS

Moderate systemic hypothermia or selective head cooling with mild systemic hypothermia are partially neuro-protective following HIE. However, despite therapeutic hypothermia, HIE continues to have a high morbidity or neurological impairment. From this review of clinical data, is the continued morbidity or neurological impairment observed with HIE related to the manner in which a proxy measure of brain temperature is measured and controlled?

Studies of direct brain temperature measurement during cooling are largely derived from anaesthetised adults during short term hypothermia, or from animal models that have limited relevance to the human neonate. There are no data on direct temperature measurement of the neonatal brain during hypothermia to guide an understanding of how core body temperatures relate to brain temperature.

Furthermore, little data exist on how different core body sites relate to each other in the human neonate during cooling. Whether rectal temperature, the routine monitoring site during therapeutic hypothermia, is relevant to temperature of the central core in the human neonate is untested.

The relative importance of BAT activity in the neonate compared to the adult suggests that relationships between body and brain temperature in neonates may be different to adults. In neonates, the anatomical distribution of BAT (centrally around the neck and chest) and the rapid transfer of warm blood centrally, allows BAT to effectively warm the thoracic core organs.

Central venous and arterial blood is warmed by BAT and blood temperature perfusing the brain is increased. Furthermore, jugular venous blood from the brain is warmed, reducing convective heat lost from the brain.

BAT has a fundamental physiological role in heating the brain, and this suggests that attempts to cool the core body may have less effect on brain temperature if BAT is active. The literature is clear that control of BAT by higher brain centres can be independent of core temperature. Therefore brain injury associated with perinatal HIE may augment BAT activity and alter brain temperature.

BAT activation also creates a metabolic stress. The effect of this stress on neuronal recovery from HIE is unknown. Thoresen et al performed 24 hours of therapeutic hypothermia on HIE piglets without sedation which failed to reduce neuropathologic damage or seizure activity.[148] The authors suggested that this finding may be due to increased stress during cooling due to the lack of sedation. They speculated that inadequate sedation of neonates undergoing therapeutic hypothermia may block the protective effects of cooling on the post-hypoxic brain.[148] The neo.nEURO.network RCT used systemic therapeutic hypothermia combined with regular sedation and the authors speculated that hypothermia with morphine as co-treatment contributed to the apparently greater benefit of cooling in their RCT compared to other cooling RCTs.[10]

In this thesis, temperature control during whole body hypothermia in human neonates is studied, with an emphasis on BAT activity and temperature gradients between core body sites.

The following hypotheses are tested:

1. Thermogenesis in BAT is active during therapeutic hypothermia for HIE.
2. BAT activity influences T_{oes} more than T_{rec}.
3. T_{rec} does not accurately reflect T_{oes}.
4. BAT activity is associated with severity of brain injury.

Clinical experiments and observational studies were conducted on human neonates using frequent and simultaneous measurements and accurate

temperature measurement techniques. Bat activity was explored using indirect calorimetry as well as direct measurements of temperature of skin overlying a region of BAT along with IR temperature measurements of the whole body during cooling. Indirect calorimetry results in a measure of oxygen consumption and respiratory exchange ratio which is designed to validate temperature changes occurring on skin overlying BAT actually reflect metabolic activity in this tissue.

This thesis has several novel aspects: (i) T_{oes} has not been correlated to T_{rec} where rectum is used as the target site for control of hypothermia; (ii) the activity of BAT during therapeutic hypothermia has not been previously studied; (iii) measurement of skin temperature changes over BAT has not been previously correlated with simultaneous oxygen consumption measurements in human neonates; (iv) thermal imaging of neonates during hypothermia is also a novel application of technology that can measure exposed skin temperatures.

The next two Chapters describe general principles of measurement of temperature and oxygen consumption to inform experimental design.

CHAPTER THREE

3 TEMPERATURE MEASUREMENT AND METHODS FOR CLINICAL EXPERIMENTS

Measurement of skin and core body temperatures is required for this thesis. Before conducting clinical experimentation, careful consideration of study design and monitoring methods were required to acquire meaningful and accurate temperature measurements.

3.1 MEASURING TEMPERATURE

Measuring temperature is performed indirectly, which is different to measuring other fundamental quantities (e.g. mass).[149] The triple point of water where solid, liquid and vapour phases of water repeatedly co-exist at 0.01°C is often used as an accurate temperature reference.[150] While the boiling point of water is approximately 100°C, it is not used as an accurate temperature reference due to slight instability and sensitivity to small fluctuations in ambient pressure.[149] Temperature references are generally used to calibrate points on a thermometer scale.

A temperature sensor is a transducer that converts the thermal state of a body of interest, to another quantity such as resistance e.g. a 'thermistor'.[149] The resistance of a thermistor can be electronically processed and conveniently presented as a reading in °C. There are two methods of measuring body temperature, contact (direct or inferred) and non-contact.

3.2 DIRECT CONTACT TEMPERATURE SENSING

In the medical setting, the most common way to measure body temperature is via direct contact between the sensor and the target tissue. Traditionally, mercury-in-glass tube thermometers were used to measure temperature. Here, the volume of the fluid changes as a function of temperature, causing the fluid to proportionally move inside a small bore glass tube coinciding with calibrated markings along the outside. Use of a mercury-in-glass thermometer to measure body temperature was first proposed in 1887.[151] Although a 'filled-system' thermometer is a straightforward way to measure temperature, their

accuracy is limited by the precision of the calibration marks along the length of the glass tube.[152] Mercury is now known to be a health hazard, and in Europe in 1990 and Australia in 1992, mercury thermometers were banned from use inside infant incubators.[153]

Electronic sensors are a convenient way to measure temperature. There are two main types, thermocouples and resistance temperature devices (RTD's). Each type has unique characteristics and properties that suit certain applications.[150] Contact temperature sensors used to measure body temperatures are usually pre-calibrated, sterilised before use, and used either to monitor skin temperature or, sheathed inside thin flexible probes to reach areas inside the body such as the rectum, nasopharynx or lower oesophagus.

3.2.1 Direct Contact: Thermocouples

Thermocouples consist of a pair of junctions (one reference, one measuring) of two dissimilar metal wires. A temperature difference between two points of a conducting wire forces free electron diffusion from the point of higher temperature to the point of lower temperature creating a voltage gradient along the wire.[149] For example, a thermocouple sensor comprising a length of copper wire alongside a length of copper-nickel (constantan) wire. One end of the wire pair is fused together and is the end used for temperature measurement. The other end of the wire pair is the reference site and connects to a sensitive volt meter. A small voltage in milli-volts (mV) between the reference wires varies in relation to temperature. The small voltage difference occurs because there is a different electron diffusion gradient for each of the different metals in the wire pair.

3.2.2 Direct Contact: Resistance type temperature devices

Metals produce a positive change in resistance for a positive change in temperature. Accurate temperature measurement is the main function of an RTD.[154] RTDs usually consist of a thin metallic layer on an electrical insulator such as plastic film and their electrical resistance varies almost linearly with temperature. Platinum is the most widely used metal due to its relatively high resistivity and its ability to withstand severe environmental conditions.

Platinum also is readily sourced with high purity which ensures repeatability and interchangeability of sensors.[149]

3.2.3 Direct Contact: Thermistors

Another form of resistance type of temperature sensor is the thermistor. The name is derived from ‘thermal resistor’ and thermistors are widely used to sense body temperature. They are made of an inexpensive metal oxide semiconductor material and thermistors used to sense body temperature often have the tip fitted inside a plastic tube or coated with a thin layer of epoxy resin.

The electrical resistance of most thermistors decreases with increasing temperature resulting in a negative temperature coefficient (NTC).[150] Not only are thermistors inexpensive, their high sensitivity to temperature change makes them popular for general use.[150] Although some thermistors are manufactured with a positive temperature coefficient (PTC), PTC thermistors are typically used in thermal protection circuits,[149] rather than for body temperature sensing.

In medicine, a common thermistor is manufactured by Yellow Springs Instrument s (YSI). Their 400 series thermistor has an electrical resistance of 2,250 ohms (Ω) at 25.0°C.[155] Medical thermistors are generally pre-calibrated to be within ±0.1°C over a temperature range of 30 to 40°C.[155]

Patient monitors typically supply a very small constant current through the thermistor that generates a small voltage across the thermistor which varies inversely to temperature. For example, a constant current of 1 mA flowing through a medical thermistor at a temperature of 36°C would have a resistance of 1411.58 ohms (Ω) and 1.41158 V across it. At a temperature of 37°C (1354.91 Ω), the thermistor would have 1.35491 V across it, a reduction of 56.67 mV per °C. This shows a high sensitivity to temperature change.

3.2.4 Direct contact: inferred temperature

An inferred, non-invasive method of estimating a deeper ‘core’ temperature, measures the surface temperature of the object and the environmental

temperature immediately surrounding the object's surface. Three examples are given below.

3.2.4.1 Direct contact: Heat Flux sensor

A 'heat flux' sensor is used to calculate temperature deeper within an object in the presence of a thermal gradient. This is based on an engineering principle where an object's core temperature can be estimated from the object's surface temperature in a known environmental temperature. Assuming that the environmental temperature is cooler than surface temperature, this becomes a measure of heat flow from core to surface to environment as a result of a thermal gradient.[156] A heat flux sensor may also be called a differential temperature sensor.

An example of a medical heat flux sensor has one side (layer) adhered to the skin of a body part (e.g. forehead) and the second (outer) layer is insulated from the inner layer and is exposed to environmental temperature. The temperature difference between the two layers over time is measured. An algorithm is used to calculate an estimate of the deeper temperature based on known thermal properties of a heat flux sensor[157] and can be used for continuous temperature monitoring with some delay in response to a change in core temperature.[158]

3.2.4.2 Direct contact: Zero Heat Flux sensor

An alternative version of the heat flux sensor is called a 'zero heat flux' sensor and it has a small heating element incorporated in between the contact surface layer and the outer environmental temperature layer. The amount of heating energy required to have a zero thermal gradient between the two surfaces is used to calculate a deeper temperature,[159] based on the thermal properties of the sensor.

3.2.4.3 Direct contact: Zero heat flow method

The principle of zero heat flow (flux) can be applied passively to estimate core temperature by placing a temperature sensor on skin in the middle of a large area in contact with a thermal insulator i.e. mattress. This concept assumes

that if there is no heat loss to the environment through a large area that is thermally insulated, then skin temperature in the centre of this area should reflect deeper tissue temperature.[160]

3.3 NON-CONTACT TEMPERATURE SENSING

Although direct contact temperature sensors are commonly used, it is possible to measure tissue temperature without direct contact. Three methods of non-contact temperature measurement are described below.

3.3.1 Non-contact: IR temperature measurement

IR energy is emitted by objects to a lower surrounding temperature and the amount of radiation increases with temperature difference.[161] Human skin emits IR radiation to a lower environmental temperature and emits 98% as much as a 'black body radiator'. Although the human eye cannot see IR radiation, an IR detector can be used to convert IR energy into °C.

The IR wavelength peak for a given temperature can be calculated by 'Wein's displacement law', λ max = 2898/T, where T is the absolute temperature in °K and λ max is the wavelength in μm at the maximum intensity. A skin temperature of 35°C has a λ max of 9.4 μm (2898/308) μm. Therefore an IR detector used to measure skin surface temperature needs to be sensitive to 'long wave' IR radiation in the region of 9 to 10 μm wavelengths. IR thermography is limited to surface temperature measurement.

3.3.2 Non-contact: Microwave temperature measurement

On the electromagnetic spectrum, microwave radiation is adjacent to IR. Microwave energy is emitted by objects at temperatures above absolute zero and proportional to the absolute temperature of the object.[162] Microwave energy can be detected using a very sensitive passive sensor tuned to a specific frequency in the microwave band. The use of multiple passive microwave sensors in an array can detect several specific microwave frequencies and this technique is called microwave radiometry (MWR).[162] MWR utilises the tissue temperature dependence of the power in the microwave region of the natural thermal radiation emitted from body tissues.[163]

At present, no commercial device is available for this purpose.

3.3.3 Non-contact: Magnetic Resonance Imaging temperature measurement

In 2012, magnetic resonance spectroscopy-thermometry (MRSt) emerged as a refined method to calculate brain temperature during an MRI scan.[164] Temperatures derived from cerebral creatine (Cr), choline (Cho) and N-acetyl-aspartate (Naa) as independent reference peaks have been combined to give a single amplitude-weighted combination temperature (Tawc).[165] While brain temperature can be measured during a scan, this method is not suitable for brain temperature monitoring in the clinical setting.

3.4 BACKGROUND SUMMARY OF TEMPERATURE MEASUREMENT

Current trends in medicine seek non-invasive or less invasive methods to measure physiological parameters for patient wellbeing and for patient comfort. Although no commercial device is currently available, Maruyama et al and Hand et al have shown that using multi band MWR can non-invasively detect deeper tissue temperature.[162, 163] MRSt can non-invasively measure brain temperature. However this is not practical to use in the clinical setting to provide ongoing monitoring of brain temperature. Heat-flux head sensors have been used to estimate measure brain temperature with some success.[92, 158] However they require stable environmental conditions, such as air temperature, humidity and air movement.

Furthermore, MWR, MRSt and ZHF methods require the subject to be perfectly still. In a busy NICU, this is not a likely prospect. Therefore a practical approach using simple methodology was required to avoid disruption to staff in the NICU during studies of critically ill neonates.

3.5 TEMPERATURE MEASUREMENT METHODS FOR CLINICAL EXPERIMENTS

There are several ways to measure body temperature so careful consideration and planning was exercised before commencing clinical studies of measuring body temperatures on human neonates.

Thermistors are commonly used in the NICU to measure skin and rectal temperature and they are available pre-sterilised and pre-calibrated. For practicality and consistency with nursery practice, thermistors were chosen to measure skin and core temperatures.

Although previously not used, an IR camera was chosen to measure exposed skin surface temperature during clinical studies. The IR camera could be mounted up high and out of the way of staff and allowed non-contact measurements of skin temperature anywhere within the field of view.

As several body sites were planned, an electronic means of measuring and recording temperatures was required. Additionally, simultaneous temperature measurements of two or more skin sites and two core sites was required to compare temperatures between sites and frequent measurements were required to observe response times at each site. Measurement of local environmental temperature was also required to provide information regarding radiant heater activity.

Therapeutic hypothermia for HIE neonates used T_{rec} to manually direct the depth of whole body cooling for 72 hours followed by at least 6 hours of slow rewarming. To observe temperatures during cooling, rewarming and for some time after rewarming, a maximum of 96 hours of temperature recording capability was required.

An electronic device that could simultaneously and frequently measure and store results from several medical thermistors was explored. To avoid any issues with electrical safety or interference to ECG monitoring, the electronic device needed to be battery operated and small.

3.5.1 A battery operated datalogger

An Omega OM-DAQPRO-5300 (Omega Engineering, MA, USA) was selected for this task as it was relatively small, battery operated, could measure up to 8 channels simultaneously along with a date and time code, accept a variety of user selectable input types including medical thermistors and measure and store results as frequently as every second (Figure 3.1). One of the user

selectable input types for this datalogger is ‘NTC-2252 thermistor’.

This is equivalent to the common YSI-400 series medical thermistor temperature sensor. The datalogger measures the resistance of the thermistor and converts the reading from Ω to °C before saving to internal memory.

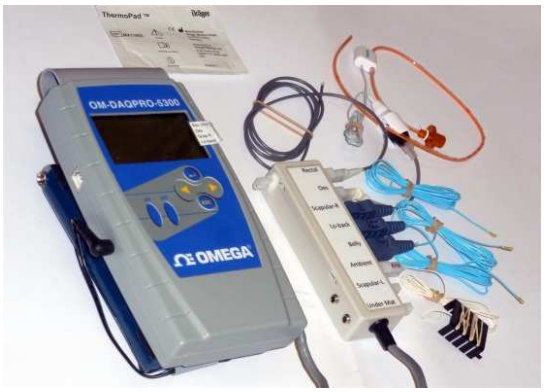


Figure 3.1 A general purpose battery operated datalogger

A battery operated datalogger was used to simultaneously record up to eight inputs, mostly from medical thermistors to measure temperature. The datalogger stored readings in °C up to a maximum of one per second along with the date and time of each measurement. Temperature sensors connect to a sensor connector box that in turn connects to the datalogger.

3.5.2 Sensor connection and rectal temperature recording

Manual control of whole body cooling is used to treat HIE neonates. The bed-side nurse observes T_{rec} on the bedside monitor and manually adjusts local environmental conditions to direct the depth of cooling to a targeted 33-34°C range. As T_{rec} was also required to be one of the datalogger inputs, an electronic interface circuit was required to interface between the T_{rec} sensor and the patient monitor to allow the datalogger to record T_{rec} without interfering with the accuracy of the bedside monitor.

As several temperatures were planned to be simultaneously recorded by the datalogger, a small sensor connector box was required to allow simple and convenient connection of all the temperature sensors. For convenience, the sensor connector box was positioned on the edge of the mattress to allow all the thermistor sensor wires to connect to one item. There was enough room inside the sensor connector box for a small battery and an electronic interface electronic. The sensor connector box connected via a single multicore cable to the datalogger, which was located out of the view of nursing staff (Figure 3.2).

The rectal thermistor probe plugged into the sensor connector box and

another cable plugged into the monitor output of the sensor connector box that connected to a bedside patient monitor. The bedside monitor needed to display T_{rec} for bed-side nurse.

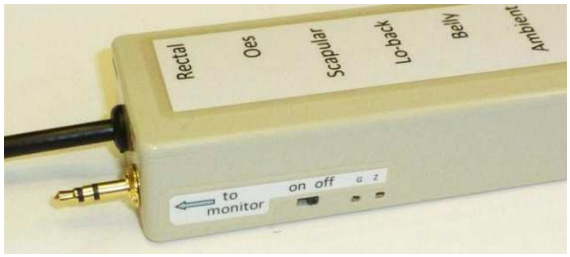


Figure 3.2 The sensor connector box
The sensor connector box allowed simple connection of all the temperature sensors and an electronic interface circuit allowed the bedside monitor to continue to display rectal temperature for the bed-side nurse.

3.5.3 Electronic interface circuit

To measure body temperature, patient monitors manufactured by Drager, GE, Philips and Siemens typically pass a small constant current of approximately 0.1 mA through a temperature sensing thermistor to measure its electrical resistance in relation to temperature. The voltage developed across the thermistor is then electronically converted inside the monitor to °C with a typical accuracy of 0.1°C. For example, passing a constant current of 0.1 mA through a medical thermistor (YSI-400, NTC-2252) would produce 166.722 mV at 32.0°C, and 130.077 mV at 38.0°C. A difference of 6°C results in a difference of 36.645 mV with an offset from zero of 130.077 mV.

For this thesis, temperature measurement accuracy was required to be better than 0.1°C. As the electronic interface circuit between the T_{rec} sensor and the bedside monitor measured the voltage developed across the thermistor, the datalogger was used to record this voltage. Apart from a 2252-NTC-thermistor, the datalogger can be set to measure voltages of either 0 to 10V, or 0 to 50 mV. The datalogger has 16 bit resolution of the input, meaning that the 0 to 10 V range equates to 0.2mV steps, or 0.04°C increments. However, using the 0 to 50 mV range equates to 3µV steps or 0.0005°C increments. The 50mV input range for the datalogger was chosen as this has ample sensitivity. However, to utilise the 0 to 50 mV input, the voltage developed across the T_{rec} sensor (32 to 38°C) needed to be offset -130mV to be within the 0 to 50mV range of the datalogger.

A second requirement was to avoid any interference with the patient monitor, particularly with the accuracy of T_{rec} being displayed on the patient monitor for

the bed-side nurse. Another requirement was to use the Philips ‘X2’ patient input module. This used a constant current of 0.2 mA for measuring temperature (twice as much as other temperature monitors). This results in an offset of 260 mV and 73.29 mV for a 6°C change with a medical thermistor. This exceeded the datalogger input range of 0 to 50mV. Hence an electronic attenuation circuit was required as well as a voltage offset circuit.

Using an electronic operational amplifier in series with the datalogger input to sense the voltage developed across the T_{rec} sensor has extremely high input resistance and avoids interfering with the accuracy of the bedside patient monitor display of T_{rec}. A second operational amplifier allowed voltage offset and attenuation or gain of the voltage from the T_{rec} sensor. This allowed many makes and models of patient monitors to be used if necessary.

The interface circuit used a miniature (3x2x1 mm) dual operational amplifier (NJU7018) capable of operating at 1.5V to achieve input resistance buffering, voltage offset, and voltage gain or attenuation to interface to several brands of patient monitor (Figure 3.3). As well as facilitating a connector for each sensor, the sensor connector box contained a AAA size battery to power the operational amplifier. A small on-off switch, an attenuation/gain potentiometer and an offset potentiometer are shown in Figure 3.3. Further details are located in Appendix A.

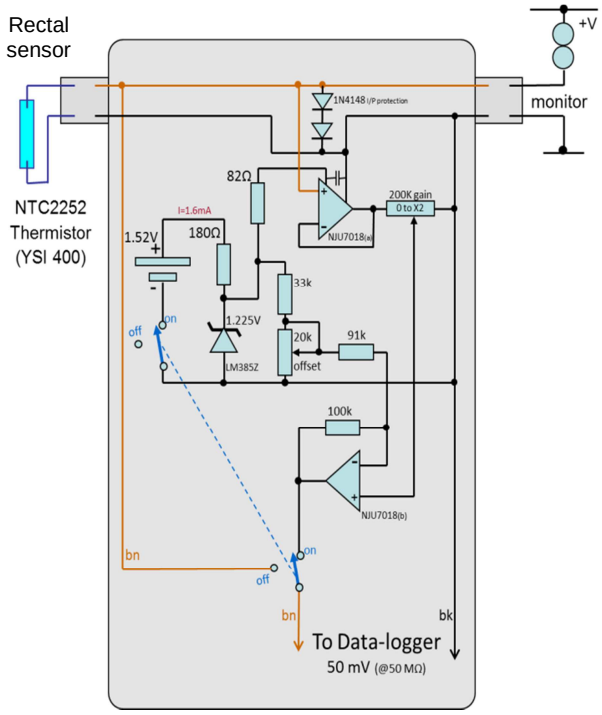


Figure 3.3 Electronic interface circuit inside the sensor connector box

The electronic circuit inside the sensor connector box allows the bedside monitor to function normally while the datalogger simultaneously records a small voltage that varied in relation to temperature. A 1.5V battery powered a dual operational amplifier to achieve no loading across the thermistor. The circuit also has and adjustable voltage offset and voltage gain or attenuation adjustment.

3.5.4 Temperature sensor and datalogger accuracy

Temperature sensors used in medicine are generally inexpensive NTC thermistors. An advantage of thermistors is sensor stability usually 0.01°C per 10 months.[155] Medical thermistors are often pre-sterilised and pre-calibrated to be accurate within ±0.1°C. While the pre-calibrated sensors are reasonably accurate, the general purpose datalogger in NTC-2252 thermistor mode is accurate to only ±0.2°C. The combined worst case accuracy of the datalogger with sensor is approximately ±0.3°C.

Bench testing was done with the datalogger in NTC-2252 thermistor mode using a fixed resistance of 1471Ω (35.0°C). This showed a worst case consistent offset of 0.2°C at 35°C. A redeeming factor of the datalogger is a specified repeatability value of ±0.1%. This equates to a measurement repeatability of 0.035°C with the datalogger. Therefore, a system calibration of the datalogger with temperature sensors attached could be used to correct measurements and achieve an accuracy of better than ±0.04°C.

3.5.5 Temperature sensors for use in studies

The temperature sensors used were medical thermistors. Where possible, the same or similar temperature probes, as used in respective NICUs, were used to measure body and environmental temperature in this thesis.

Initially, a Covidien ‘Mon-a-therm’ 9 Fr probe was used to measure T_{oes}. This was replaced by a Philips ‘Innersense’ combined gastric feeding tube size 8 Fr. A Dräger 7.5 French probe was generally used to measure rectal temperature. While Tyco ‘IncuTemp 5’ skin probes were used to measure skin temperatures, a Fisher & Paykel disk type skin probe was attached to a small black anodized heatsink to record radiant heater (on-off) activity. Response time (from zero to 90% of final value) for the oesophageal and rectal sensor was 20 seconds, and less than 5 seconds for all other sensors.

3.5.6 Temperature calibration of sensors and datalogger

Calibration of the temperature sensors and datalogger occurred shortly after each study. All the sensors were still connected to the sensor connector box

and datalogger. A reference electronic thermometer (Fluke model 1523, Fluke Corporation, Everett, WA, USA) was bundled together with the sensors and placed in a 1L stirred water bath heated to several temperatures. The electronic reference thermometer had an accuracy of ±0.01°C. After 20 seconds of thermal steady state, a minimum of 10 measurements at each calibration temperature was recorded by the datalogger. Calibrations were made at 32, 33, 34, 35, 36, 37 and 38°C.

Datalogger measurements were downloaded via a USB cable to a computer running MS Excel 2010 (Microsoft Corporation, WA, USA). The average reading of each calibration temperature was used to create a correction equation for each sensor used. As T_{rec} was recorded in mV and in proportion to the negative temperature coefficient of the thermistor, a 3rd order polynomial correction equation was used to accurately convert measurements from mV to °C. For other readings recorded in °C on the datalogger, a 2nd order polynomial correction equation was used to improve measurement accuracy.

While all calibration readings had similar accuracy to the reference thermometer, the datalogger's repeatability value added further uncertainty (±0.035°C). Overall, corrected temperatures of the system used to measure and store results on the datalogger achieved better than ±0.05°C accuracy. Data were analysed as corrected temperatures and progressively averaged over one minute that served to smooth the data and reduce noise.

3.5.7 Extension battery for datalogger

During cooling studies, up to 96 hours of datalogging could be required. As the datalogger is specified to run for only 24 hours from an internal rechargeable battery, an external large capacity battery was required to keep the datalogger running.

Instead of using one large external battery, two smaller extension batteries were constructed that could each keep the datalogger running and fully charged for 48 hours. Each battery was designed to fit underneath the datalogger and be replaced after 48 hours. A second extension battery could be used if required.

Each extension battery consisted of eight “4/3 A” size Nickel Metal Hydride rechargeable cells. This was chosen to give a nominal 9.6 Volts with 3.8 amp-hour (A-h) capacity. As a safety precaution, the extension battery was fitted with a 65°C thermal cut-out switch in case of an unexpected cell failure (Figure 3.4).

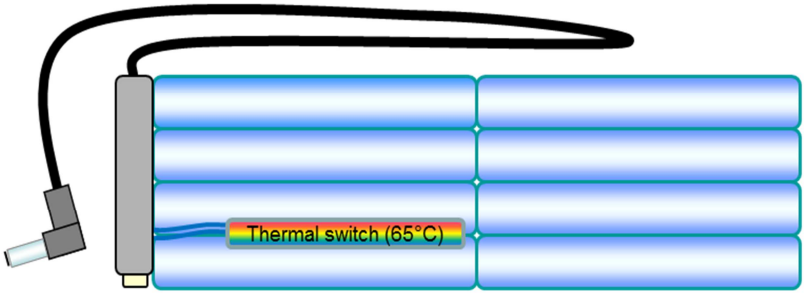


Figure 3.4 An extension battery pack

An extension battery pack was constructed to extend the run time of the datalogger by 48 hours and consisted of eight 4/3A rechargeable nickel metal-hydride cells and protected with a thermal cut-out switch.

The charger input to the datalogger is specified as 9 to 12 V. The extension battery simply plugged into the charger input of the datalogger, which ensured that the datalogger’s internal battery was kept fully charged for 48 hours. The dropout voltage of the datalogger’s charger input is 7.5 V. With the extension battery connected to the datalogger, this limited the discharge voltage of each cell in the extension battery to 0.9 V when flat and avoided cell reversal. The same charger used to charge the datalogger was also used to charge the extension battery. This could be done either individually or while connected to the datalogger. Further technical details are in Appendix B.

3.5.8 Infra-red (IR) temperature measurement

During hypothermia treatment, the temperature of exposed skin could be measured using non-contact IR thermography. A FLIR model P660 IR camera (FLIR Systems Inc, OR, USA) was chosen for this role. While the absolute temperature accuracy is only ±0.35°C, the sensitive micro-bolometer sensor array (640 x 480 pixels) can detect very small temperature differences (0.05°C) within each image. The camera uses a memory card to store IR

images. Stored images were analysed after each study using FLIR 'Thermacam Researcher 2.9' software (FLIR Systems Inc, OR, USA).

A mounting arm was constructed that attached either to a Dräger open radiant warmer or to a Dräger enclosed infant incubator. This held the IR camera approximately 1m above the subject and out of the way during clinical studies.

Thermal images of each subject were automatically recorded every 10 to 30 seconds for the duration of each clinical study. The skin temperature sites chosen for measurement were: nape of neck, above the heart, upper arm, forearm, thigh, lower leg, mattress surface near the head and mattress surface near feet. The average temperature of a 1 cm diameter circle was used for each measurement site except for the nape of neck where the temperature was averaged from a 1 cm line positioned above the neck crease.

3.6 CONCLUSION

A small and portable battery operated datalogger was planned to frequently and simultaneously measure and record temperatures for long study periods.

A sensor connector box was added to the datalogger to allow simple connection of the temperatures sensors. Inside the sensor connector box was an electronic interface circuit. The circuit allowed T_{rec} to be displayed on the patient monitor while simultaneously recording a measure of T_{rec} on the datalogger without interfering with the patient monitor. The electronic interface circuit needed to be very reliable and accommodate several brands of patient monitor.

Chapters 5 to 8 involve experiments and observational studies with neonates where core and skin temperatures will be concurrently measured using direct contact thermistors and IR thermography.

CHAPTER FOUR

4 INDIRECT CALORIMETRY FOR CLINICAL EXPERIMENTS

4.1 INTRODUCTION

In this thesis, temperature control during whole body hypothermia in human neonates is studied, with an emphasis on BAT activity and temperature gradients between core body sites. The first hypothesis tested is that BAT thermogenesis is active during whole body cooling. In Chapter 5 an experiment will be described where skin surface temperatures over BAT, core temperature changes, and oxygen consumption in response to mild environmental cooling in healthy term babies are measured. Skin surface temperatures over BAT are used because direct invasive measurement is not feasible in the human neonate.

As described in the literature review in Chapter 2, Dawkins and Scopes showed that oxygen consumption (VO_2) increased during experiments with human neonates when exposed to cooling and the authors suggested that this was due to non-shivering thermogenesis (NST) in BAT.[130] In this Chapter, the development of the method for measuring oxygen consumption used in Chapter 5 is described.

Within the body, VO_2 is the product of cardiac output and arterio-venous oxygen difference and can be calculated via the direct Fick method.[166] While this method requires invasive measurement, VO_2 can also be defined as the amount of oxygen extracted from inspired air in a given period of time.[167] This non-invasive method of calculating VO_2 is called indirect calorimetry.[168]

Indirect calorimetry has been used in adult and paediatric intensive care.[169] However the use of indirect calorimetry for research in neonates has challenges, such as complete collection of expired gas and measurement error due to small gas volumes.[169, 170] Measurement of VO_2 and carbon dioxide production (VCO_2) can be used to calculate the respiratory exchange ratio (RER). RER is the ratio of VCO_2/VO_2 and provides information on which substrate e.g. fat, glucose is being metabolised.[169]

4.2 MEASUREMENT AND CALCULATION OF OXYGEN CONSUMPTION

VO_2 is measured in ml of O_2 per minute or in ml/kg/min when referenced (normalised) to body weight.[167] To calculate VO_2, the following parameters require accurate measurement: fractional inspired oxygen and carbon dioxide concentration (FiO_2, $FiCO_2$), fractional expired oxygen and carbon dioxide concentration (FeO_2, $FeCO_2$), environmental pressure, temperature and dry expired volume.[167, 169, 171-174] If expired gas is measured, then using the combined gas law, inspired volume (Vi) can be calculated from expired volume (Ve) and the above fractional measurements of inspired and expired O_2 and CO_2 using Haldane's transformation equation ($Vi * FiN_2 = Ve * FeN_2$).[175] FiN_2 is the fraction of inspired nitrogen and FeN_2 is the fraction of expired nitrogen. Further details are given in the following section (4.3.2). Haldane's transform assumes that the virtually insoluble nitrogen gas is stable within capillary blood.[175]

There are two types of indirect calorimeter used to measure VO_2 in the clinical setting; the 'breath-by-breath' method, and the 'open circuit mixing chamber' method.[176] The breath-by-breath method measures air volume and gas concentrations at the mouth during each breath, calculates the difference between oxygen intake and outtake and gives a final VO_2 value by averaging the values obtained on complete breaths over a fixed time interval e.g. 1 minute.[176] The open flow mixing chamber method uses suction to control a flow of air that entrains the subject's expired breath and passes through a chamber that mixes the gas before measurement. VO_2 is calculated from airflow rate and FiO_2 and FeO_2 measurement.[167] A dual O_2 analyser or a single O_2 analyser that samples FiO_2 and FeO_2 at selected intervals can be used. Both types of indirect calorimeter use either a pneumotachograph or mass flow sensor to measure gas volume or airflow.[167] The open circuit mixing chamber systems are capable of accurate metabolic rate measurements.[176]

Indirect calorimetry used to measure VO_2 in the newborn has either used custom built equipment,[167, 169-174, 177-181] or commercially manufactured systems.[182] Evans et al used a simplified VO_2 open circuit mixing chamber method with spontaneously breathing neonates based on knowledge of room

air O_2 concentration and measuring oxygen concentration in expired breath. Expired breath was captured using a special funnel that was gently applied to the face and a constant air flow was created by suction that drew in room air and expired breath. After passing through the mixing chamber, the mixed gas was measured by a sensitive oxygen analyser.[170] Although Evans et al calculated an approximation of oxygen consumption, the authors did not measure carbon dioxide in the expired gas.[170] Therefore the authors could not calculate respiratory exchange ratio (RER) to analyse substrate utilisation.

To calculate RER, both O_2 and CO_2 levels in inspired and expired gas are required.[167, 169, 171-174, 177, 178, 181, 183] RER gives an indication of substrate utilisation which is an important factor for metabolic analysis.[169, 171, 177, 184] However, many indirect calorimeters are complex and are limited to specific research applications such as the system used by Thureen et al.[169]

Expanding on Evans' basic concept[170] and simplifying Thureen's complex system,[169] a practical alternative system was considered to measure VO_2 and VCO_2 with spontaneously breathing neonates using an open circuit mixing chamber. The next section looks at validating methods and equipment available to measure the parameters necessary to calculate VO_2 and RER with 5% accuracy or better as recommended by Thureen et al.[169]

4.3 OXYGEN CONSUMPTION EQUIPMENT AND METHODS

For spontaneously breathing neonates, a non-invasive open circuit flow method of indirect calorimeter allows the neonate to sleep during an experiment. This type of indirect calorimeter uses suction to cause an overall gas flow rate that is approximately double the neonate's maximum expired breath rate to ensure total capture of expired breath. The neonate's expired breath is entrained into the system flow along with room air from the immediate environment. Inspired and expired oxygen and carbon dioxide concentrations need to be measured along with ambient temperature, ambient pressure and gas moisture content.

A calculation is required to compensate for ambient temperature and ambient pressure with saturated moisture content (ATPS) in the measured gas so that

results are in a standardised format called ‘standard temperature, pressure and dry’ (STPD). Standard temperature (T_{STD}) = 273.15°K (0°C) and standard pressure (P_{STD}) = 760 mmHg.

To determine a suitable and practical method for calculating VO_2 and VCO_2 for experiments planned in Chapter 5, three designs and methods of an open circuit mixing chamber indirect calorimeter were investigated. These were authored by Evans et al,[170] Hill,[172] and Thureen et al.[169] To explore these methods, a mathematical exercise using specified values was performed that incorporated each author’s calculation formula. See section 4.4. Results were compared to a reference formula for calculating VO_2 and VCO_2.that was based on a blend of the combined gas law equation and Haldane’s transform.

Parameters typically measured to calculate VO_2 and VCO_2 :

FiO_2	fraction of inspired oxygen
$FiCO_2$	fraction of inspired carbon dioxide
FeO_2	fraction of expired oxygen
$FeCO_2$	fraction of expired carbon dioxide
Flow	open circuit gas flow rate containing expired breath
Ta	ambient temperature
Pa	atmospheric (ambient) pressure
PH_2O	water vapour pressure (unless totally removed)

Abbreviations:

P_{STD}	Standard Pressure = 760 mmHg
T_{STD}	Standard Temperature = 273.15°K (0°C)
ATPS	ambient pressure with saturated moisture content
STPD	standard temperature, pressure and dry

Variables:

FiO_2	fraction of inspired oxygen
FeO_2	fraction of expired oxygen
$Fe'O_2$	fraction of expired oxygen (CO_2 free)
$FiCO_2$	fraction of inspired carbon dioxide
FiN_2	fraction of inspired nitrogen

FeN_2 fraction of expired nitrogen

Ve volume expired per minute (STPD)

Vi volume inspired per minute (STPD)

4.3.1 Calculating VO_2 and VCO_2 using first principles

The combined gas law is useful for deriving an unknown gas quantity if other quantities are known. Two quantities that need to be derived to calculate VO_2 are: (i) gas volume at a standard temperature, pressure and dry; (ii) inspired gas volume. Both can be derived from the combined gas law:

$$\frac{P_1 * V_1}{T_1} = \frac{P_2 * V_2}{T_2} \quad (1)$$

Using first principles, subscripts 1 and 2 in equation (4) were substituted by STPD and ATPS respectively such that

$V_{STPD} = V_{ATPS} * [(Pa - P_{H2O}) / 760] * [273.15 / (Ta + 273.15)]$. (2)

Calculating inspired volume

With spontaneously breathing neonates, measuring inspired volume is a challenge. As measuring expired volume is simpler, inspired volume was calculated using the combined gas law and incorporating Haldane's transform.[175]

4.3.2 Haldane's transform

Haldane's transform assumes that nitrogen (N_2) is physiologically inert[175] such that:

$Vi * FiN_2 = Ve * FeN_2$ (3)

$FiN_2 = 1 - FiO_2 - FiCO_2$ (4)

$FeN_2 = 1 - FeO_2 - FeCO_2$ (5)

Substituting equations (4) and (5) into equation (3) gives:

$Vi = Ve *(1 - FeO_2 - FeCO_2) / (1 - FiO_2 - FiCO_2)$ (6)

Oxygen consumption based on Fick's principle:

$VO_2 = [Vi * FiO_2] – [Ve * FeO_2]$ (7)

Reference formula to calculate VO_2

Using the combined gas law with Haldane's transform to substitute Vi in equation 7 results in the formula to calculate VO_2:

$VO_2 = [Ve *(1 - FeO_2 - FeCO_2) / (1 - FiO_2 - FiCO_2)] * FiO_2 – [Ve * FeO_2]$ (8)

Reference formula to calculate VCO_2

VCO_2 formula based on Fick's principle:

$VCO_2 = [Ve * FeCO_2] – [Vi * FiCO_2]$ (9)

Using the combined gas law with Haldane's transform to substitute Vi in equation 9 results in the formula to calculate VCO_2:

$VCO_2 = [Ve*FeCO_2] – [Ve*(1- FeO_2 - FeCO_2) / (1- FiO_2 - FiCO_2)]* FiCO_2$ (10)

4.4 MATHEMATICALLY COMPARING THREE VO_2 CALCULATION METHODS

Three different methods of calculating VO_2 were mathematically compared to the reference method shown in equation 8 based on values given in an example below.

Method 1 (Evans et al's VO_2 calculation formula)

Evans et al's method[170] was adapted from Fick's principle where:

$VO_2 = Flow * (FiO_2 – FeO_2)$ (11)

Method 2 (Hill's VO_2 calculation formula)

Hill's method[172] calculated VO_2 and assumed that CO_2 had been removed from the expired gas before oxygen concentration measurement, as CO_2 in the sample gas has a diluting effect on O_2 measurement. Hill offered 3 slightly different methods to calculate VO_2. Hill's third equation for calculating VO_2 was the most applicable for this study and is shown below where the measurement of $Fe'O_2$ is CO_2 free:

$$VO_2 = Vi * [FiO_2 – (1 – FiCO_2) * Fe'O_2] \textbf{ / } [1- Fe'O_2] \quad (12)$$

Method 3 (Thureen et al's VO_2 calculation formula)

Thureen et al's[169] equation for VO_2 was based on the Fick principle and incorporated the combined gas law with Haldane's transform:

$$VO_2 = [Ve *(1 - FeO_2 - FeCO_2) \textbf{ / } (1 - FiO_2 - FiCO_2) * FiO_2] – [Ve * FeO_2] \quad (13)$$

Example 1. Representative gas measurement values

> *5 litres of expired gas with 20.0% oxygen and 0.8% carbon dioxide in a balance of 79.2% nitrogen at an environmental pressure and temperature of 750 mmHg at 25ºC with no moisture content. Inspired gas with 20.96% oxygen and 0.04% CO_2 in a balance of nitrogen with no moisture content.*

Example 1 in parametric terms: Ve = 5 Lt, FeO_2 = 0.2, $FeCO_2$ = 0.008, FeN_2 = 0.792, Pa = 750mmHg, Ta = 25°C, FiO_2 = 0.2096, $FiCO_2$ = 0.0004 and P_{H20} = 0 (due to moisture absorption from in line desiccant before measurement).

First step

The first step converts ambient temperature and pressure with saturated moisture content volume (ATPS) to STPD volume using equation (2):

$$Ve_{STPD} = 4.5205 \text{ Lt} \quad (14)$$

Second step

The second step calculates Vi from Ve_{STPD} using equation (6):

$$Vi = 4.532 \text{ Lt} \quad (15)$$

Reference method result (equation 8)

$$VO_2 = 45.8 \text{ ml/min} \quad (16)$$

Method 1 result (Evans et al, equation 11)

$$VO_2 = 43.4 \text{ ml/min} \quad (17)$$

Method 2 result (Hill, equation 12)

Using Hill's method, the O_2 reading in Example 1 would be slightly higher if

CO_2 was removed prior to measurement. Removing the 0.8% CO_2 before gas measurement would produce a ratio of 99.2/100. Therefore to restore to 100%, Fe'O_2 would equal 20.16129%O_2.

VO_2 = 45.7 ml/min (18)

Method 3 result (Thureen et al, equation 13)

VO_2 = 45.8 ml/min (19)

Comparing the three VO_2 calculation formulas using values in Example 1

Method 1

Evans et al's formula[170] did not include CO_2 measurement and did not calculate Vi. Thus, Evans et al's method is only accurate when the RER = 1.0.

Evans et al's simplified method showed a -5.2% discrepancy when compared to the reference method (equation 8).

The difference between FiO_2 and FeO_2 in Example 1 was 0.8%. If this difference was smaller, the error would have been greater using this method.

Method 2

Using Hill's 3rd formula,[172] the difference in VO_2 calculation compared to the reference (equation 8) was 0.2%.

Method 3

Thureen et al's VO_2 calculation formula[169] was identical to the derived combined gas law that incorporated Haldane's transform. The difference in VO_2 calculation compared to the reference (equation 8) was 0%.

Comparing VCO_2 calculation formulas using values in Example 1

The methods of Evans et al and Hill et al did not measure CO_2 therefore VCO_2 was not calculated. Only Thureen et al's method was evaluated.

VCO_2 reference method calculation (equation 10)

VCO_2 = 34.351 ml/min (20)

VCO_2 calculation method based on Thureen et al

Thureen et al's formula calculated VCO_2 as:

$VCO_2 = Ve * (FeCO_2 - FiCO_2)$ (21)

$VCO_2 = 34.356$ ml/min (22)

Comparing VCO_2 calculation results using values from Example 1

Thureen et al's method (equation 21) was similar to the reference method (equation 10). While Thureen et al did not include FiO_2 or FeO_2 values in their VCO_2 calculation, the difference in VCO_2 calculation compared to the reference (equation 10) was only 0.015%.

Mathematically calculating RER using example 1

RER $= VCO_2 / VO_2$

Reference RER $= 0.7501$ (23)

Thureen et al RER $= 0.7502$ (24)

Comparing RER calculation results using values from Example 1

Because Thureen et al's method of calculating VCO_2 (equation 22) showed a slight difference, the same discrepancy exists in the RER calculation (0.015%).

4.4.1 Evaluation of three methods used to measure VO_2 and VCO_2

This thesis considered that the most accurate formula to calculate VO_2 and VCO_2 was a blend of the combined gas law formula with Haldane's transform (equations 8 and 10). These equations were used to compare the results from three methods in a mathematical exercise. The three methods had different levels of complexity to measure VO_2.[169, 170, 172]

Based on data used in example 1, the method of Thureen et al showed no difference in VO_2 calculation and a VCO_2 calculation difference of only 0.015%. However, Thureen et al used a complex arrangement of equipment to measure VO_2 and VCO_2.[169]

Although the simpler calculation method used by Evans et al showed potential for discrepancy, their basic concept could be adapted to incorporate CO_2

measurement and improved to be more sensitive and responsive. By using the combined gas law formula with Haldane's transform, a suitable system could be built to accurately measure VO_2 and VCO_2.

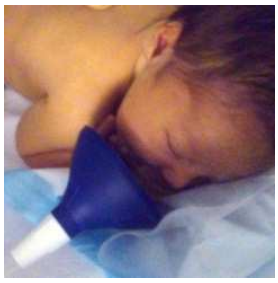
4.5 EQUIPMENT USED TO BUILD AN INDIRECT CALORIMETER

Based on devices available to measure air flow, O_2 and CO_2 concentrations, ambient temperature and pressure, a custom design open circuit mixing chamber style indirect calorimeter was assembled. This was based on the simple concept of Evans et al yet adapted to include CO_2 measurement along with design improvements to be more responsive and sensitive. The combined gas law equation combined with Haldane's transform was used in this thesis to calculate VO_2 and VCO_2 from measured parameters.

Thureen et al recommended that an indirect calorimeter should have an overall accuracy of 5% or better.[169] Thureen et al's recommendation was adopted for this thesis. Another important requirement of a custom built indirect calorimeter was sufficient sensitivity and response time to detect small changes in VO_2 and RER occurring in response to step-changes in environmental temperature.

4.5.1 System air flow

Evans et al suggested that a system air flow (lpm) of approximately 1.3 times the neonate's birth weight (kg) was sufficient to ensure capture of all expired breath into the system flow.[170] Evans et al's suggestion was adopted for this thesis.

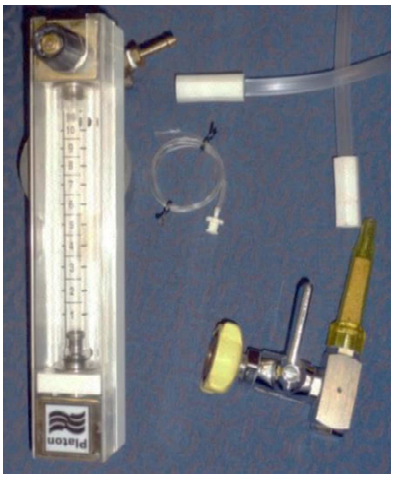


A soft rubber funnel was chosen to collect expired breath by being placed close to the neonate's mouth and nose. This allowed the neonate to sleep during an experiment (Figure 4.1). The funnel connected to a 1.5 metre length of 6.5 mm internal diameter flexible silicone hose that attached to the indirect calorimeter.

Figure 4.1 A soft rubber funnel was used to collect expired breath

A system air flow of approximately 1.3 times the neonate's birth weight was used to collect expired breath and some room air via a soft rubber funnel. A small (4 mm) safety hole was placed near the base of the funnel to avoid a potential risk of the funnel sealing over the neonate's face.

Wall suction was required to control a flow of air into the soft rubber funnel.



A 0 to 10 l/min positive pressure air flowmeter was dismantled and reassembled upside down. Adjusting the flow control knob of this flowmeter controlled overall suction flow rate from 0 to 10 l/min as well as give an approximate visual indication of flow rate. A standard 5 m length of suction hose was used to connect to the wall outlet suction tap at one end, while the other end connected to a fitting on the top of the suction flowmeter (Figure 4.2).

Figure 4.2 Inverting an air flowmeter to control suction flow

A positive pressure air flowmeter arranged upside down was used to control overall suction flow rate from 0 to 10 l/min as well as give an indication of flow rate. One end of a 5 m long flexible suction hose connected the wall suction outlet tap, and the other end connected to the top of the flowmeter. A 1.5 m length of 0.5 mm bore PVC tubing connected to the suction fitting to produce a steady suction flow of 50 ml/min for each gas sensor.

4.5.2 Mixing chamber and water vapour removal

Accurate measurement of moisture content in a gas is particularly challenging. So instead of measuring moisture content in sampled gas, a 500ml in line desiccant chamber filled with blue indicating anhydrous calcium sulphate desiccant (W.A. Hammond Co., Drierite, stock # 26800, OH, USA) was used to remove all moisture from the gas before measurement (Figure 4.3). The desiccant chamber also served as a gas mixing chamber.

Figure 4.3 Using desiccant to dry and mix gas before measurement

An air-tight 500 ml polycarbonate chamber containing blue indicating desiccant dried the flow of gas before gas concentration measurement and also acted as

a gas mixing chamber. A pink colour indicated moisture absorption in the desiccant.

4.5.3 VO_2 calculation error

Gore et al published a table of VO_2 calculation error based on sensor inaccuracy for individual components used in an indirect calorimeter when using the combined gas law equation with Haldane's transform.[166] Table 4.1 shows the specified accuracy of available devices for use in this thesis and a corresponding potential VO_2 calculation error.

Measurement parameter	Device accuracy	Error in VO_2
Volume or flow	1.75%	1.75%
O_2 concentration	0.01%	-0.07%
CO_2 concentration	0.02%	-0.01%
Ambient pressure	0.7%	0.7%
Ambient temperature	2.0%	-0.14%

Table 4.1 VO_2 calculation error in relation to sensor accuracy
The specified accuracy of available devices to use in a custom built indirect calorimeter. Based on the results of Gore et al[166] a corresponding potential VO_2 calculation error is shown.

Gore et al showed that if an oxygen analyser in an indirect calorimeter had a measurement inaccuracy of 1%, this would result in a VO_2 calculation error of -6.46%.[166] As O_2 measurement requires the most accuracy of all the components of an indirect calorimeter, Gore et al recommended that an O_2 analyser has a measurement accuracy of at least 0.05%.[166] This recommendation was adopted for this thesis.

4.5.4 Gas measuring equipment

Oxygen sensor

An AEI model S-3A-II analyser (AEI Technologies, Inc. PA, USA) with a specified accuracy of 0.01% and dual inputs was chosen. One input measured inspired O_2 concentration from room air and the second input measured expired O_2 concentration.

Carbon dioxide sensor

An AEI model CD-3A analyser (AEI Technologies, Inc. PA, USA) with a specified accuracy of 0.02% was chosen to measure expired CO_2 concentration and intermittent measurement of inspired CO_2 concentration in room air.

4.5.5 Suction flow for gas sensors

To generate a suction flow for sample gas to be drawn through a gas sensor, Evans et al and Thureen et al used in-line air pumps. An airflow pump manufactured by AEI (model R2) was trialled for use in the custom built indirect calorimeter to draw sample gas through the gas sensors with an approximate flow rate stability of 2%. However this device was expensive, contained a vibrating bellows and was rated unacceptable as it was both large and noisy.

Considering that wall suction was available in the post-natal ward where experiments were planned to be carried out, a simple alternative was used. When connected to wall suction at a pressure of -540 mmHg, a 1.5 m length of 0.5 mm bore PVC tubing produces a high resistance to air flow through the PVC tubing. This results in a silent, stable suction flow rate of 50 ml/minute through the tube (Figure 4.3). A 1.5 m length of small bore tubing was coiled up to keep it out of the way and one was used for each gas sensor.

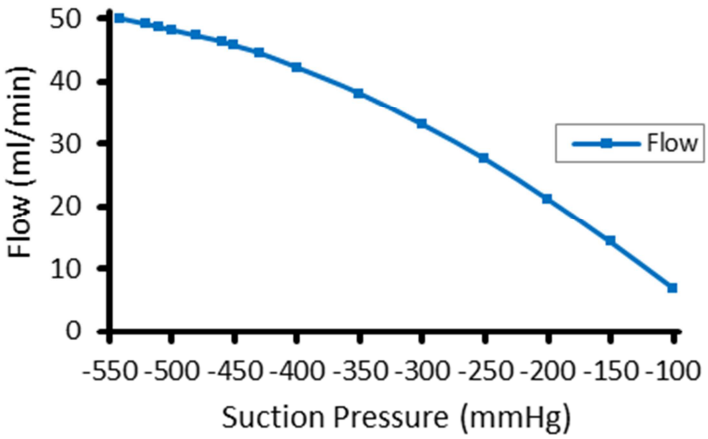


Figure 4.3 Using small bore tubing to control suction air flow through sensors
Results of flow versus suction (vacuum) pressure for a 1.5 m length of small bore (0.5 mm) PVC tubing from creating a resistance to suction air flow. A 1% change in wall outlet vacuum pressure at -540 mmHg, produced only a 0.5% change in suction flow through the PVC tubing. This allowed a steady flow of sample gas to be drawn

through each gas analyser.

4.5.6 System air flow and barometric pressure

A Puritan-Bennett model PTS-2000 (TSI Incorporated, MN, USA) mass type flow meter measured system gas flow with a specified accuracy of 1.75%. The PTS-2000 also measured atmospheric pressure with a specified accuracy of 0.7%.

4.5.7 Ambient temperature

Ambient temperature was measured using a red dye in alcohol thermometer (Vickers Instruments, UK) with an accuracy of 2%.

4.5.8 Uninterruptible power supply

The AEI oxygen analyser uses a high temperature (750°C) zirconium oxide sensor that requires 24 hours of operation to fully stabilise. Therefore the O_2 analyser was kept running for at least 24 hours before an experiment. To avoid measurement drift from switching off the O_2 analyser when travelling to the post natal ward to conduct an experiment, a 240 V, 1,000 W uninterruptible power supply (UPS) kept the AEI O_2 analyser running.

The AEI carbon dioxide analyser uses an infra-red light beam to pass through a sample gas window to measure CO_2 gas concentration and requires at least 4 hours to fully stabilise. Another 240V, 1,000 W UPS kept the AEI CO_2 analyser running while the system was taken from one location to the location of the experiment.

4.5.9 Recording gas analyser measurements

A second datalogger was used to directly measure analogue voltage outputs from the gas analysers. The oxygen analyser output was normally 2.1 V for 21% O_2 (corresponding to inspired air) and 2.05 V for 20.5% O_2 (corresponding to expired air), representing a range of 0.05 V for a typical experiment. Although the datalogger has 16 bit input resolution, a typical change of 0.05 V for the 0 to 10 V range lacked suitable precision. As the datalogger also had a 0 to 50 mV input range, a resistive voltage divider was used on the output of the O_2 analyser to allow use of the 0 to 50 mV

datalogger input, such that the maximum expected change in O_2 concentration equalled 50 mV.

The CO_2 analyser output was approximately 0.02 V with 0.04% CO_2 (corresponding to inspired air) and 0.25 V for 0.5% CO_2 (corresponding to an average of system gas containing expired breath), representing a range of 0.23 V for a typical experiment. Once again, the 0 to 10 V input range was not suitable. Using a resistive voltage divider on the output of the CO_2 analyser allowed use of the 0 to 50 mV datalogger input such that the maximum expected change in CO_2 value corresponded to 50 mV. Each measurement recorded on the datalogger was given a corresponding time code. This allowed data synchronising with other measured parameters such as core and body temperatures that were recorded on another datalogger during the same experiments.

4.5.10 Gas calibration

To ensure optimum accuracy of results from each experiment, a calibration of the gas sensors used in the custom built indirect calorimeter was required. Two certified calibration gases were used to cover the range of expected oxygen and carbon dioxide concentrations encountered in experiments. Calibration gas mix 1 was 21.09 ±0.02% O_2, 0.0305 ±0.0002% CO_2 balance N_2. This was similar to inspired air gas concentrations. Calibration gas mix 2 was 20.445 ±0.01% O_2, 0.507 ±0.01% CO_2 balance N_2. This was similar to measurement concentrations seen during experiments.

Two point gas calibrations for both O_2 and CO_2 were performed after each experiment with no change to the system air flow setting, as well as using the same flexible gas collecting hose to entrain the calibration gas. The same datalogger recorded a minimum of ten calibration values for each measurement when steady state was reached. This was repeated for the second calibration gas. Results were downloaded from the datalogger to a computer and imported to a MS Excel spreadsheet. The calibration values were averaged and then converted to % gas concentration using a linear conversion equation (y=mx+b) where x is the measured value in mV and y is the output value in $\%O_2$ or $\%CO_2$.

The accurate calibration gases of O_2 and CO_2 along with highly accurate O_2 and CO_2 gas sensors ensure high measurement accuracy of gas concentration. The mathematical formula for calculating VO_2 and VCO_2 has also been verified in the preceding pages.

While the Datex Deltatrac metabolic monitor has been used in published studies of spontaneously breathing neonates,[185-188] this device was withdrawn from sale in Australia in the mid-1990s and none was available to compare results. Indirect calorimetry metabolic results from published studies of healthy spontaneously breathing neonates have shown similar magnitude to the values obtained with this custom built indirect calorimeter.[169, 185, 186, 189]

4.6 CONCLUSION

A non-invasive, open-circuit mixing chamber type indirect calorimeter was custom assembled for experiments in the next Chapter. This style of indirect calorimeter allows spontaneously breathing newborns to sleep during each experiment. Therefore any changes in metabolism are not likely to be due to crying or gross movement. The combined gas law equation merged with Haldane's transform was used in this thesis to calculate VO_2 and VCO_2 from measured parameters.

The components of the custom built open circuit indirect calorimeter are shown in the next Chapter (Figure 5.1). Experiments in the next Chapter investigate body temperatures during periods of mild warming and cooling as well as simultaneous measurement of oxygen consumption.

CHAPTER FIVE

5 EXPERIMENTS TO MEASURE BROWN ADIPOSE TISSUE ACTIVITY IN HEALTHY TERM GESTATION NEONATES

5.1 INTRODUCTION

In this Chapter, the relationship between temperature in the lower oesophagus (T_{oes}), rectal temperature (T_{rec}), interscapular skin temperature (T_{scap}) and oxygen consumption is investigated during mild environmental warming and cooling with healthy neonates.

I speculated that T_{scap} reflected the temperature of BAT, although this has never been confirmed in the literature by comparison with direct BAT temperature measurement. The aim of this experiment was to determine if changes in oxygen consumption during warming and cooling were reflected by changes in T_{scap}.

Prior to this study, an enclosed infant incubator (Dräger Medical, Incubator 8000, Lubeck, Germany) was used to warm and cool 20 healthy term neonates to study metabolism and thermoregulatory responses. Five subjects were placed prone on the mattress and due to substantial heat loss from the back of the subjects during cooling, T_{scap} also cooled greatly, masking any sign of thermogenic activity. With the fifteen subjects place supine on the mattress, T_{scap} was observed as being influenced by continued heating under the mattress of the incubator during the 30 minute cooling period. Modifications to the incubator were unable to eliminate heating of the mattress base, therefore changes in T_{scap} were uninterpretable and results from these experiments were not used.

To avoid any interference of T_{scap} from heating under the mattress, an open radiant warmer (Dräger Medical, Model Babytherm, Lubeck, Germany) was used to control subject temperature. Healthy term gestation neonates were studied to avoid the influence of illness or medications on thermoregulation. Neonates were studied supine because critically ill neonates undergoing hypothermia are routinely positioned on their backs. T_{scap} was compared to a low-back reference skin temperature (T_{lobak}) as both of these sites were in

contact with the mattress and T_{lobak} is away from BAT.[122, 127] Temperature beneath the mattress (T_{submat}) was measured to document heat gain/loss through the insulating mattress. Absolute changes in T_{scap} and changes in gradients between T_{scap} and reference sites were analysed. VO_2 and RER were measured to relate observed temperature changes to metabolic activity.

The novel aspects of this study are: (i) T_{scap} has not previously been compared to both T_{oes} and T_{rec} simultaneously; (ii) changes in T_{scap} with mild environmental cooling have not previously been correlated with oxygen consumption in human neonates; (iii) high resolution infra-red (IR) imaging has not been utilised to explore BAT activation and the influence of limb temperatures on core temperature sites.

5.2 METHODS

Trial design and study setting

Spontaneously breathing, healthy term gestation neonates aged within 2 days of birth and with a stable and normal axillary temperature (36.2 – 37.2°C) when clothed and admitted to the Flinders Medical Centre post natal ward were eligible for enrolment.

Institutional ethics approval was granted by the Southern Adelaide Clinical Human Research Ethics Committee and informed consent was obtained from a parent.

System for varying environmental temperature

The equipment set-up for experiments is shown in Figure 5.1. At the start of each 90 minute experiment, the neonate was undressed (except for a standard disposable nappy) and placed supine on an open radiant warmer mattress. The overhead radiant warmer was initially set to manual heater control at 60% heater output. An exposed abdominal skin temperature (T_{belly}) sensor was applied to the subject and when T_{belly} reached approximately 36.0°C, the radiant warmer operating mode was altered to run on servocontrol to a skin temperature set point of 36.0°C (standard nursery practice). This avoided the radiant heater running at 100% when first switched on. Servo-control of T_{belly} was used for 30 minutes of initial warming and later for 30 minutes of rewarming.

After the 30 minute initial warming period, the radiant warmer was turned off for 30 minutes (cooling period) to expose the subject to room temperature (approx. 25°C). The final 30 minutes (rewarming period) was used to re-warm the subject using the same method as for initial warming.

The author and a neonatal research nurse were in attendance for the duration of each experiment.

Three litre saline bag test

To determine how changes in radiant heating affect the temperature of a surface in contact with the mattress, in order to interpret changes in T_{scap} in neonates during simulated normothermia, a 3 litre clear PVC bag filled with normal saline was placed on an open incubator mattress. The upper surface of the bag was exposed to the radiant heater and had a skin sensor applied with an adhesive hydrogel cover (standard nursery practice). This temperature measurement site was termed '$T_{bag\text{-}surf}$'. The lower surface of the bag in contact with the mattress had a skin sensor applied with an adhesive hydrogel cover. This temperature measurement site was termed '$T_{bag\text{-}mat}$'. Core temperature of the bag was also measured using a medical temperature sensor. This temperature measurement site was termed '$T_{bag\text{-}core}$'.

After reaching steady state temperature (approx. 37°C), the incubator heater output was adjusted from 50% to either 100% or 0%, to simulate a heater-on or heater-off step transition respectively. Measurements were recorded on a datalogger every second.

Metabolic monitoring

A non-invasive, open-circuit mixing chamber type indirect calorimeter was custom built for the experiments in this Chapter as detailed in Chapter 4. This style of indirect calorimeter allows spontaneously breathing newborns to sleep during each experiment. To avoid post prandial thermogenesis, experiments commenced approximately one hour after feeding.

There were intermittent short periods of no expired breath collection due to the neonates stirring and head movement which resulted in lost data. During these intermittent short periods of no expired breath measurement, room air was sampled. This allowed measurement of room CO_2 concentration.

Due to irregular minute to minute RER and VO_2 values (Figure 5.5), results from each of the three main 30 minute periods were subdivided into three 7 minute segments for each 30 minute period. This resulted in nine 7 minute segments for each experiment. For uniformity, all simultaneously measured parameters were averaged over the same 7 minute segments and VO_2 and VCO_2 calculations were normalised to birth weight.

Gas calibration

Shortly after each experiment, the indirect calorimeter was operated at the same system flow rate that was used during the experiment and a two point calibration for both O_2 and CO_2 was performed as outlined in Chapter 4.5.10.

Heater-off step down transition

After approximately 30 minutes of initial warming, the radiant heater was turned off to produce a step down transition. To closely examine the data associated with the heater-off step event, results from the last initial warming period (W3) were subtracted from the same parameters in the first cooling segment (C1) immediately following the step down transition. The rationale for this process was to observe T_{scap} during a period in which exposed skin temperature began to fall sharply while changes in core temperature remained small.

Temperature measurement and recording

Simultaneous temperature measurement and data storage occurred every second using a small, electronic datalogger as outlined in Chapter 3.5.

Core temperature sites

There were two core temperature measurement sites: (i) rectum (T_{rec}), using a Dräger 7.5 Fr probe inserted 6 cm from the anal verge; (ii) lower oesophagus (T_{oes}) using a Philips 'Innersense' combined feeding tube 5 Fr inserted nasally so that the temperature sensor was at the approximate level of T7 or T8 using the formula body length cm/4 +3 cm.

Skin temperature sites

The following temperature measurement sites used a Tyco 'IncuTemp 5' skin sensor with a Dräger hydrogel adhesive cover: (i) inter-scapular skin in contact with the mattress; left and right of the spine (T_{scap-l}, T_{scap-r});

(ii) lumbar skin in contact with the mattress but away from BAT (T_{lobak});
(iii) exposed abdominal skin (T_{belly}).

Inter-scapular skin was expected to be in contact with the mattress and the side most in contact with the mattress was used for analysis as some subjects repositioned themselves with one shoulder not in full contact with the mattress resulting in heat loss. This was evident when one inter-scapular temperature was lower than the other.

Occasional patient movement or sensor detachment artefact that resulted in a rapid reduction of core/skin temperature (< 32°C) was removed from analysis.

Environmental temperature

Environmental temperature (T_{amb}) was measured with a Fisher and Paykel skin temperature sensor attached to a small aluminium (black anodised) heat sink placed on the mattress near the feet. Temperature beneath the middle of the mattress was measured with Tyco 'IncuTemp 5' sensor.

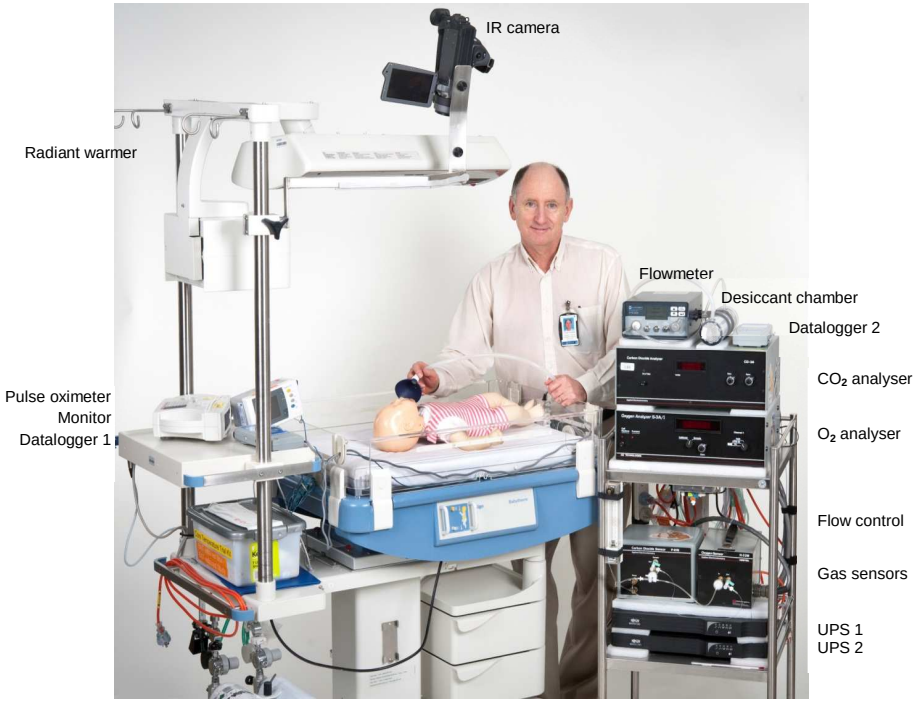


Figure 5.1 Equipment used for experiments

The equipment setup included an open radiant warmer, patient monitor, pulse oximeter, infra-red camera, air collection hose with soft rubber funnel, gas mixing and drying chamber, air flow meter, air flow regulator, CO_2 and O_2 analysers, two dataloggers and two uninterruptible power supplies (UPS).

Temperature sensor and datalogger calibration

Calibration of temperature sensors and the datalogger occurred shortly after each experiment using methodology described in Chapter 3.5.6. Based on calibration results, all temperatures were corrected before analysis.

Thermal imaging

An infra-red (IR) camera was mounted approximately 1m above the subject and automatically recorded thermal images of exposed skin every 10 seconds for the duration of each experiment. IR images allowed non-contact skin temperature measurements at multiple sites post experiment using FLIR thermal imaging software. Sites chosen for measurement were: (i) nape of the neck; (ii) skin above heart; (iii) upper arm; (iv) lower arm; (v) thigh; (vi) lower leg; (vii) mattress near the head; (viii) mattress near the feet. The average temperature of a 1 cm diameter circle was used for each measurement site except for the nape of the neck where an average was used of a 1 cm line positioned above the neck crease (Figure 5.2).

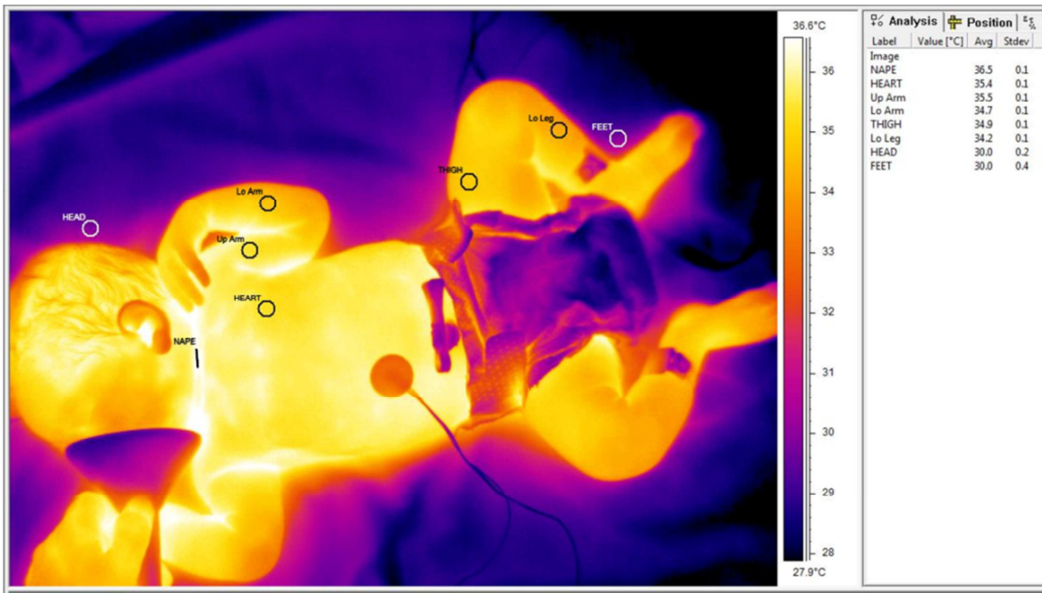


Figure 5.2 Infrared (IR) imaging method

An example of an IR image of a subject lying supine at the end of the cooling phase. A colour scale to the right of picture represents temperature. Sites measured: (i) nape of the neck, (ii) skin above heart, (iii) upper arm, (iv) lower arm, (v) thigh, (vi) lower leg, (vii) mattress near the head and (viii) mattress near the feet. The average of a 1 cm diameter circle (or 1 cm line in the case of the nape) was used to measure temperature at sites using FLIR Thermacam Researcher 2.9 software.

Heart rate measurement

Heart rate was analysed to determine if any changes seen in VO_2 during cooling were related to cardiac work. A Masimo pulse-oximeter (Masimo Model Radical 7, Masimo, CA, US) with an adhesive wrap sensor was placed on the subject's foot to provide a simple method of heart rate measurement. A 1V analogue output signal corresponded to a heart rate (HR) of 250 beats per minute (bpm). This signal was divided down (1/20) to suit a 50 mV input to a second datalogger. The pulse oximeter averaged readings over an 8 second period while a second datalogger recorded the analogue output every second.

Heart rate calibration

A pulse oximeter tester (Pronk Technologies, OxSim-1, CA, USA) was used to calibrate the datalogger connected to the pulse oximeter. The OxSim-1 provided a simulated HR output accurate to ±1 digit at 80 and 140 bpm. A linear equation converted measurements from mV to bpm.

Statistical methods

Data were analysed using IBM SPSS version 22 (IBM Corp. NY, USA). Measurements from each subject were averaged every 1 minute using all 1 second data. For comparison between parameters, an independent samples T-test was used to calculate mean difference and statistical significance. Where a difference was made with a particular parameter at two different times, a paired samples T-test was used to calculate mean difference and statistical significance. During warming and cooling, a Spearman's ranked correlation (r_s) was used to assess the association between VO_2 and: RER, HR and T_{scap}. Data are presented as mean ±standard error (SE) unless indicated otherwise. The alpha level for statistical significance was chosen as $p<0.05$.

5.3 RESULTS

Three litre saline bag test

A 3 litre bag filled with normal saline was placed on the open incubator mattress. The bag core temperature was in thermal steady state for at least 15 minutes at approximately 37°C before the incubator heater output was adjusted from 50% to either 100% or 0%, to simulate a heater-on or heater-off step transition. Results from 15 minutes before and 30 minutes after a step transition are shown in Figure 5.3.

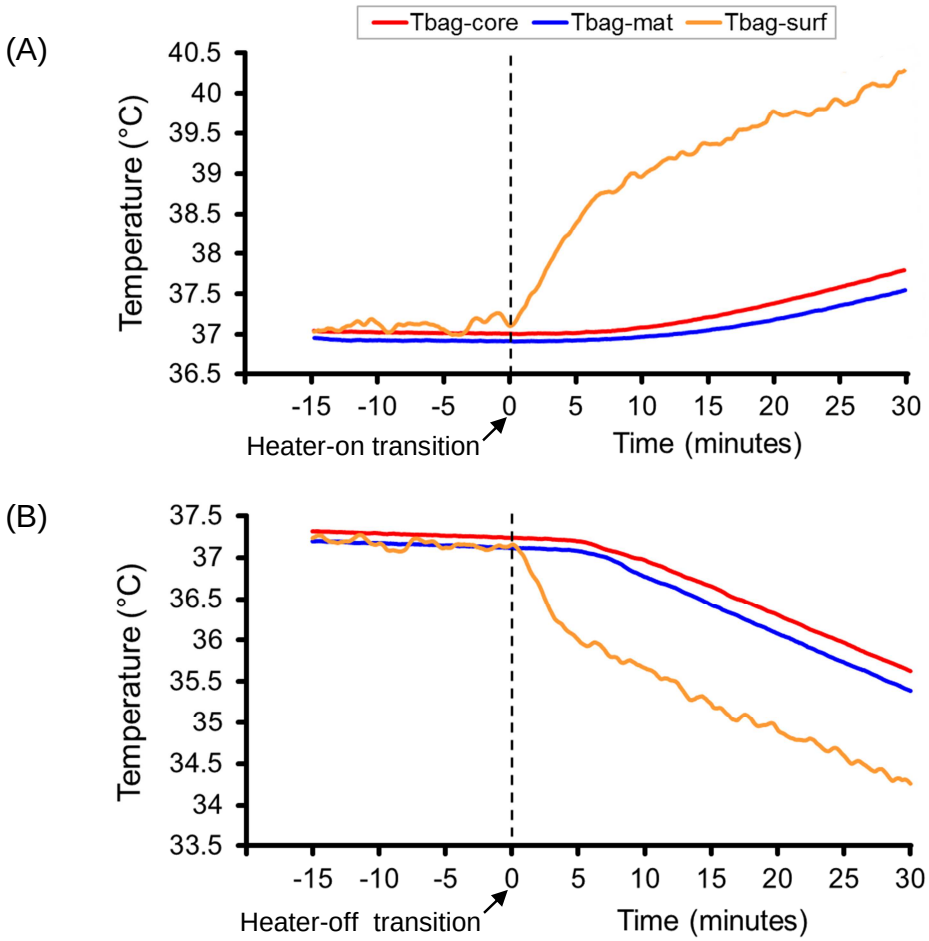


Figure 5.3 3 litre saline bag temperatures on an open incubator mattress

To simulate heater-on and heater-off step transitions, a 3 litre saline bag was maintained in thermal steady state (approx. 37°C) with 50% radiant heater output. (A) The radiant heater output was suddenly increased to 100%, rapidly warming the upper bag surface ($T_{bag\text{-}surf}$). The lower bag surface was in contact with the mattress ($T_{bag\text{-}mat}$). Both $T_{bag\text{-}mat}$ and the bag core temperature ($T_{bag\text{-}core}$) slowly increased at approximately 10 minutes after the heater increase. (B) The heater output was reduced from 50% to 0%.

The data show that with 50% heater output, $T_{bag\text{-}core}$ was maintained near thermal equilibrium and that $T_{bag\text{-}mat}$ was slightly cooler than $T_{bag\text{-}core}$. After the heater-on step transition, $T_{bag\text{-}mat}$ and $T_{bag\text{-}core}$ showed a lag time of approximately 10 minutes before increasing. After the heater-off step transition, $T_{bag\text{-}mat}$ and $T_{bag\text{-}core}$ showed a lag time of approximately 7 minutes before decreasing.

Experiments with healthy term neonates

Five male subjects were studied with the demographics, environmental conditions and study parameters shown in Table 5.1.

	Mean	Range
Gestational age (weeks)	39.14	38.57 – 39.57
Postnatal age (days)	1.4	1 - 2
Birth weight (g)	3716	3420 - 4120
Room air temperature (°C)	25.1	24.8 – 25.5
Atmospheric air pressure (mmHg)	759.8	756.1 – 763.8

Table 5.1 Demographics and study conditions of five healthy normothermic neonates

Environmental and body temperatures

A summary of the results of all parameters measured is presented in Table 5.2. Figures 5.4 (A, B and C) show temperatures for the three initial warming periods along with the three cooling periods followed by three rewarming periods using a progressively narrower temperature range to better view the progression of specific temperatures. SE was less than ±0.05°C for all body temperatures and therefore not included in Table 5.2. SE was less than 0.02 for RER, less than 0.25 ml/kg/min for VO_2, and less than 2.4 bpm for HR in Table 5.2.

During the initial warming period, T_{scap} and T_{lobak} took approximately 10 minutes to stabilise as the open radiant warmer mattress was not preheated before experiments.

PERIOD	W1	W2	W3	C1	C2	C3	RW1	RW2	RW3
VO_2 (ml/kg/min)	6.63	6.11	6.02	6.23	7.39	7.92	7.33	7.00	6.52
RER	0.76	0.77	0.80	0.80	0.81	0.81	0.78	0.76	0.74
T_{oes} (°C)	37.33	37.36	37.37	37.34	37.24	37.18	37.20	37.27	37.34
T_{rec} (°C)	37.10	37.13	37.13	37.16	37.11	37.03	36.97	37.00	37.04
T_{scap} (°C)	36.98	37.04	37.10	37.17	37.19	37.17	37.12	37.14	37.18
T_{lobak} (°C)	36.85	36.95	37.04	37.05	37.06	37.03	36.98	37.01	37.05
T_{belly} (°C)	36.44	36.45	36.43	35.97	34.87	34.72	36.44	36.41	36.40
T_{amb} (°C)	32.76	33.46	32.91	31.60	26.94	26.31	32.55	32.67	32.92
T_{submat} (°C)	25.61	25.61	25.68	25.67	25.65	25.61	25.57	25.61	25.73
HR (BPM)	130	121	128	130	133	132	124	128	131

Table 5.2 Mean results of indirect calorimetry and body temperatures from healthy normothermic neonates

Mean values of indirect calorimetry, body and environmental temperature, and heart rate (HR) during nine 7 minute stages: initial warming (W1 – W3); heater off (cooling, C1 – C3); re-warming (RW1 – RW3) from five healthy full term newborn neonates lying supine on an open radiant warmer mattress.

Mean temperature differences between sites

Over all stages (W1 - RW3), T_{oes} was 0.22 ±0.01°C ($p<0.001$) warmer than T_{rec}, and T_{scap} was 0.12 ±0.02°C ($p<0.001$) warmer than T_{lobak} (Figure 5.4).

Mean temperature differences at the end of each section

At the end of initial warming (W3) the $T_{oes} – T_{rec}$ difference was 0.24 ±0.03°C ($p<0.001$). This temperature difference reduced to 0.15 ±0.05°C ($p=0.001$). At the end of cooling (C3) the T_{oes} - T_{rec} difference increased to 0.30 ±0.04°C ($p<0.001$) at the end of re-warming (RW3).

At the end of initial warming (W3) the $T_{oes} – T_{scap}$ difference was 0.27 ±0.05°C ($p<0.001$). There was no significant temperature difference at the end of cooling (C3), i.e. 0.01 ±0.05°C ($p=0.90$). The $T_{oes} – T_{scap}$ difference increased to 0.16 ±0.05°C ($p=0.005$) at the end of re-warming (RW3).

At the end of initial warming (W3) there was no significant difference $T_{rec} – T_{scap}$ i.e. 0.03 ±0.05°C ($p=0.5$). At the end of cooling (C3) the temperature difference $T_{rec} – T_{scap}$ inverted (T_{scap} warmer than T_{rec}) to -0.14 ±0.04°C ($p<0.001$) and this temperature difference continued at -0.14 ±0.05°C ($p=0.01$) at the end of re-warming (RW3).

At the end of initial warming (W3), T_{scap} was not significantly warmer than T_{lobak} i.e. 0.05 ±0.06°C ($p=0.38$). However T_{scap} was significantly warmer than T_{lobak} at the end of cooling (C3) i.e. 0.15 ±0.05°C ($p=0.003$). The $T_{scap} – T_{lobak}$ difference remained similar (0.14°C ±0.05°C, $p=0.01$) at the end of re-warming (RW3).

Correlation between VO_2 and T_{scap}

From warming to cooling (W1 to C3), there was a moderate, positive correlation between VO_2 and T_{scap} ($r_s = 0.36$, $p<0.001$).

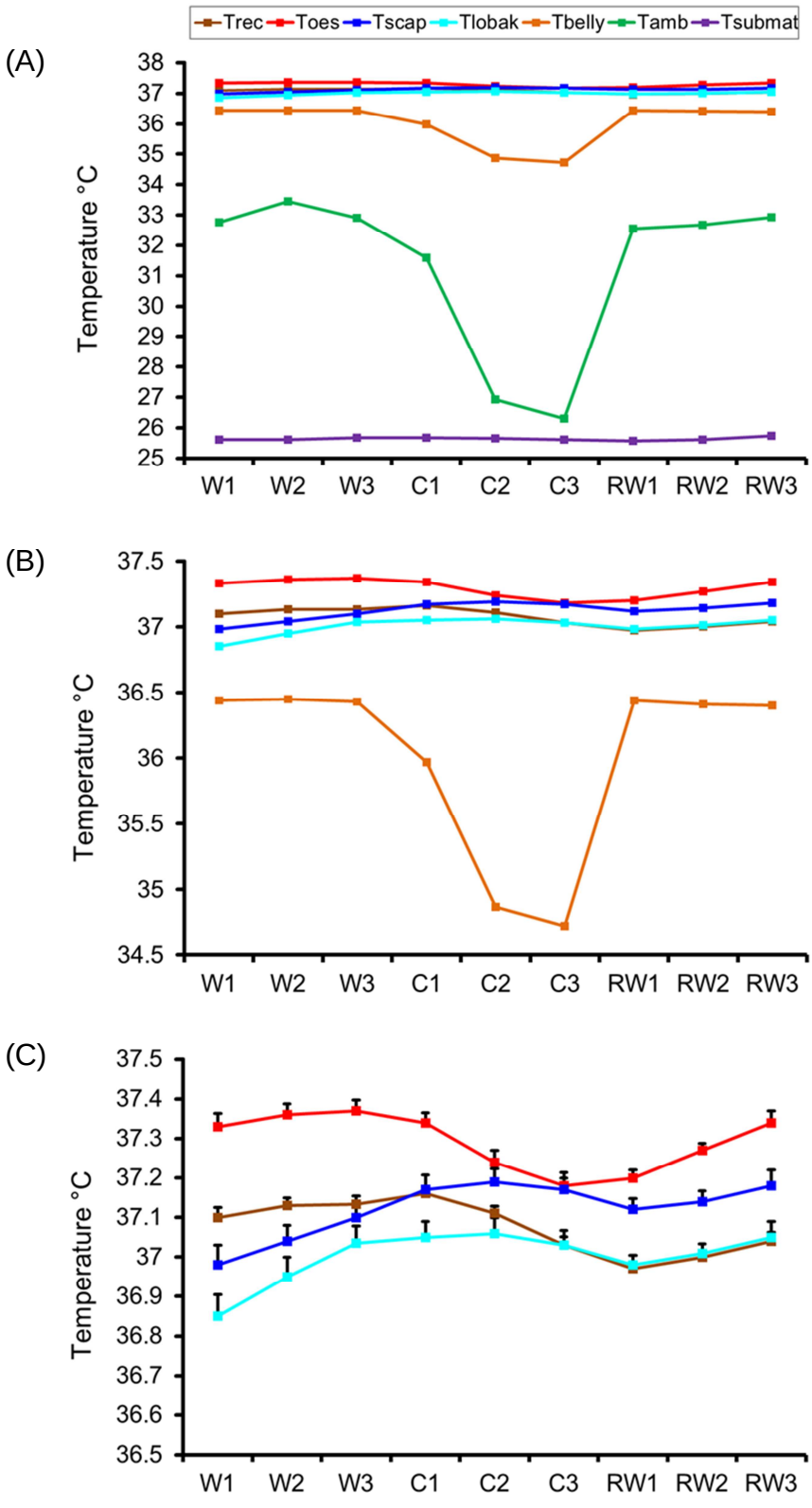
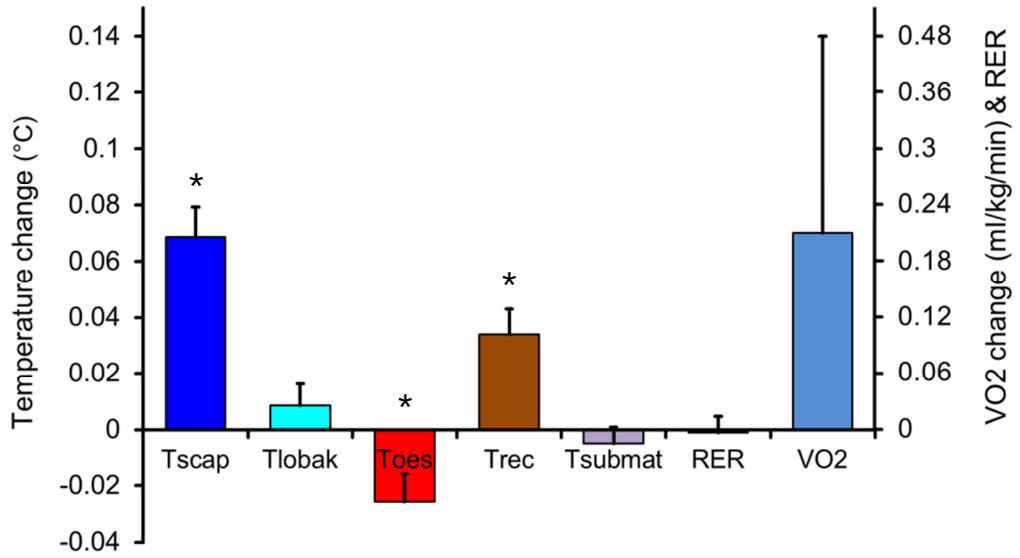


Figure 5.4 Mean temperatures during three 7 minute stages

Environmental and body temperatures from nine 7 minute sequential periods beginning with initial warming (W1 – W3), followed by no warming (mild cooling, C1 – C3), and then re-warming (RW1 – RW3) from five subjects lying supine on an open radiant warmer mattress. Figures (A, B and C) show the same data presented with a progressively narrower temperature scale with +SE shown in (C).

Heater-off step down transition

After 30 minutes of initial warming, the radiant heater was turned off to produce a step-down transition (W3 to C1). During this step-down transition T_{belly} and T_{oes} decreased by 0.47 ±0.07°C ($p<0.001$) and 0.03 ±0.01°C ($p=0.02$) respectively. The following parameters increased: T_{scap} by 0.07 ±0.01°C ($p<0.001$), and T_{rec} by 0.03 ±0.01°C ($p=0.001$) as shown in Figure 5.5. T_{lobak}, T_{submat}, RER and VO_2 showed no significant change with this transition i.e. 0.01 ±0.01°C ($p=0.26$), -0.01 ±0.01°C ($p=0.32$), 0.00 ±0.02 ($p=0.87$) and 0.21 ±0.21 ml/kg/min ($p=0.34$) respectively.



***** $p<0.05$

Figure 5.5 Parametric changes as a result of turning the heater off

Parameters for each subject were averaged every minute for 7 minutes immediately before the radiant heater was turned off (W3), and subtracted from the same parameters for 7 minutes following the 'heater off' transition (C1). Mean (+SE) differences are shown for inter-scapular skin temperature (T_{scap}), low-back skin temperature (T_{lobak}), lower oesophageal temperature (T_{oes}), rectal temperature (T_{rec}), and the temperature beneath the incubator mattress (T_{submat}), respiratory exchange ratio (RER) and oxygen consumption (VO_2). RER and VO_2 are shown with a separate scale so all results can be compared on the same graph.

Infra-Red temperatures

Temperature values of IR images during warming showed that some of the IR radiation emitted from the radiant heater was reflected from the skin surface, resulting in elevated IR skin temperatures rendering measurements unreliable (Table 5.3, W3). Hence IR temperatures were only used for analysis from the three cooling segments (C1 to C3).

Mean (±SE) temperatures from IR images are shown in Table 5.3 and include the end of initial warming (W3) through to the end of cooling (C3). During the progression of cooling (C1 to C3), all temperature sites showed a gradual decrease, except for T_{nape} which increased (in all subjects) by a mean ±SE of 0.22 ±0.07°C (p=0.04) from mid cooling (C2) to the final cooling segment (C3). The mean ±SE difference between nape and heart was initially 0.90 ±0.17°C (C1, p=0.006), then 0.88 ±0.14°C (C2, p=0.003) and finally 1.20 ±0.10°C (C3, p<0.001). There was no significant temperature difference between the mattress surface near the head to near the feet i.e. 0.50 ±0.29°C (C2, p=0.16).

Experiment period	W3	C1	C2	C3
Nape of neck (°C)	37.90 (0.17)	36.26 (0.13)	36.08 (0.23)	36.30 (0.16)
Skin above heart (°C)	37.56 (0.23)	35.36 (0.09)	35.20 (0.17)	35.10 (0.16)
Upper arm (°C)	37.20 (0.14)	35.50 (0.23)	35.38 (0.27)	35.14 (0.19)
Forearm (°C)	36.86 (0.29)	34.84 (0.07)	34.62 (0.15)	34.40 (0.16)
Thigh (°C)	37.08 (0.25)	34.76 (0.07)	34.18 (0.17)	33.78 (0.21)
Lower leg (°C)	36.30 (0.22)	34.48 (0.17)	34.34 (0.19)	34.02 (0.31)
Mattress near head(°C)	36.02 (0.59)	29.60 (0.31)	28.44 (0.37)	28.06 (0.42)
Mattress near feet (°C)	35.74 (0.56)	29.94 (0.27)	28.94 (0.38)	28.26 (0.52)

Table 5.3 Mean IR temperature values from healthy normothermic neonates during cooling

Mean IR temperatures (±SE) from eight sites (n=5) at the end of initial warming (W3) followed by initial cooling (C1), mid cooling (C2) and at the end of cooling (C3).

Indirect Calorimetry

RER showed wide fluctuations when sequentially averaged over 30 seconds (Figure 5.6). The consequence of these fluctuations was to use averaging over a 7 minute period to reduce potential error.

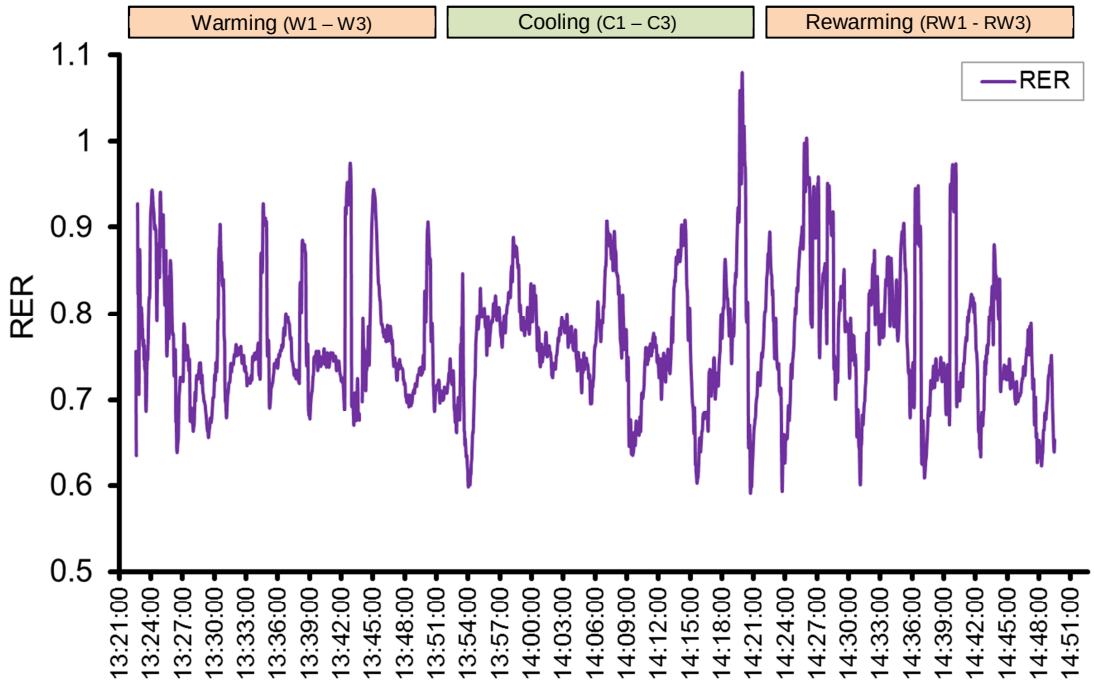


Figure 5.6 Example of a ninety minute experiment showing variation in RER
An example from one, 90 minute experiment of respiratory exchange ratio (RER) showing short term fluctuations when sequentially averaged over 30 seconds. Local time is shown along the x axis. The subject was lying on their back (supine) on an open radiant warmer mattress.

VO_2 and RER along with other parameters were averaged over nine, 7 minute sequential periods (Figure 5.7). VO_2 decreased 9% during initial warming (W1 - W3), and increased 27% during cooling (C1 - C3). VO_2 then decreased 11% during re-warming (RW1 - RW3). The mean RER increased 5% during initial warming (W1 - W3), with a further 1% increase during cooling (C1 - C3). RER then decreased 5% during re-warming (RW1 - RW3).

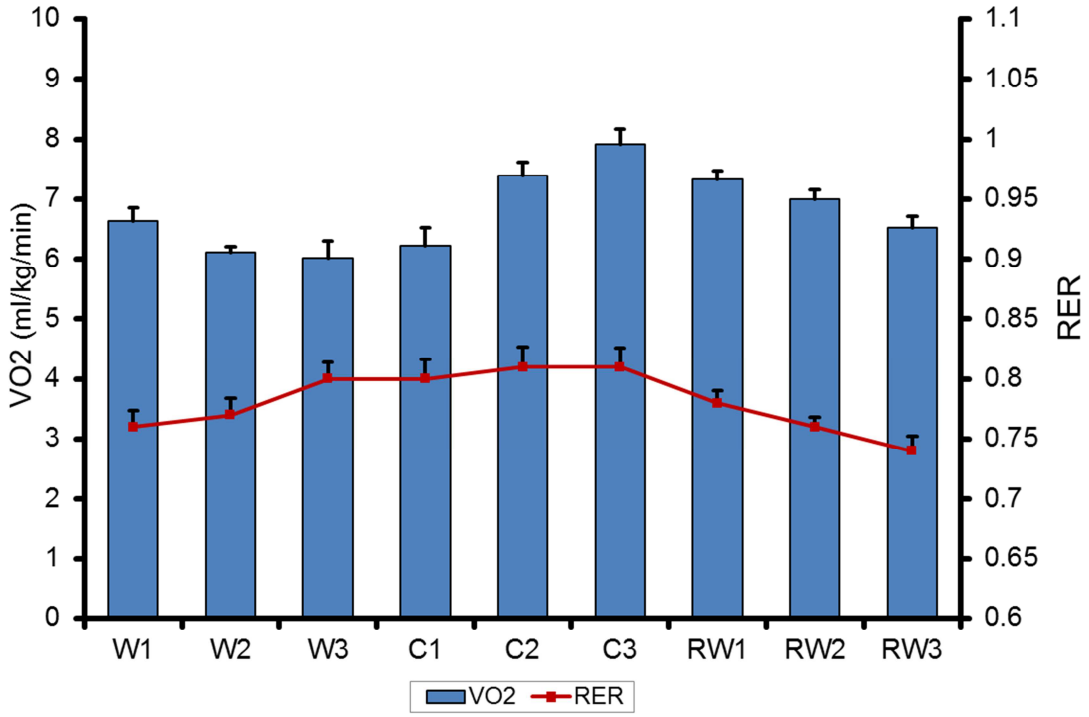


Figure 5.7 Combined plot of VO_2 and RER

Combined plot of mean (+SE) oxygen consumption (VO_2) and respiratory exchange ratio (RER) from five subjects who were placed on their back (supine) on the open radiant warmer mattress. Results were arranged into discrete seven minute periods with three initial warming periods, followed by three cooling periods and finally three rewarming periods.

Correlation between VO_2 and RER

From warming to cooling (W1 to C3), there was no correlation between VO_2 and RER (r_s = 0.01, p=0.88).

Heart Rate

HR showed variability when sequentially averaged over 30 seconds (Figure 5.8). The consequence of these fluctuations was to use averaging over a 7 minute period to reduce potential error.

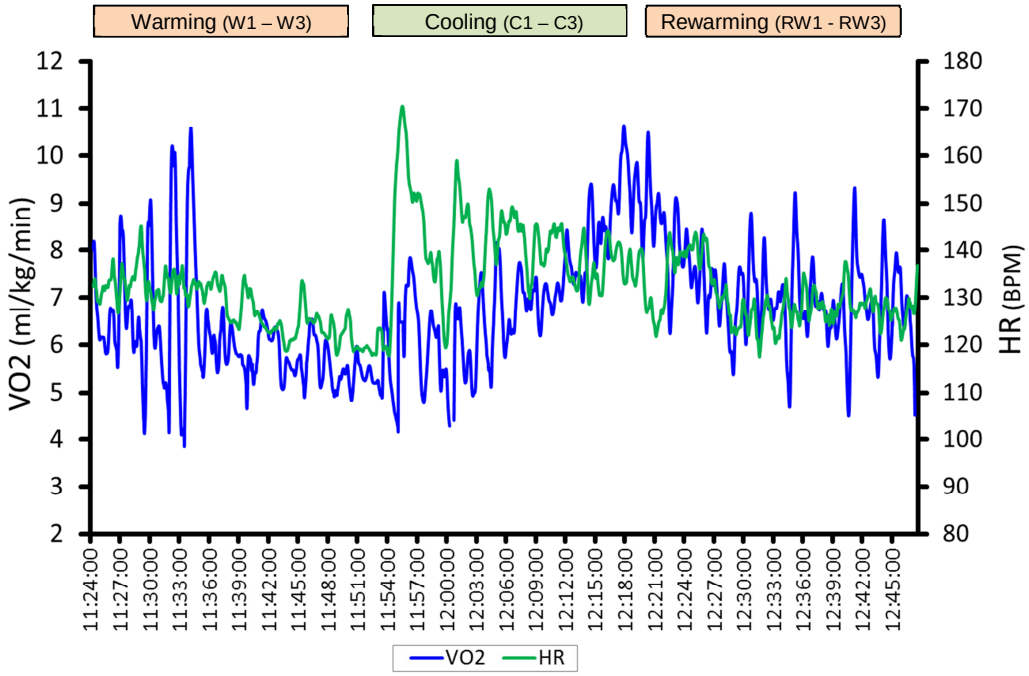


Figure 5.8 An example of HR and VO_2 averaged over 30 seconds from one experiment

An example of heart rate (HR) and oxygen consumption (VO_2) sequentially averaged over 30 seconds from one subject during an experiment during initial warming, cooling and then rewarming. Local time is shown along the x axis. The subject was lying on their back (supine) on an open radiant warmer mattress.

Correlation between VO_2 and HR

From warming to cooling (W1 to C3), there was a weak, negative correlation between VO_2 and HR (r_s = -0.17, p=0.03).

5.4 DISCUSSION

Results of baseline heater-on and heater-off step transitions with a 3 litre saline bag on an open incubator mattress showed that $T_{bag\text{-}mat}$ was slightly cooler than $T_{bag\text{-}core}$ during thermal equilibrium (50% radiant hearing). Therefore some heat loss occurred from the bag to the environment through the mattress. Although $T_{bag\text{-}mat}$ responded similarly to $T_{bag\text{-}core}$, both measures were slower to respond than $T_{bag\text{-}surf}$. At no time did $T_{bag\text{-}surf}$ transiently decrease or increase shortly after a heater-on or heater-off step transition respectively. These results form a baseline response to step changes in radiant heater output.

With healthy neonates immediately after the heater was turned off, exposed skin surfaces began to cool while T_{scap}, T_{lobak} and T_{rec} continued to increase in temperature. The behaviour of the back and core sites is likely to reflect slower response times to the drop in environmental temperature. However, T_{scap} increased to a greater extent than both T_{lobak} and T_{rec} when the heater was first turned off. During the 30 minutes of cooling T_{oes}, T_{rec} and T_{loback} reduced while T_{scap} remained relatively stable. As a result, the temperature gradient between T_{scap} and T_{loback} increased, that between T_{scap} and T_{oes} narrowed, and the gradient between T_{scap} and T_{rec} reversed. The data showed a moderate, positive correlation between T_{scap} and $VO_{2,}$ and that T_{scap} behaved differently to other sites. These results are consistent with local thermogenesis in the inter-scapular region and are similar to the findings of other authors who have studied skin and rectal temperatures during mild environmental cooling of neonates where the back is exposed.[133, 135] Interpretation of Silverman et al's and Grausz's results require caution because exposure of the back may result in differences in regional skin temperature due to variations in vasoconstriction and skin blood flow. In the current study, neonates were placed supine with their backs on the mattress. Under these experimental conditions, results should be interpreted with caution because of the possible interaction between the temperatures of the mattress surface in contact with the back.

The potential for heat retention in the mattress surface is likely to invalidate comparisons of T_{scap} to core temperatures under dynamic conditions, because the mattress could keep the skin of the back warm while the rest of the body

cooled. Interpretation of insulated skin temperature and core temperature gradients would require the system to be in steady state. However, warmth retained in the mattress close to the skin of the back is an unlikely explanation for both the sharp increase in T_{scap} when the heater was first turned off, and for the lesser initial increase and subsequent fall in T_{loback}. This is because IR imaging shows the mattress surface temperature near the head and shoulders when the heater was turned off, and mattress surface temperatures near the feet were similar over the 30 minute cooling period. Nonetheless, T_{scap} is in close proximity to the warmer upper body (head, arms, T_{oes}), while T_{loback} is close to the cooler lower body (legs, T_{rec}) and an effect of heat transmission between sites adjacent to T_{scap} and T_{loback} cannot be excluded.

IR analysis showed that the nape of neck was slightly warmer than skin above the heart with the maximum difference of 1.2°C occurring at the end of cooling (C3). Also during C3, the nape of the neck increased in all subjects (0.22°C) following the mid-cooling (C2) segment. As the exposed nape is not influenced by the mattress, measuring temperature of the nape (T_{nape}) may indicate thermogenic activity in BAT (not necessarily in the neck).

If the increases observed in T_{nape} and T_{scap} are indicative of BAT thermogenesis, then such increases should be accompanied by an increase in oxygen consumption reflecting increased metabolic activity in BAT. The data show that VO_2 increased with cooling in the absence of visibly increased motor activity or shivering, consistent with non-shivering thermogenesis in BAT and thus supporting the suggestion that the rise in T_{nape} and T_{scap} is due to BAT activity. However, interpretation of VO_2 requires careful consideration of methodological limitations and other possible causes of increased metabolic rate.

These methods are limited because an objective measurement tool to assess gross motor activity and EMG measurement of shivering activity were not used. An effect of subtle increased motor activity or subclinical shivering cannot be excluded as a cause of increased VO_2 during cooling.

An increase in heart rate alone could also possibly account for an increase in VO_2, however heart rate remained relatively stable (1% change) over the cooling period. While increased heart rate will lead to greater cardiac work and

an increase in VO_2, increased cardiac work can occur without change in heart rate. Stroke work is the product of stroke volume and mean arterial pressure, and cardiac work is the product of heart rate and stroke work. An effect of cooling on sympathetic tone and mean arterial blood pressure was not evaluated to determine a possible effect of cooling on stroke work. However, increased cardiac work is unlikely to cause the increased VO_2 noted in the cooling phase based on studies of haemodynamics during therapeutic cooling to 33-34°C.[190] These studies show that stroke volume and cardiac output are modestly reduced with cooling when compared to normothermia.

VO_2 is known to change with sleep state, being higher in REM than non-REM sleep, and this difference is exaggerated in a cool environment.[180] Sleep phase was not assessed in the current study and so an increase in REM sleep during the cooling period cannot be excluded. However, this is an unlikely explanation for the higher VO_2 based on other data that show the frequency of REM, non-REM and total sleep duration is not altered by environmental temperature.[191]

Another possible explanation for the data is a post-prandial increase in VO_2 as all neonates were studied after settling post-feeding. A post-prandial increase in VO_2 in neonates has been associated with the specific dynamic action of food.[181, 192] However, this is also unlikely to explain the observations in this study because oxygen consumption data obtained in healthy post-prandial neonates using the same VO_2 measurement methods have shown little change in VO_2 for up to 135 minutes after feeding (Dasireddy V, Carlisle T, Morris S. Postprandial oxygen consumption in newborn neonates using indirect calorimetry. Perinatal Society of Australia and New Zealand, Adelaide 2013).

While VO_2 data support the assumption that the rise in T_{nape} and T_{scap} is due to BAT thermogenesis, the behaviour of the RER data are less clear. RER would be expected to fall if BAT thermogenesis was active, due to increased fatty acid metabolism.[193] However, RER was constant during cooling indicating no change in the rate of fatty acid oxidation. This finding suggests either that RER measurements were erroneous, that an increase in fatty acid metabolism in BAT was present but not detected due to concurrent glucose metabolism, or

that the increased VO_2 is not due to BAT thermogenesis but rather metabolic activity in another tissue.

The measurement of RER was noted to be unstable, and hence the short periods of averaging (7 minutes) could have introduced error. The cause of the minute to minute variation in RER is uncertain but is a recognised phenomenon and longer averaging periods have been recommended (Gerhard Heldmaier, Marburg Uni, December 2011, personal communication). A systematic measurement error is unlikely to have prevented accurate measurement of CO_2 excretion due to calibration of the instrument and validation of sensitivity. Although ambient CO_2 levels increased over the course of the study due to the CO_2 expired by adults in the study room, F_ECO_2 and F_ICO_2 were compared simultaneously allowing accurate measurement of CO_2 excretion in the subjects.

Failure to detect a change in RER with cooling may have occurred if fatty acid metabolism in BAT was already active in the warming phase when the neonate was placed naked on a cool mattress. Alternatively, fatty acid oxidation in BAT may be masked if glucose is the predominant metabolic substrate as reported by Dawkins and Scopes.[130] A decline in RER was observed during the re-warming period at a time when BAT should be less active. These periods commenced at least 60 minutes after feeding and may reflect ketone and fatty acid substrate utilisation associated with fasting. This in turn may suggest that glucose was still a preferred metabolic substrate during the earlier period of cooling. A change in RER may therefore not be detected unless a more stressful or prolonged cooling phase caused greater stimulation of BAT such that fatty acid metabolism became predominant process.

This study found that T_{oes} was overall significantly warmer (0.22 ±0.01°C, $p<0.001$) than T_{rec} in healthy normothermic neonates and is consistent with the results of Sarkar et al.[194] During the heater-off and heater-on transitions, T_{rec} responded slower than T_{oes}. Hoque et al reported T_{rec} undershoot during four different methods of inducing therapeutic hypothermia in HIE neonates using T_{rec} to target the depth of cooling.[65] This finding may be due to T_{rec} being slow to respond. As noted in the Literature Review (Chapter 2), T_{oes} has a greater

correlation to aortic blood temperature and is more likely therefore to reflect brain temperature than T_{rec}. Therefore, both core sites should be measured on neonates requiring rescue hypothermia to allow comparative analysis.

5.5 CONCLUSION

Interpretation of skin temperatures on the back must take into consideration the potential effect of heat retention on the mattress surface. However, T_{scap} behaved differently to T_{lobak} and T_{scap} showed a moderate, positive correlation with VO_2. T_{scap} is a worthy site to be used for further investigation into detecting local thermogenesis in this region.

Comparisons of T_{scap} to core temperature sites under dynamic conditions will have limited validity because of the potential influence of mattress heat retention. However, comparisons of T_{scap} to core temperature sites may be valid under conditions of steady state.

Under dynamic conditions, an increase in T_{scap} in the first 7 minutes after ceasing overhead radiant warming, and an increase in T_{nape} measured by IR thermography after approximately 20 minutes, suggest activation of BAT. The absence of a definable fall in RER counts against BAT activity under the experimental conditions applied. However, this finding does not exclude BAT activity because the short averaging period used in this study may have introduced error.

The next Chapter investigates the effect of environmental temperature changes on the neck nape, T_{scap}, T_{oes} and T_{rec} in critically ill normothermic term gestation neonates nursed on an open radiant warmer mattress using the same body sites and methodology for temperature measurement as in this Chapter.

CHAPTER SIX

6 MEASUREMENT OF OESOPHAGEAL AND RECTAL TEMPERATURE AND BROWN ADIPOSE TISSUE ACTIVITY IN INTUBATED NORMOTHERMIC NEONATES

6.1 INTRODUCTION

The paucity of data regarding the relationship between lower oesophageal and rectal temperature (T_{oes} and T_{rec}) in the newly born human neonate was identified in the Literature Review section of this thesis. In Chapter 5, T_{oes} was observed to be higher than T_{rec} in spontaneously breathing normothermic neonates. However, in Chapter 5 neonates were studied over a short time period and, because of differing response times in T_{oes} and T_{rec}, the steady state relationship between these two sites could not be determined. An exploration of the thermal steady state behaviour of these two core sites during normothermia in critically ill ventilated neonates may assist the understanding of core temperature measurements during therapeutic hypothermia.

In the Literature Review, the potentially important influence of BAT activation on body core and brain temperature was discussed, and the gap in our knowledge regarding the behaviour of BAT in the human neonate was emphasised. Chapter 5 concluded that BAT activity may be detected by analysis of T_{scap} following step-up and step-down environmental temperature events when a neonate is nursed supine with the back insulated by a standard incubator mattress. In thermal steady state, heat loss from the back must be close to zero when the back is thermally insulated.[195] Steady state T_{scap} should therefore reflect a combination of local thermogenesis and conduction of heat from the thoracic core. Based on this principle, changes in T_{scap} relative to T_{oes} in steady state should indicate local thermogenesis.

In addition to direct skin temperature measurement, IR imaging data in Chapter 5 suggested that BAT activity or warmer blood flow from the brain may be reflected by T_{nape}. This requires IR imaging after a step-down in environmental temperature because assessment of skin temperature by IR

imaging during step-up events (radiant heater turned on) is unreliable due to reflected IR radiation from the surface of the skin.

This Chapter is based on an observational study tha

37.2°C. The overhead heater skin temperature set point was adjusted manually if axilla temperature was outside these limits.

Temperature monitoring

Two core temperature measurement sites were used. Rectum (T_{rec}) and lower oesophagus (T_{oes}). A Dräger 7.5 Fr or Philips 9 Fr probe was inserted 5-6 cm from the anal verge to measure T_{rec}. A Tyco 9 Fr oesophageal probe or Philips 'Innersense' combined feeding tube 8 Fr was inserted nasally to the level of T7 or T8 using the formula body length cm/4 +3 cm to measure T_{oes}. Three skin temperature measurement sites were used. Abdominal skin (T_{belly}) and inter-scapular skin to the left and right of the spine (T_{scap-l}, T_{scap-r}). All three sites used a Tyco 'IncuTemp 5' sensor with a hydrogel adhesive cover.

Inter-scapular skin was expected to be in contact with the mattress and the side most in contact with the mattress was used for analysis as some subjects repositioned themselves with one shoulder not in full contact with the mattress resulting in heat loss. This was evident when one inter-scapular temperature was lower than the other.

Environmental temperature (T_{amb}) was measured with a Fisher and Paykel disk style skin temperature sensor attached to a small aluminium (black anodised) heatsink placed on the mattress near the feet.

Measurement of skin temperature overlying the lower back (T_{lobak}, away from BAT), to control for potential heat retention in the mattress contacting the skin of the back and influencing T_{scap}, was not included in the present study. The rationale is that in Chapter 5 it was demonstrated that, under similar experimental conditions, T_{scap} changes showed no clear relation to T_{lobak}, implying that heat retention in the mattress did not sway T_{scap}.

A sensor connector box was placed at the edge of the mattress to allow connection of all the temperature sensors. The sensor connector box was joined by a single cable to a datalogger that was located out of the view of nursing staff and recorded temperature measurements every 10 seconds for the duration of the study.

Occasional patient movement or sensor detachment artefact that resulted in a

rapid reduction of core/skin temperature (< 30°C) was removed from analysis.

Temperature sensor and datalogger calibration

Calibration of temperature sensors and the datalogger occurred shortly after each study using methodology described in Chapter 3.5.6. Based on calibration results, all temperatures were corrected before analysis.

Thermal imaging

As in Chapter 5, an infra-red (IR) camera was mounted approximately 1m above the subject and automatically recorded thermal images of exposed skin every 10 seconds for the duration of each experiment. IR images allowed non-contact skin temperature measurements at multiple sites post experiment. Based on the results from Chapter 5, the site chosen for temperature measurement in the present study was the nape of the neck. IR measurements were made during periods of no radiant heater activity. The first measurement occurred 1 minute after the radiant heater was turned off and subsequent measurements occurred at 5 minute intervals up to 30 minutes.

Heater on and off step transitions

The overhead radiant warmer was servo-controlled to a set-point on exposed abdominal skin unless the bed-side nurse manually turned the radiant heating on or off if axilla temperature was below 36.2°C or above 37.2°C respectively. This happened occasionally and caused a step increase or decrease in T_{belly} followed by an increase or decrease in both T_{rec} and T_{oes} respectively. Response time was measured in minutes from when T_{belly} increased/decreased 0.1°C during a step change to a corresponding 0.1°C increase/decrease in T_{rec} and T_{oes}.

The first 30 minutes following the step down transition along with the prior 15 minutes was used for analysis of T_{oes}, T_{scap} and T_{nape}. If there was more than one step down event per subject, the event with the longest preceding steady state period immediately before the step event was used. A maximum of one step down event per subject was used to avoid potential bias.

Steady state core temperature

A steady thermal state was used to compare absolute temperatures from

different body sites. A steady state period was defined as having less than 0.2°C change over a continuous 60 minute period in three measurement sites simultaneously: T_{oes}, T_{rec} and T_{scap}. The last 30 minutes of steady state temperature comparisons were chosen to minimise the influence of variations in temperature lag times in different parts of the body following a change in environmental temperature. If there was more than one steady state period per subject, the longest steady state period was used with a maximum of one steady state period per subject to avoid potential bias.

Statistical methods

Data were analysed using Stata version 13.0 (Statacorp, Texas, USA). For analysis of steady state temperatures, the longest common steady state period observed across all subjects during both warming (heater-on) and cooling (heater-off) states was used. Steady state temperature recordings of longer than 30 minutes were truncated at 30 minutes to avoid bias from subjects with longer steady state recordings. One 30-minute steady state period was used for each subject in each heater state, using all 10-second temperature recordings. Mean temperature differences relative to oesophageal temperature were determined using mixed-effects linear regression to account for within-subject correlation with body site, heater status and time as the main fixed effects and a random-intercept used for the random effect of the neonate (i.e. to allow for the overall mean differences in temperatures between neonates). Two-way interactions were also included for (heater status x site), (heater status x time) and (site x time) in order to ensure that the model fitted the observed data well.

IBM SPSS version 22 (IBM Corp. NY, USA) was used to analyse temperature difference made at a particular site (T_{scap}, T_{nape}) at different times using paired samples T-tests to calculate mean difference and statistical significance. An independent samples T-test was used to calculate mean difference and statistical significance in lag time between T_{rec} and T_{oes} after a step change in environmental temperature. Results are presented as mean ±standard error (SE) unless otherwise indicated.

The alpha level for statistical significance was chosen as $p<0.05$.

6.3 RESULTS

Thirteen neonates were studied, 10 male and 3 female, with a median (range) birth weight 3135g (2310-4530g), gestation 38 weeks (34-40 weeks), and postnatal age 3 days (1-5 days). Primary diagnoses were hyaline membrane disease (6), viral infection (1), hypoxic-ischaemic encephalopathy (1), apnoea (1), anaemia (1), meconium aspiration (1), plural effusion (1) and congenital pneumonia (1). Medications administered included phenobarbitone (2), and infusions of opioid (10), midazolam (5) and dopamine (5). All neonates were initially intubated for airway support and received humidified warmed inspired gases. Three neonates were extubated during the study.

Heater-on and heater-off step transitions

Of the 13 neonates, 11 contributed step transition events for analysis. Four subjects contributed to a heater-on and a heater-off step event, 4 subjects contributed to only a heater-on step event while 3 subjects contributed to only a heater-off step event.

Figure 6.1 displays the observed means for T_{oes}, T_{scap} and T_{rec} during heater-on and heater-off step transitions at 5 minute intervals for 30 minutes after the step transition and for 15 minutes preceding the step transition.

Increases or decreases in T_{belly} caused by radiant heater on/off activity were followed by gradual increases or decreases in the same direction in T_{oes} and T_{rec}, with a tendency for T_{oes} to respond more rapidly. However T_{scap} behaved differently to each core site with an initial decrease of 0.05 ±0.007°C ($p<0.001$) ten minutes after an increase in T_{belly} when the radiant heater was turned-on, with an initial increase of 0.03 ±0.003°C ($p<0.001$) ten minutes after a decrease in T_{belly} when the radiant heater was turned-off.

Step changes in environmental temperature

The lag time was measured in minutes between a change in T_{belly} and a corresponding change in T_{rec} and T_{oes}. Mean ±SE lag times for T_{rec} and T_{oes} were 23.7 ±2.9 minutes and 15.3 ±1.6 minutes respectively. T_{rec} was 8.4 ±3.3 minutes (50%) slower than T_{oes} ($p=0.02$).

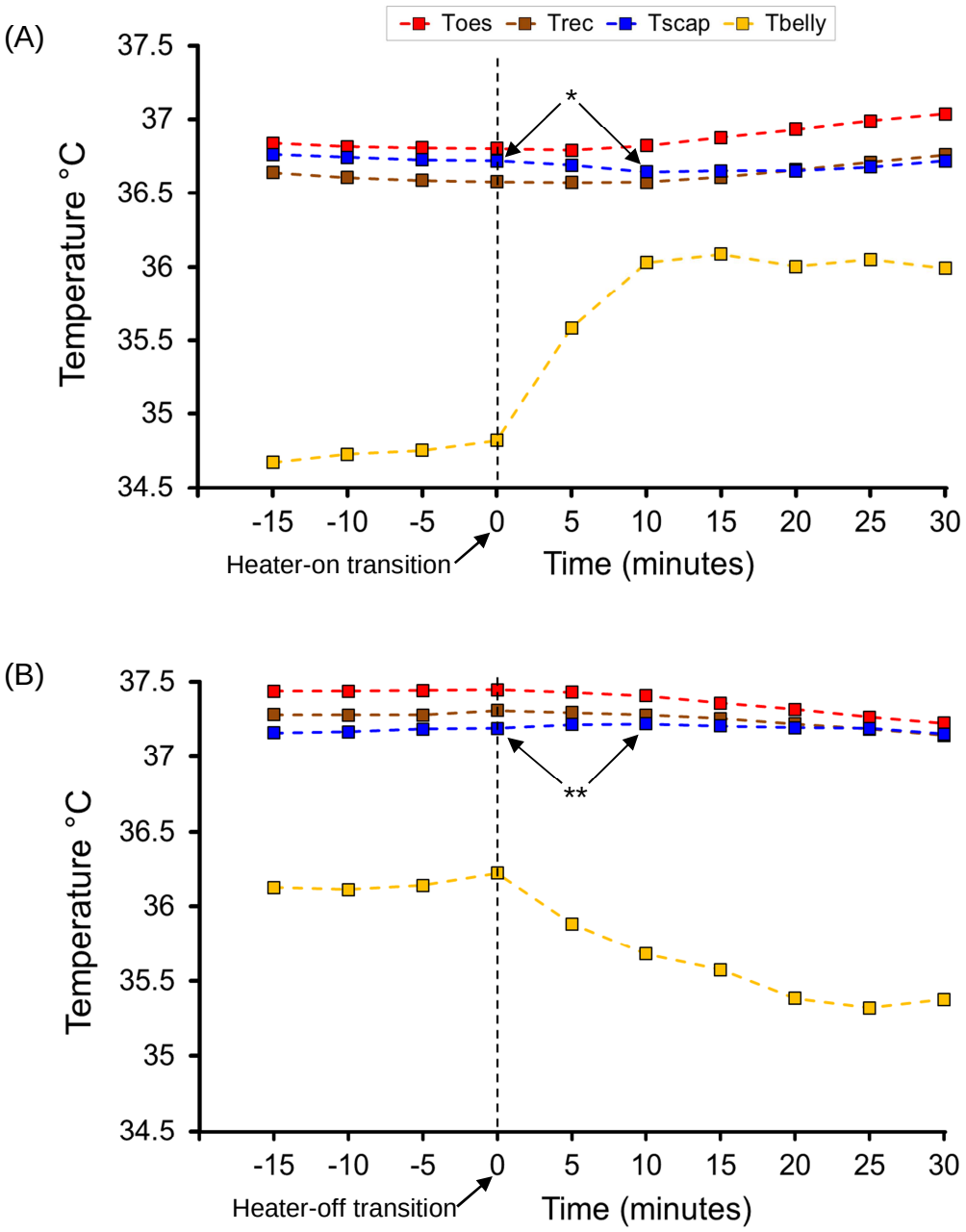


Figure 6.1 Mean temperatures during heater on/off step transitions

Mean temperatures showing: (A) a response to a step increase in radiant heater activity indicated by an increase in T_{belly} (n=8). (B) a step down in radiant heater activity shown by a fall in T_{belly} (n=7). SE was less than 0.05°C for all temperatures and therefore not shown. The overhead radiant warmer was servo-controlled to a set-point on exposed abdominal skin unless the bed-side nurse manually turned the radiant heating on or off if axilla temperature was below 36.2°C or above 37.2°C respectively. Note: *10 minutes after heater-on transition, T_{scap} decreased of 0.05 ±0.007°C ($p<0.001$). **10 minutes after heater-off transition, T_{scap} increased 0.03 ±0.003°C ($p<0.001$).

Infra-Red temperatures after heater-off

Temperatures of the neck nape were measured from five subjects using thermal images taken shortly after the radiant heater was turned off. Temperatures were averaged at approximately 5 minute intervals over the range of 1 to 30 minutes. The response of T_{nape} is shown on a combined plot with T_{oes} and T_{scap} in Figure 6.2.

When the radiant heater was turned-off, T_{nape} showed an initial decrease mean ±SE temperature by 0.15 ±0.03°C (p=0.01) ten minutes after the heater-off transition. Although not statistically significant, T_{scap} showed an increase 0.13 ±0.05°C (p=0.08) from 10 to 20 minutes after the heater-off transition.

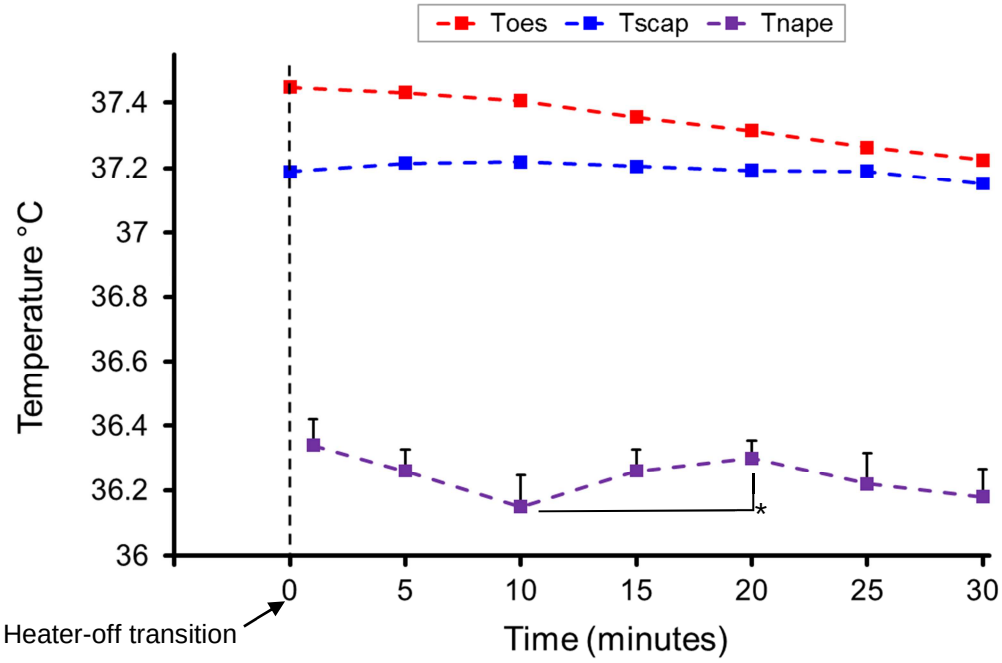


Figure 6.2 Contact and non-contact temperatures after heater-off

Observed mean temperatures of the lower oesophagus (T_{oes}), inter-scapular skin (T_{scap}) and the nape of the neck (T_{nape}) showing the response to a heater-off transition. T_{oes} and T_{scap} sensors were placed in contact with body tissue (n=7), whereas T_{nape} was measured using infra-red thermography (non-contact) from the average of a 1 cm line positioned over the neck nape (n=5). The overhead radiant warmer was servo-controlled to a set-point on exposed abdominal skin unless the bed-side nurse manually turned the radiant heating off if axilla temperature was above 37.2°C. T_{nape} (+SE) is shown whereas SE was less than 0.05°C for T_{oes} and T_{scap} and therefore not shown. Note: * although not statistically significant, T_{scap} increased 0.13 ±0.05°C (p=0.08) from 10 to 20 minutes after the heater-off transition.

Steady state temperature

Of the 13 neonates; 8 subjects contributed both heater-on and heater-off steady state periods; 1 subject contributed a heater-on steady state period, and 4 subjects contributed a heater-off steady state period. Figure 6.3 depicts observed mean steady state temperatures for T_{oes}, T_{scap} and T_{rec} during heater-on and heater-off steady thermal states.

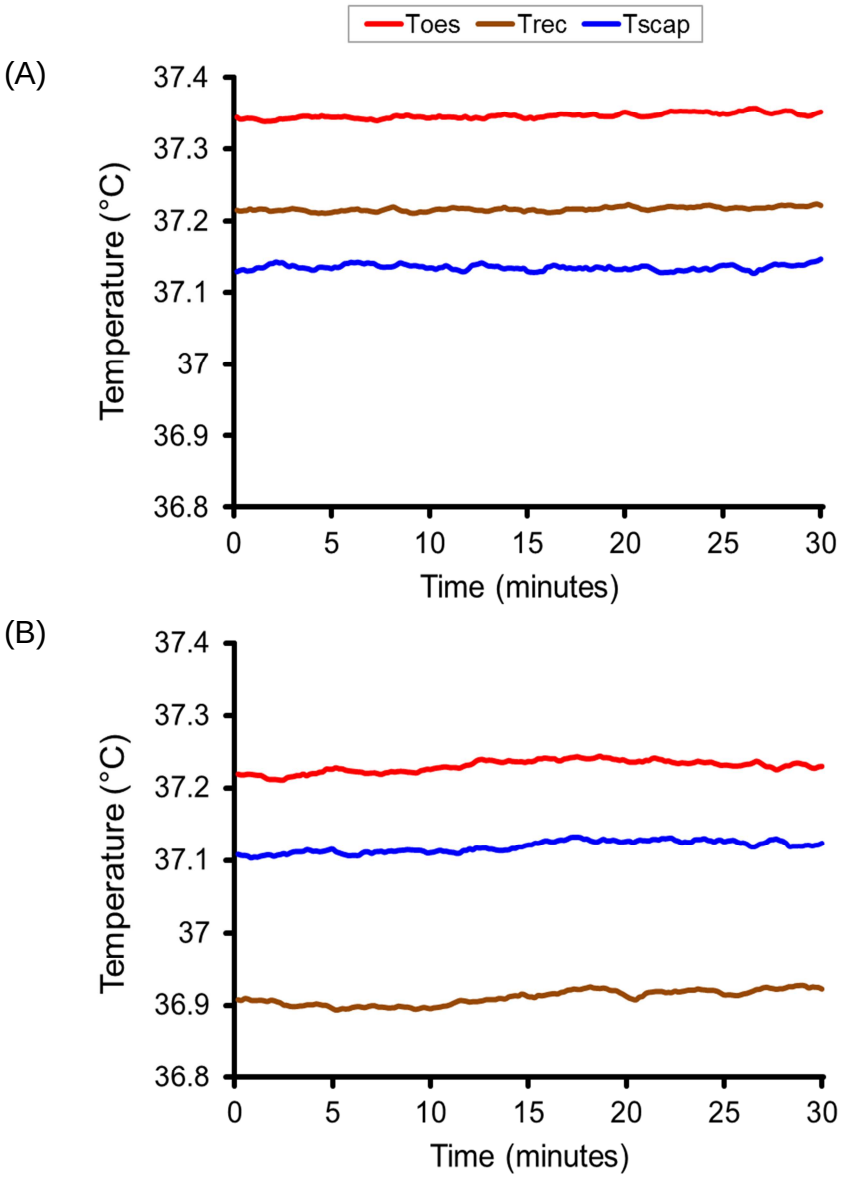


Figure 6.3 Temperatures during steady state heater-on and heater-off periods
Observed mean temperatures of the lower oesophagus (T_{oes}), rectum (T_{rec}) and inter-scapular skin (T_{scap}) during normothermia in continuous 30 minute steady state periods from thirteen subjects showing: (A) thermal equilibrium with radiant heater-on (n=12); (B) thermal equilibrium with radiant heater-off (n=9). There was a considerable time difference between the heater-on and heater-off states.

Table 6.1 shows mean differences in temperature during thermal steady state at each site relative to T_{oes} (reference) in each heater on-off state.

Site	Heater state	Observed mean temperature	Temperature difference from T_{oes} (reference)	Change in relative temperature difference heater-on to heater-off
T_{oes}	On	37.35 (0.004)		
T_{rec}	On	37.22 (0.005)	0.13 (0.004)*	
T_{scap}	On	37.13 (0.004)	0.21 (0.004)*	
T_{oes}	Off	37.23 (0.006)		
T_{rec}	Off	36.91 (0.005)	0.32 (0.005)*	-0.19 (0.007)**
T_{scap}	Off	37.12 (0.004)	0.11 (0.005)*	0.10 (0.007)**

*p≤ 0.001 versus T_{oes} **p≤ 0.001 versus heater-on

Table 6.1 Temperatures and differences during heater on/off steady states
Observed mean (± SE) temperatures (°C) of the lower oesophagus (T_{oes}), rectum (T_{rec}) and inter-scapular skin (T_{scap}) and temperature differences between sites and heater states over 30 minute normothermic steady state periods for heater-on (n=12) and for heater-off (n=9) from 13 normothermic subjects.

Core temperature for T_{oes} and T_{rec} were within the normal range of 36.5-37.5°C in both steady heater states, with T_{oes} warmer than T_{rec} under both steady state conditions ($p<0.001$, Table 1).

With the radiant heater-on and skin warmed, T_{oes} was 0.21 ±0.004°C higher than T_{scap} ($p<0.001$) and this difference reduced to 0.11 ±0.005°C ($p<0.001$) with the heater-off. The change in temperature difference ($T_{oes} – T_{scap}$) from heater-on compared to heater-off was 0.10 ±0.007°C ($p<0.001$).

During steady heater-on thermal state, the difference between T_{oes} and T_{rec} was 0.13 ±0.004°C ($p<0.001$) and this difference increased to 0.32 ±0.005°C ($p<0.001$) with heater-off. The change in temperature difference ($T_{oes} –T_{rec}$) from heater-on compared to heater-off was -0.19 ±0.007°C, ($p<0.001$).

During steady heater-on thermal state, T_{rec} was higher than T_{scap} ($T_{rec} – T_{scap}$ 0.08 ±0.004°C, $p<0.001$) whereas T_{rec} was lower than T_{scap} during steady heater-off thermal state ($T_{rec} – T_{scap}$ -0.21 ±0.005°C, $p<0.001$).

6.4 DISCUSSION

In this study of normothermic term or near term gestation neonates receiving intensive care, T_{scap} was compared to T_{oes} and T_{rec} during periods when all three temperature sites were in steady state. With mild cooling of exposed skin on the front of the body (radiant heater-off state) T_{scap} was lower than but close to T_{oes} (reference temperature). However with mild warming of exposed skin (radiant heater-on state) the difference between T_{oes} and T_{scap} increased. Although the principle of zero heat flux suggests that T_{scap} should closely follow T_{oes} under steady state conditions[160], data show that T_{scap} did not passively equilibrate with T_{oes} as predicted. The data suggest that in the heater-off state, the smaller difference between T_{oes} and T_{scap} is due to local heat production in the inter-scapular skin region.

The behaviour of T_{scap} relative to T_{oes} in steady state in this study may have been a result of heating or cooling of the mattress in contact with the back. Under-mattress temperature was measured in open radiant warmers in Chapter 5 using the same incubators. The radiant heat source and was shown not to vary and IR imaging of mattress temperature around the neonate did not show heat retention in the mattress. Additionally, experimental studies noted in Chapter 5 with a 3 kg saline bag showed that the mattress had no significant thermal storage capacity that would sway T_{scap}.

The observed differences in T_{scap} relative to T_{oes} in heater-on and off states can be explained if the steady state definition did not truly reflect steady state. However, any continued settling of temperature is likely to have been small and the maximum length of steady state was determined by servo-control of abdominal skin temperature and nursing intervention based on axillary temperature measurements. Furthermore, because the same definition of steady state was consistently applied at all sites simultaneously, any further temperature settling was unlikely to influence relative differences between T_{scap} and T_{oes}.

At the outset this study assumed that during thermal steady state, T_{scap} should reflect a combination of local thermogenesis and transmission of heat towards or from the thoracic core. Continuous temperature recordings over time in individual subjects showed that increases in T_{scap} occurred shortly after sudden cooling of exposed skin that is consistent with BAT thermogenesis. However with continued cooling, while heat from local thermogenesis would be expected to be transferred to the core, continued heat loss from other exposed surfaces exceeded heat gain as indicated by a decline in T_{oes}. The reduction in T_{oes} was then reflected in T_{scap} as temperature equilibration occurred, T_{scap} started to fall after a lag period. These dynamics were mirrored with sudden skin warming. T_{scap} initially reduced, consistent with decreased BAT thermogenesis, before an increase occurred that was reflected in the rise in T_{oes}. Observation of T_{rec}, T_{oes} and T_{scap} during thermal steady state allowed interpretation of thermal gradients during of steady thermal state and the real-time data highlight the complexities of attempting to interpret core-skin gradients in non-steady state.

Compared to the heater-on steady thermal state, T_{rec} reduced considerably relative to T_{oes} during heater-off steady state. In this study the movement of T_{rec} from above T_{scap} in the heater-on state to below T_{scap} in the heater-off state could equally be explained by rectal cooling rather than heating of $T_{scap.}$ Bazzett et al reported that leg skin cooling reduces T_{rec} in adult subjects.[69] Bazzett et al's results support observations in this thesis because skin temperature in the legs of neonates dropped considerably more than in the upper limbs. Therefore comparison to T_{oes} is required to interpret changes in T_{scap}. Steady state temperature is also important for correct interpretation of core-inter-scapular surface skin temperature differences because of the complex relationship between T_{scap} and T_{oes} noted previously.

Ootsuka et al directly and simultaneously measured inter-scapular BAT, brain and body temperatures in rats and reported that BAT temperature started to increase 2.3 minutes before brain temperature and 3 minutes before body temperature.[54] The authors also showed that the temperature increases were greatest in BAT, followed by brain and body, suggesting that activated BAT heats brain. Considering that both T_{scap} and T_{nape} increased maximally 10 and

20 minutes respectively after sudden cooling of exposed skin, it might be speculated that BAT thermogenesis is reflected by T_{scap}, and the delayed increase in T_{nape} may reflect warm blood flow from the brain in the great veins of the neck. Alternatively thermogenesis in BAT deposits around the great veins may be occurring.

This study was limited because temperature in BAT was not directly measured and an assumption was made that inter-scapular skin temperature reflects underlying BAT activity. Oxygen consumption data presented in Chapter 5 support this assumption. The data also cannot quantify the heat putatively generated by BAT. While the observed absolute changes in T_{scap}, and relative changes in T_{scap} compared to T_{oes} were small, heat generated in BAT is likely to be rapidly transferred to central venous blood and so surface temperatures would not be expected to closely reflect quantities of heat produced. Direct temperature measurement by invasive temperature probes in BAT or measurement of blood temperature entering and leaving BAT are required to address this limitation.

A potential effect of endotracheal intubation or spontaneous breathing of ambient air on T_{oes} can also not be excluded as three neonates were extubated during the study. However an effect of intubation status is unlikely as tracheal temperature would be expected to be the same in both intubated and extubated states. This is because warmed humidified gas circuits in intubated neonates mimic the normal gas temperatures and humidity generated by upper airway with spontaneous breathing. Medications administered to the neonates may influence thermoregulatory responses[106] however this reflects a clinical reality in the population of intensive care neonates studied that cannot be controlled.

6.5 CONCLUSION

The change in the temperature difference between lower oesophagus and inter-scapular skin with mild warming and cooling of exposed skin surfaces in term gestation neonates during steady thermal states suggests that heat is generated in the inter-scapular area. Absolute changes in T_{scap} and T_{nape}

measured by skin probes and IR imaging respectively also support local thermogenesis. The data do not indicate the amount of heat generated in the inter-scapular area, only that heat generation appears to be active. BAT is the likely source of this thermogenesis.

T_{nape} increased 10 minutes after a rise in T_{scap} following a heater-off transition. The significance of this finding is uncertain and may represent warmer venous blood from the brain, or increased skin blood flow consequent to increased BAT activity in the neck and/or inter-scapular regions.

The next Chapter begins to explore core temperatures in neonates undergoing therapeutic hypothermia.

CHAPTER SEVEN

7 OESOPHAGEAL AND RECTAL TEMPERATURE: DYNAMIC DIFFERENCES DURING THERAPEUTIC HYPOTHERMIA

7.1 INTRODUCTION

Whole body hypothermia for HIE neonates is currently considered the standard of care for neuro-protection in Australia and most other countries. Whole body hypothermia may be administered by either manual cooling, where nursing staff cool the environment and manually adjust temperature based on a target core temperature range, or by using a commercial servo-controlled cooling blanket. Manual cooling may be passive, where an absence of radiant heating allows the neonate to become hypothermic, or active where additional measures are used to facilitate cooling such as cool packs, wet cloths or fans. Manual cooling is the only mode used in South Australia at the present time, and although servo-controlled cooling blankets are now commonly used in clinical practice elsewhere, manual cooling remains a common and accepted standard practice.

During whole body hypothermia for neonatal HIE, core body temperature (T_{core}) is maintained at 33-34°C in either rectum (T_{rec}) or lower oesophagus (T_{oes}) as a neuro-protective strategy.[4, 8-10] However, T_{rec} and T_{oes} differ under normal thermal conditions as shown in Chapters 5 and 6 and by other authors,[69, 70, 194] and these differences are magnified during hypothermia.[59, 120, 194, 196] When environmental temperature is suddenly increased or decreased, differences between T_{rec} and T_{oes} are exaggerated because T_{oes} responds more quickly (has a shorter response or lag time) than T_{rec}.[59, 120, 196] As dynamic environmental temperature conditions are the norm during manual or servo-control of prolonged whole body hypothermia, considerable differences between lower oesophageal temperature (T_{oes}) and rectal temperature (T_{rec}) might be expected.

The only study in the literature that compared T_{oes} and T_{rec} during therapeutic hypothermia is that of Sarkar et al who showed that during whole body hypothermia, where the lower oesophageal temperature (T_{oes}) is servo-

controlled between 33 to 34°C using a cooling blanket, rectal temperature (T_{rec}) is variable and lower than T_{oes}.[194] T_{rec} is however the most commonly used target site for temperature control during whole body hypothermia where either cooling blankets or manually controlled methods are used.[4, 8, 10] Servo-controlled blanket cooling devices currently used in Australia are required by the Therapeutic Goods Administration (TGA) to use T_{rec} monitoring.[65] The behaviour of T_{oes} when T_{rec} is controlled at 33-34°C has not previously been studied in human neonates during therapeutic hypothermia. It is predicted that T_{oes} would be higher than T_{rec} with T_{rec} targeting, and tested this hypothesis by continuously and simultaneously measuring both T_{oes} and T_{rec} during manually controlled whole body hypothermia.

7.2 METHODS

This was an observational cross-sectional study conducted on neonates admitted to the Flinders Medical Centre and the Women's and Children's Hospital NICUs in Adelaide, which provide tertiary neonatal services in South Australia. Institutional ethics approval was granted by the Southern Adelaide Clinical Human Research Ethics Committee and the Children Youth and Women's Health Service Human Research Ethics Committee. Informed consent was obtained from parents.

Participants

Twenty one neonates receiving whole body hypothermia for HIE were enrolled and recruited sequentially. Subjects were 35 to 40 weeks gestation and nursed on their back on an open incubator mattress with an overhead radiant heater. Nursery ambient temperature was kept at approximately 25°C. Subjects were studied for up to 78 hours, depending on how early informed consent was obtained in the course of the standard 72 hour therapeutic hypothermia period followed by rewarming. Due to time delays in obtaining consent, the period of induction of hypothermia was not studied.

Temperature regulation

Whole body hypothermia for a 72 hour period is the standard practice for HIE following birth asphyxia. Hypothermia was induced and maintained by manual

control. Servo-controlled cooling blankets, a standard practice used elsewhere, were not used in the present study. The radiant heater was initially turned off and a bed-side nurse adjusted radiant heating based on the displayed T_{rec} with a therapeutic target of 33-34°C. This mode of cooling was termed 'passive'. Where turning off the radiant heater was insufficient to cool the neonate to the T_{rec} target, either a fan was turned on, or wet cloths or cool packs applied to the skin. This mode of cooling was termed 'active'.

Response (lag) times from step changes

When using manual methods to control the targeted depth of cooling, there were intermittent changes made to environmental temperature. Some of these step changes in environmental temperature produced pronounced step changes in skin temperature and were generally followed by step changes in T_{rec} and T_{oes}. Response time was measured in minutes when exposed abdominal skin temperature (T_{belly}) increased/decreased 0.1°C during a step change, to a corresponding 0.1°C increase/decrease in T_{rec} and T_{oes}.

Temperature monitoring

Two core temperature measurement sites were used: rectum (T_{rec}) and lower oesophagus (T_{oes}). A Dräger 7.5 Fr or Philips 9 Fr probe was inserted 5-6 cm from the anal verge to measure T_{rec}. A 9 Fr Tyco oesophageal probe, or 8 Fr Philips 'Innersense' combined feeding tube was inserted nasally to the level of T7 or T8. The depth of insertion was determined using the formula: body length in cm/4 +3 cm to measure T_{oes}.

Three skin temperature measurement sites were used. Abdominal skin (T_{belly}), and inter-scapular skin to the left and right of the spine (T_{scap-l}, T_{scap-r}). All three sites used Tyco 'IncuTemp 5' sensors with a hydrogel adhesive cover.

Some subjects repositioned themselves with one shoulder not in full contact with the mattress resulting in heat loss. Therefore the inter-scapular skin (either left or right) that was most in contact with the mattress was used for analysis.

Environmental temperature (T_{amb}) was measured with a Fisher and Paykel

disk style skin temperature sensor attached to a small aluminium (black anodised) heatsink placed on the mattress near the feet.

Measurement of skin temperature overlying the lower back (T_{lobak}, away from BAT), to control for potential heat retention in the mattress contacting the skin of the back and influencing T_{scap}, was not included in the present study. The rationale is that in Chapter 5 it was demonstrated that, under similar experimental conditions, T_{scap} changes showed no clear relation to T_{lobak}, implying that heat retention in the mattress did not sway T_{scap}.

A sensor connector box was placed at the edge of the mattress to allow connection of all the temperature sensors. The sensor connector box was joined by a single cable to a datalogger that was located out of the view of nursing staff and recorded temperature measurements every 10 seconds for the duration of the study.

Occasional patient movement or sensor detachment artefact that resulted in measurements with a rapid reduction of core/skin temperature (< 30°C) were removed from analysis.

Temperature sensor and datalogger calibration

Calibration of temperature sensors and the datalogger occurred shortly after each study using the methodology described in Chapter 3.5.6. All temperatures were corrected before analysis, based on the calibration results.

Statistical methods

Data were analysed using Stata version 13.0 (Statacorp, Texas, USA). Only the first 24 hour study period for each subject was analysed (the longest common study period) to avoid statistical bias from subjects with a larger number of observations. The mean of temperature differences in neonates cooled actively was compared to those passively cooled by independent samples T-test. Standard deviations of temperature differences were compared between active and passively cooled neonates with an F-test. The percentages of time that T_{oes} and T_{rec} exceeded 34°C were compared within active and passively cooled neonates using the Wilcoxon matched-pairs signed-ranks test. Wilcoxon's rank sum test was used to compare the

percentages of time that T_{oes} and T_{rec} exceeded 34°C between active and passively cooled neonates. Data are presented as mean ±SE unless indicated otherwise. The alpha level for statistical significance was chosen as $p<0.05$.

7.3 RESULTS

Twenty-one neonates were studied with moderate to severe HIE receiving therapeutic whole body hypothermia (15 male, 6 female). The mean (range) birth weight was 3379 (2150 to 4970) g, and gestation 39.5 (35 to 42) weeks. Medications administered included phenobarbitone (12), phenytoin (2), dopamine (13), opioids (13) and midazolam (14). Fourteen neonates were intubated for airway support and received humidified warmed inspired gases. Seven neonates breathed spontaneously throughout the study in room air at ambient nursery temperatures (25°C) and without either nasal continuous positive airway pressure or high flow intranasal cannula support. Fourteen neonates received active cooling measures of which nine were intubated, and seven neonates were given passive cooling of which five were intubated.

Lag times in T_{rec} and T_{oes} after step changes in environmental temperature

The lag time between a change in T_{belly} and a corresponding change in core temperature was longer for T_{rec} than for T_{oes}. Observed means ±SE lag time in minutes were 37.7 ±2.7 and 27.4 ±1.9 for T_{rec} and T_{oes} respectively ($p=0.004$).

Temperature differences between T_{rec} and T_{oes}

T_{oes} was higher than T_{rec} for the majority of the study periods in all neonates with a maximum and minimum temperature difference of +1.8°C and -0.27°C, and a mean 24-hr difference of 0.47 ±0.001°C ($p<0.001$). The difference between T_{oes} and T_{rec} fluctuated within and between each subject as shown in Figure 7.1.

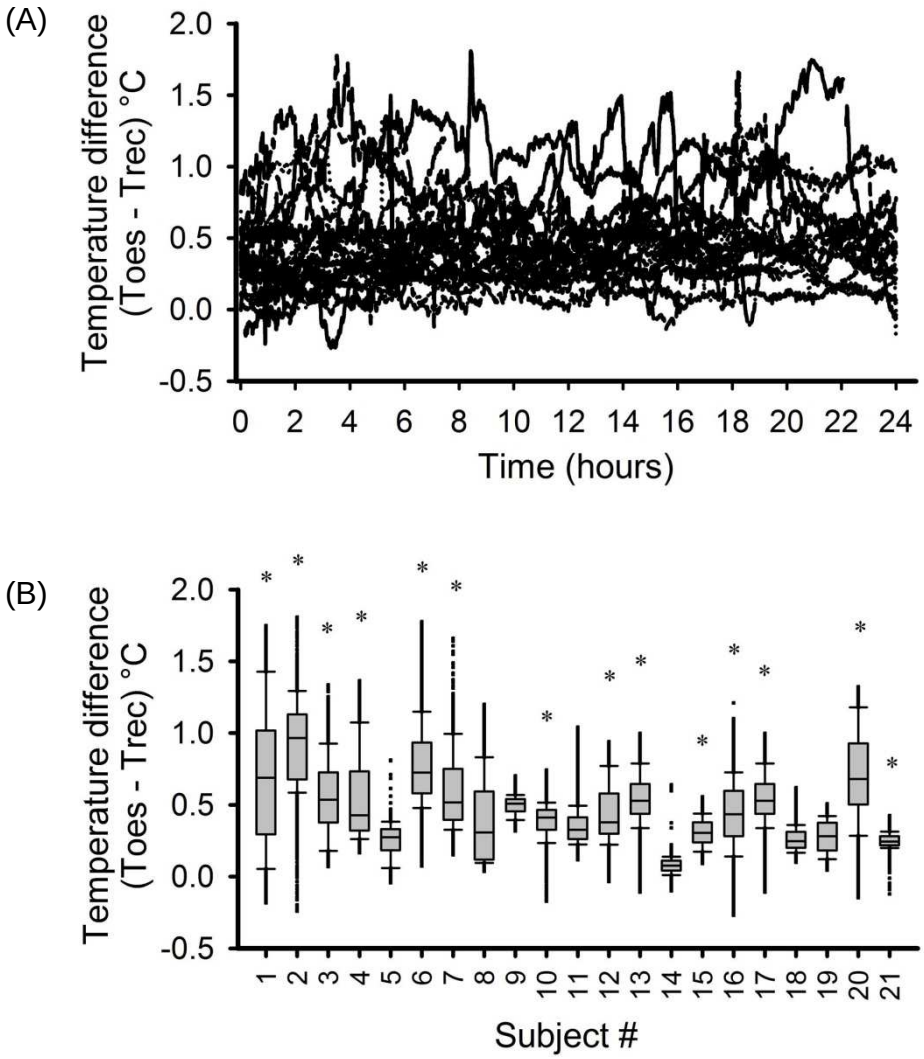


Figure 7.1 24 hours of oesophageal temperature compared to rectal

Observed temperature differences in the lower oesophagus (T_{oes}) using rectal temperature (T_{rec}) as a reference, recorded every 10 seconds over 24 hours of hypothermia in 21 subjects showing: (A) raw serial data for all neonates; (B) box plot of temperature differences for each subject. Note, * depicts neonates who received active cooling.

Manual cooling methods

Three different manual methods (heater on-off, fan on-off, and cool packs on-off) to cool subjects were used to target T_{rec} within a temperature range 33 to 34°C and an example of each method is shown in Figure 7.2.

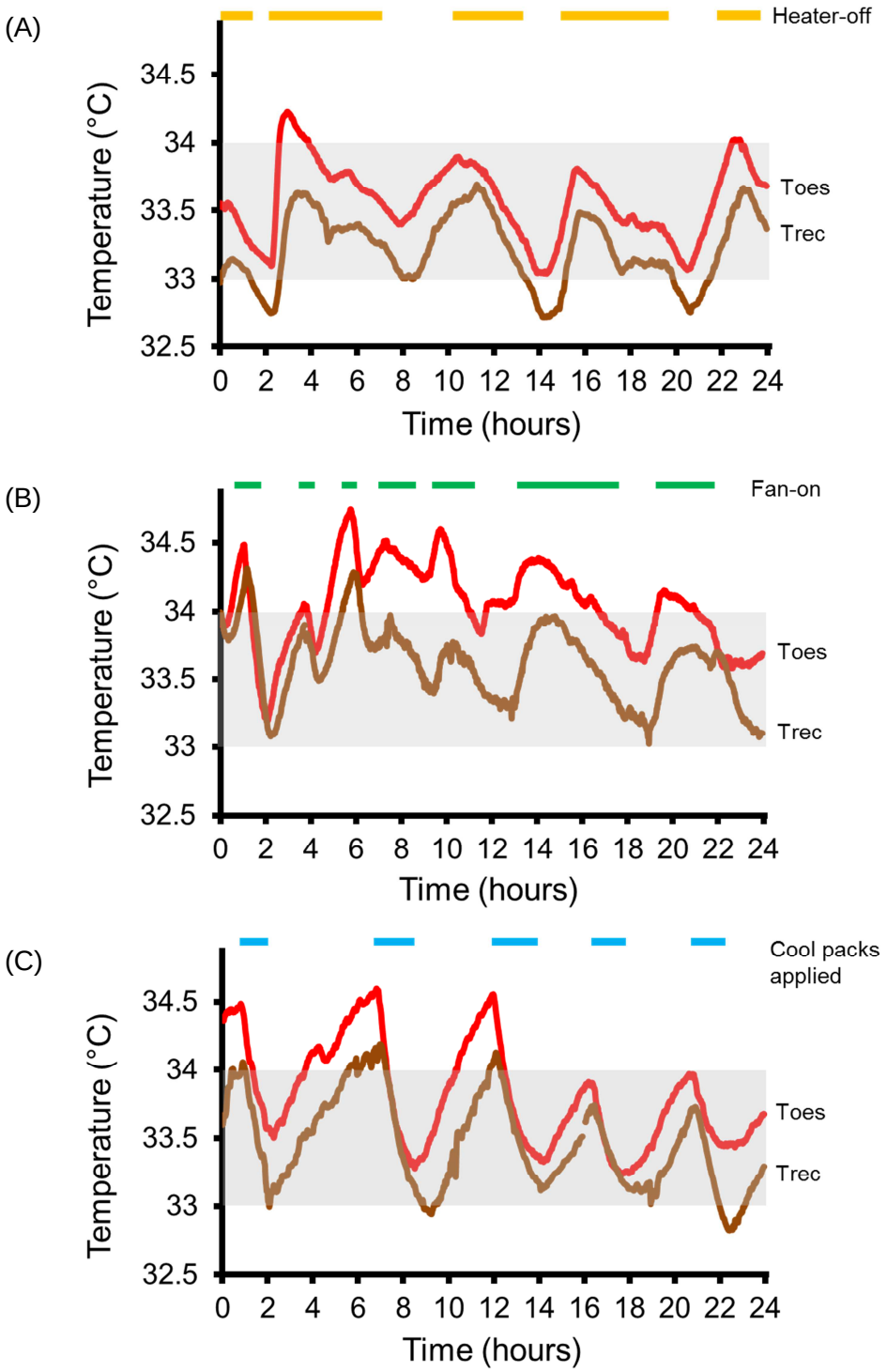


Figure 7.2 Examples of three manual cooling methods

Examples from three subjects showing lower oesophageal temperature (T_{oes}) and rectal temperature (T_{rec}) for a 24 hour period during whole body hypothermia. Three different manual cooling methods used to target T_{rec} within a 33 to 34°C (shaded) range were: (A) operating the incubator radiant heater off/on to lower/raise T_{rec}; (B) switching a fan on/off to lower/raise T_{rec}; (C) applying/removing cool packs to lower/raise T_{rec}. The coloured segments above each plot indicate when an attempt was made to reduce T_{rec}.

Notably, passively cooled neonates spontaneously allowed a fall in their body temperature (anapyrexia) and the attending nurse was required to apply intermittent radiant heating to keep T_{rec} within the target range. The examples in Figure 7.2 demonstrate qualitatively that with more aggressive cooling, larger swings occurred in both T_{rec} and T_{oes}.

Active cooling vs passive cooling

The mean difference between T_{oes} and T_{rec} was greater in subjects who received active cooling when compared to passive cooling (0.54 ±0.001°C vs 0.29 ±0.001°C, p<0.001). The standard deviation (SD) of difference between T_{oes} and T_{rec} was also greater for active compared to passive cooling (0.32°C vs 0.17°C, p<0.001). Figure 7.3 shows that neonates who received active cooling to maintain T_{rec} in the target range had T_{oes} above 34°C for 58% of the time while T_{rec} was above 34°C for 18% of the time (p=0.001). Subjects receiving passive temperature control had T_{oes} above 34°C for 9% vs T_{rec} 1% of the time (p=0.022). The proportion of time T_{oes} was above 34°C was greater with active vs passive cooling methods (58% vs 9%, p=0.0003).

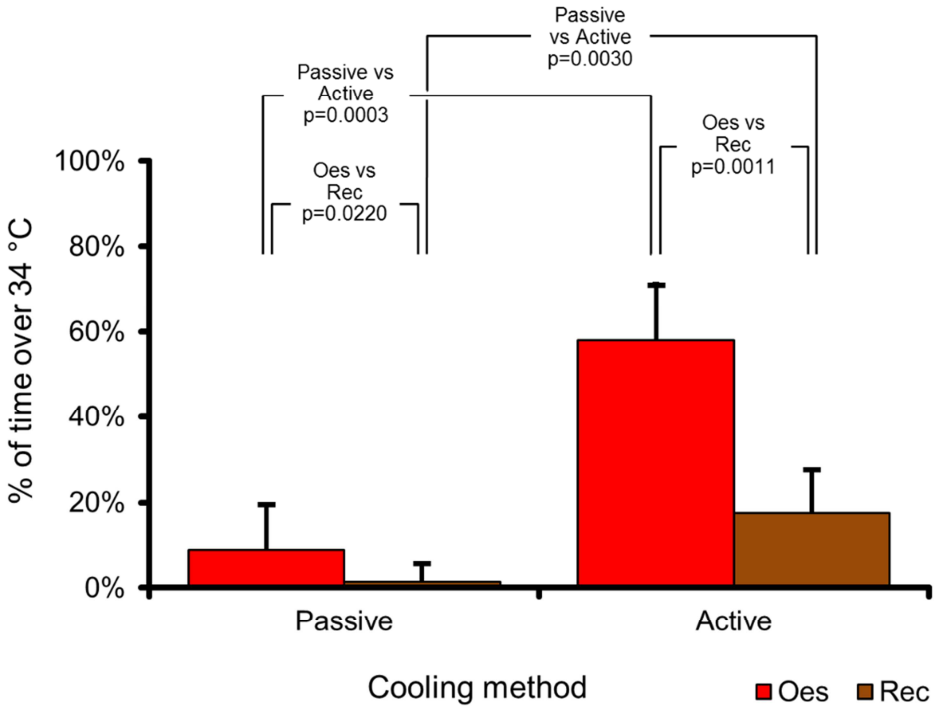


Figure 7.3 Percentage of time core temperature spent above 34°C

Bar chart of the percentage of time lower oesophageal temperature (T_{oes}) and rectal temperature (T_{rec}) were above 34°C during manual whole body cooling for both passive and actively cooled neonates.

7.4 DISCUSSION

In this study temperatures were recorded every 10 seconds in the rectum and lower oesophagus during manual cooling. When T_{rec} was controlled within the target range, T_{oes} was a mean of 0.47°C warmer. However, variability in temperature difference between the two sites was observed within and between subjects, with T_{oes} a maximum of up to 1.8°C above T_{rec}. The variability in the temperature difference between T_{rec} and T_{oes} and the frequency with which T_{oes} was above 34°C were both greatest in neonates where active cooling interventions were administered by the bed-side nurse. During active cooling, T_{rec} showed larger swings within the 33-34°C target window and T_{oes} measurements were frequently above range before environmental temperature was adjusted. The relatively slow response time of T_{rec} following heating and cooling of skin compared to the more rapid response of T_{oes}, contributes to large and unsuspected swings in T_{oes}. Consequently a stable and controlled T_{oes} is unlikely to be reliably achieved with manually controlled cooling and T_{rec} monitoring, even if T_{rec} is kept largely within target range.

T_{oes} closely reflects aortic blood temperature in adult humans.[74, 120] T_{oes} also correlates with non-invasive measurements of brain temperature in asphyxiated human neonates.[92] Therefore the fluctuations in T_{oes} above 34°C observed in this study suggest that aortic blood temperature (and the temperature of blood perfusing deep brain) may show similar temperature instability.

In contrast, T_{rec} has been shown to vary substantially from brain temperature in adult humans following head injury during hypothermia.[53] T_{rec} can be lowered by cooling of the skin of the legs and buttocks due to cooler venous return.[196] Based on data in the present study, choosing to monitor T_{rec} may result in warmer than expected T_{oes}, and therefore warmer than expected deep brain blood temperatures.

This is an important clinical concern given that small temperature differences of 1-2°C in brain influence neuronal survival in animal models of brain ischaemia.[197, 198] However, the clinical significance of the observations from

this study remains uncertain. The influence of choice of monitoring site, either T_{oes} or T_{rec}, on neurological outcome in human neonates is unknown as the question has not been addressed by clinical trials. However, the study of Jacobs et al which used comparable methods to the present study,[8] would be expected to have the same issues with T_{oes} instability, and showed similar neurological outcomes to the study of Shankaran et al that controlled T_{oes} within the 33-34°C range.[9]

The behaviour of T_{oes} during blanket cooling where T_{rec} is targeted has not been described. However, a similar physiological response in T_{oes} is expected to those observed with manual control, because both modalities heat and cool the skin in response to T_{rec} which is a slowly reacting measure of T_{core}. Further studies are required to determine the magnitude of T_{oes} fluctuations using such devices. However, as servo-controlled devices result in less fluctuation in T_{rec} than manual control,[65] T_{oes} fluctuations are likely to be less.

Sarkar et al have presented data on the mean temperature difference between T_{oes} and T_{rec} during whole body hypothermia.[194] They used T_{oes} targeting to 33-34°C and blanket servo-control rather than T_{rec} targeting and manual temperature control as in the present study. The authors presented mean and standard deviations of temperatures, and mean temperature differences for their study group at intermittent time intervals (1 hourly for the first 12 hours and then 4 hourly during the subsequent 60 hours of cooling). The authors found that T_{rec} was cooler than T_{oes}, with the median of the mean differences 0.78°C. This is similar to the mean temperature difference found in the present study of 0.54°C with actively cooled subjects. However, the methodology of Sarkar et al does not allow comment about fluctuations over time within subjects, meaning that although average temperature differences might suggest that T_{rec} monitoring is satisfactory, the study data may not have captured the much larger temperature differences that occur for short periods as found in the present study. The wider standard deviation of T_{rec} compared to T_{oes} in their data may suggest that tight servo-control of T_{oes} results in fluctuations in T_{rec} similar to those seen in T_{oes} in the present study. However their fluctuations shown in T_{rec} would not result in unexpected brain warming, in contrast to the potential for brain warming where T_{rec} is the basis of temperature control.

Limitations

The present study has several limitations. The behaviour of T_{oes} during manually controlled cooling using T_{oes} as the target was not studied. There are also no published data describing the behaviour of T_{oes} during manually controlled cooling using a T_{oes} target. While T_{oes} monitoring is likely to result in greater T_{oes} stability than T_{rec} monitoring during manual control, this assertion cannot be made with confidence.

Additionally, all subjects received anticonvulsants, sedatives or inotropes, and our sample of neonates had variable degrees of brain injury, all of which may influence thermoregulatory responses to cooling. The effect of endotracheal intubation or spontaneous breathing of ambient air on the observed instability in T_{oes} cannot be excluded as study numbers are too small to allow a meaningful analysis. Despite these limitations, the sample of neonates is typical of normal clinical management and results of the present study are likely to be generalisable.

7.5 CONCLUSION

In conclusion, T_{oes} is warmer than T_{rec} during whole body hypothermia when a T_{rec} of 33-34°C is maintained by manual cooling. Some of the temperature difference is related to different lag times at the two core sites, such that T_{rec} and T_{oes} are out of phase following a change in environmental temperature. T_{oes} is frequently above 34°C, especially when active cooling procedures are required that result in larger swings in T_{rec}. Monitoring and controlling T_{oes} may be preferable to T_{rec} to maintain a stable central core body temperature. However, the impact of monitoring T_{oes} on neurological outcomes after cooling requires investigation in a larger prospective study. More data are required during servo-controlled blanket cooling to assess the clinical relevance of these findings to changing neonatal practices.

The study also shows that neonates with HIE can broadly be divided into those requiring active cooling measures (hypothermic neonates who are defending a normal core temperature set-point) and those that allow passive

cooling (anapyrexic neonates who have a lower core temperature set-point). Anapyrexic neonates require intermittent radiant heating to maintain T_{rec} at 33-34°C. The significance of the anapyrexic response of some neonates is uncertain. In the next Chapter the mechanisms of anapyrexia and core temperature defence with HIE will be explored further by measurement of BAT activity during cooling, using changes in T_{scap} as an indication of BAT activity.

CHAPTER EIGHT

8 BAT ACTIVITY AND CORE TEMPERATURES DURING HYPOTHERMIA IN HIE HUMAN NEONATES

8.1 INTRODUCTION

The data presented in Chapter 7 showed that T_{oes} was warmer than T_{rec} and that larger swings in T_{rec} that occurred with active manual cooling exacerbated instability in T_{oes}. Some of the temperature difference between T_{oes} and T_{rec} was related to different lag times at the two core sites. Other possible influences contributing to a warmer T_{oes} than T_{rec} include warmer central venous blood returning to the heart from the head and colder venous return from the legs. In support of these possibilities, the data in Chapter 5 show that leg skin temperature is lower than arm skin temperature in a cool environment. A contribution of BAT to warming central venous blood during hypothermia is also possible based on what is known of the physiology of BAT (see Literature Review in Chapter 2 for a full discussion). The larger differences between T_{oes} and T_{rec} during hypothermia (Chapter 7) compared to normothermia (Chapters 5 and 6) are consistent with a larger degree of rectal cooling and/or BAT activity.

BAT activity during therapeutic hypothermia has not been previously studied in the human neonate. It may have an important role in defending brain temperature and acting against therapeutic efforts to cool the brain. In Chapters 5 and 6, evidence was presented from normothermic neonates suggesting that the response of T_{scap} following step changes in environmental temperature, and the difference between T_{scap}, T_{oes} and T_{rec} during steady state with skin warming or cooling, indicated BAT activity.

This Chapter aims to describe the relationship between T_{oes} and T_{rec} in steady state during manually controlled therapeutic hypothermia to exclude the influence of different lag times on temperature differences. A further aim investigated the activity of BAT by observing the effect of environmental temperature changes on T_{scap}, T_{oes} and T_{rec} during thermal steady state and non-steady state conditions with the skin of the back mostly insulated from

heat loss. IR temperatures (non-contact) of the neck nape were also investigated as T_{nape} increased shortly after commencement of environmental cooling in Chapters 5 and 6.

This study tests the hypotheses that: (i) steady state T_{oes} is higher than T_{rec} during manually controlled whole body hypothermia; (ii) BAT contributes to warming T_{oes}; (iii) BAT is less active in neonates exhibiting an anapyrexic response to cooling.

8.2 METHODS

Trial design, study setting

The study was conducted on the same cohort of subjects described in Chapter 7. For ease of reading, some material is repeated from Chapter 7. Institutional ethics approval was granted by the Southern Adelaide Clinical Human Research Ethics Committee and the Children Youth and Women's Health Service Human Research Ethics Committee and informed consent was obtained from parents.

Participants

Twenty one neonates receiving whole body hypothermia for HIE were enrolled and recruited sequentially. Subjects were 35 to 40 weeks gestation and nursed on their back on an open incubator mattress with an overhead radiant heater. Nursery ambient temperature was kept at approximately 25°C. Subjects were studied for up to 78 hours, depending on how early informed consent was obtained in the course of the standard 72 hour therapeutic hypothermia period followed by rewarming. Due to time delays in obtaining consent, the period of induction of hypothermia was not studied.

Temperature regulation

Whole body hypothermia for a 72 hour period is the standard practice for HIE following birth asphyxia. Hypothermia was induced and maintained by manual control. Servo-controlled cooling blankets, a standard practice used elsewhere, were not used in the present study. The radiant heater was initially turned off and a bed-side nurse adjusted radiant heating based on the

displayed T_{rec} with a therapeutic target of 33-34°C. This mode of cooling was termed ‘passive’. Where turning off the radiant heater was insufficient to cool the neonate to the T_{rec} target, either a fan was turned on, or wet cloths or cool packs were applied to the skin. This mode of cooling was termed ‘active’.

Temperature monitoring

Two core temperature measurement sites were used: rectum (T_{rec}) and lower oesophagus (T_{oes}). A Dräger 7.5 Fr or Philips 9 Fr probe was inserted 5-6 cm from the anal verge to measure T_{rec}. A 9 Fr Tyco oesophageal probe, or 8 Fr Philips ‘Innersense’ combined feeding tube was inserted nasally to the level of T7 or T8. The depth of insertion was determined using the formula: body length in cm/4 +3 cm to measure T_{oes}.

Three skin temperature measurement sites were used. Abdominal skin (T_{belly}), and inter-scapular skin to the left and right of the spine (T_{scap-l}, T_{scap-r}). All three sites used Tyco ‘IncuTemp 5’ sensors with a hydrogel adhesive cover.

Some subjects repositioned themselves with one shoulder not in full contact with the mattress resulting in heat loss. Therefore the inter-scapular skin (either left or right) that was most in contact with the mattress was used for analysis.

Environmental temperature (T_{amb}) was measured with a Fisher and Paykel disk style skin temperature sensor attached to a small aluminium (black anodised) heatsink placed on the mattress near the feet.

Measurement of skin temperature overlying the lower back, (T_{lobak}, away from BAT) to control for potential heat retention in the mattress contacting the skin of the back and influencing T_{scap}, was not included in the present study. The rationale was that in Chapter 5 it was demonstrated that, under similar experimental conditions, T_{scap} changes showed no clear relation to T_{lobak}, implying that heat retention in the mattress did not affect T_{scap}.

A sensor connector box was placed at the edge of the mattress to allow connection of all the temperature sensors. The sensor connector box was joined by a single cable to a datalogger that was located out of the view of

nursing staff and recorded temperature measurements every 10 seconds for the duration of the study.

Occasional patient movement or sensor detachment artefact that resulted in measurements with a rapid reduction of core/skin temperature (< 30°C) were removed from analysis.

Temperature sensor and datalogger calibration

Calibration of temperature sensors and the datalogger occurred shortly after each study using the methodology described in Chapter 3.5.6. All temperatures were corrected before analysis, based on the calibration results.

Heater-on and heater-off step transitions

Whole body hypothermia for 72 hours was induced and maintained by manual control. The bed-side nurse adjusted radiant heating based on the displayed T_{rec} with a therapeutic target of 33-34°C. Manual adjustment to the radiant heater output often caused a step increase/decrease in T_{belly} followed by an increase/decrease in both T_{rec} and T_{oes}.

Compared to normothermic neonates, cooled neonates showed a slower response to heater-on and heater-off transitions. Therefore the analysis period was extended to 60 minutes (rather than 30 minutes) after the step transition. The first 60 minutes following a step up or down transition along with the preceding 15 minutes, were used for non-steady state analysis. Where there was more than one step up or step down event per subject, the step up or step down event with the longest previous steady state period immediately before the step event was used. A maximum of one step up and one step down event per subject was used to avoid bias.

Thermal imaging

As in Chapters 5 and 6, an IR camera was mounted approximately 1m above subjects and automatically recorded thermal images of exposed skin every 30 seconds during each study. IR images allowed non-contact skin temperature measurements at multiple sites post study period. The IR temperatures during radiant heating were found to be inaccurate in Chapter 5 because reflected IR radiation obscured changes in subcutaneous temperature. Therefore, only IR

skin temperatures during radiant heater off periods were studied. The same sites that were used for measurement in Chapter 5 were used in this study, namely: nape of the neck (T_{nape}), skin above the heart (T_{heart}), upper arm (T_{up-arm}), lower arm (T_{lo-arm}), thigh (T_{thigh}), lower leg (T_{lo-leg}), mattress surface near the subject's head (T_{mat-H}) and mattress surface near the subject's feet (T_{mat-F}).

IR measurements were made during periods immediately following heater-off transitions. The first measurement occurred 1 minute after the radiant heater was turned off and subsequent measurements occurred at 5 minute increments up to 60 minutes. If there was more than one 60 minute heater-off event per subject, the event with the longest preceding warming period was used. A maximum of one heater-off event per subject was used to avoid bias.

Endotracheal extubation during cooling

HIE neonates undergoing whole body hypothermia were also likely to require mechanical ventilation. Heated humidification is required to warm and moisten inspired gases from the ventilator as the naso-pharynx is bypassed by an endotracheal tube.

During cooling, T_{rec} and T_{oes} were observed before and after extubation as the temperature of tracheal gas (37°C for intubated neonates, compared to 33-34°C in spontaneously breathing neonates in room air) may influence T_{oes}. Heated humidification at both NICU's was pre-set to deliver 37°C inspiratory gas during hypothermia and this temperature was not altered.

Steady state core temperature during cooling

A steady thermal state was used to compare absolute temperatures from different body sites. A steady state period was defined as having less than 0.2°C change over a continuous 60 minute period at three measurement sites: T_{oes}, T_{rec} and T_{scap}. Two types of thermal steady state identified in Chapter 6, heater-on and heater-off were both used in the present study.

To minimise the influence of variations in temperature lag times in different parts of the body following a change in environmental temperature, the last 30 minutes of steady state temperature comparisons were chosen for analysis. If there was more than one steady state period per subject, the longest steady state period was used with a maximum of one heater-on and one heater-off

steady state period per subject to avoid bias.

In Chapter 7, two different methods of manual cooling were identified: passive and active. The need for passive or active cooling reflected the vigour with which individual neonates defended core temperature. Analysis of steady state temperatures used overall observations and also subgroup analysis of cooled neonates who received passive cooling (anapyrexic neonates) and active cooling (hypothermic neonates).

Steady state core temperature after rewarming

Where temperature monitoring continued for some time after rewarming, heater-on and heater-off steady state periods were compared to steady state temperatures from a cohort of critically ill normothermic subjects who had not previously been exposed to hypothermia therapy (Chapter 6).

Statistical methods

Data were analysed using IBM SPSS version 22 (IBM Corp. NY, USA). For analysis of steady state temperatures, the longest common steady state period observed across all subjects during both warming (heater-on) and cooling (heater-off) states was used. Steady state temperature recordings of longer than 30 minutes were truncated to the last 30 minutes to avoid bias from subjects with longer steady state recordings. One 30-minute steady state period was used for each subject in each heater state. Analysis comprised all 10-second temperature recordings from each subject.

Mean temperature differences between sites or between groups were determined using independent samples T-tests to calculate mean difference and statistical significance. Where a temperature difference was made with a particular site at two different times, a paired samples T-test to calculate mean difference and statistical significance.

Data are presented as mean ±SE unless otherwise indicated. The alpha level for statistical significance was chosen as $p<0.05$ for single comparisons, or $p<0.05/n$ for multiple comparisons (n=number of comparisons).

8.3 RESULTS

The study cohort was described in Chapter 7. Twenty-one neonates (15 male, 6 female) were studied with moderate to severe HIE and treated with whole body hypothermia for 72 hours. The mean (range) birth weight was 3379 (2150 to 4970) g, and gestation 39.5 (35 to 42) weeks. Medications administered included phenobarbitone (12), phenytoin (2), dopamine (13), opioids (13) and midazolam (14).

Fourteen neonates were intubated for airway support and received humidified and warmed (37°C) inspired gases. Three of these neonates were extubated during whole body hypothermia. Seven neonates breathed spontaneously throughout the study in room air at ambient nursery temperatures (25°C) without either nasal continuous positive airway pressure, or high flow intranasal cannula support.

Fifteen neonates received active cooling measures (10 intubated). Six anapyrexic neonates received passive cooling measures (2 intubated).

Heater-on and heater-off step transitions

Of the 21 neonates: 17 contributed step transition events for analysis; 6 subjects contributed to a heater-on and a heater-off step event; 8 subjects contributed to only a heater-on step event and 3 subjects contributed to only a heater-off step event.

Increases/decreases in T_{belly} caused by radiant heater on/off activity showed gradual increases/decreases in the same direction in T_{oes} and T_{rec}, with a tendency for T_{oes} to respond more rapidly (Figure 8.1). However T_{scap} showed an initial decrease of 0.085 ±0.003°C ($p<0.001$) twenty minutes after an increase in T_{belly} when the radiant heater was turned on. T_{scap} showed an initial increase of 0.085 ±0.006°C ($p<0.001$) fifteen minutes after a decrease in T_{belly}, when the radiant heater was turned-off.

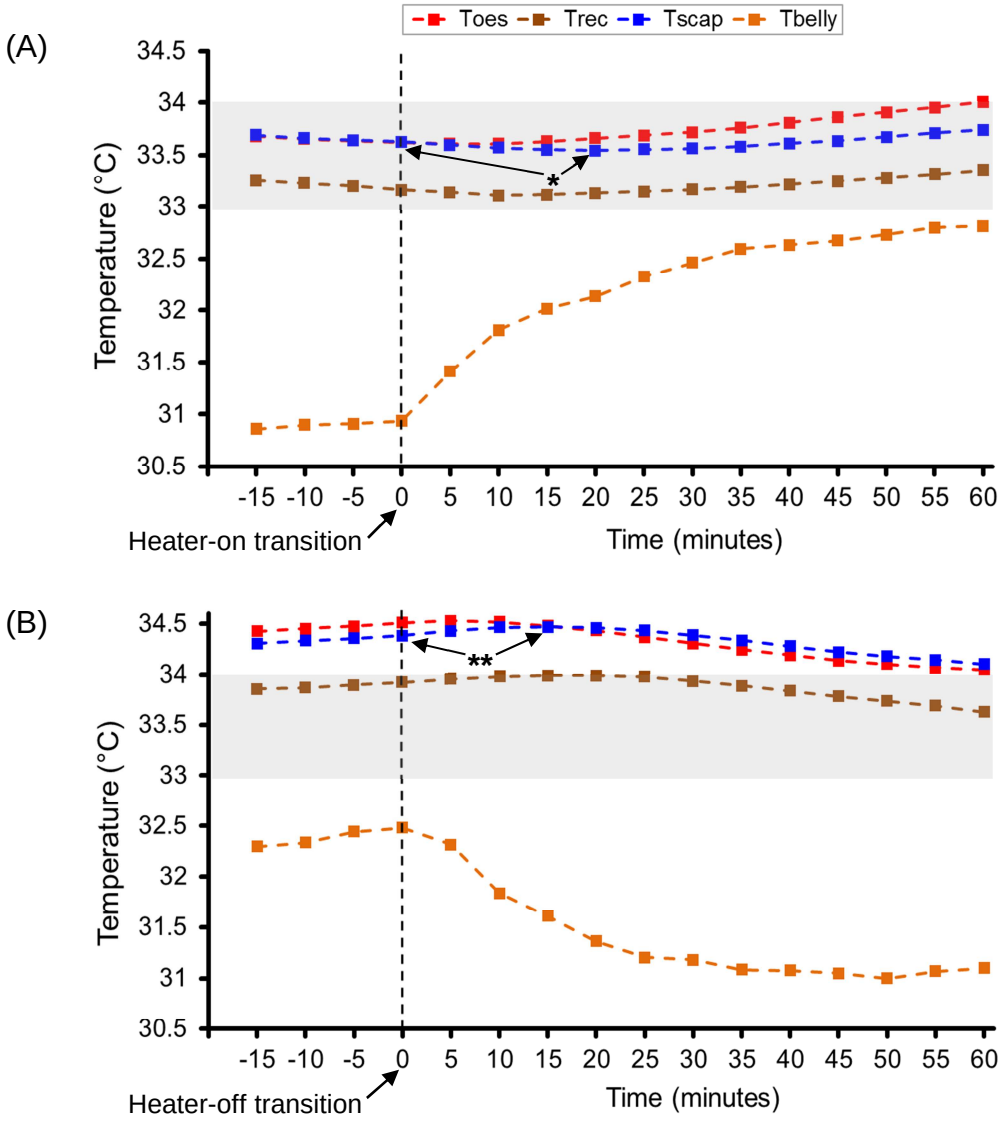


Figure 8.1 Mean temperatures during heater on and off step transitions

Observed mean temperatures from the lower oesophagus(T_{oes}), rectum (T_{rec}), inter-scapular skin (T_{scap}) and exposed abdominal skin (T_{belly}) from 17 subjects showing: (A) the response to a step increase in radiant heater activity, indicated by an increase in T_{belly} (n=14); (B) the response to a step decrease in radiant heater activity, shown by a decrease in T_{belly} (n=9). SE was less than 0.08°C for all temperatures and therefore not shown. The overhead radiant warmer was manually adjusted by the bed-side nurse when T_{rec} approached, or was outside the target range of 33-34°C. Note: *20 minutes after heater-on transition, T_{scap} decreased 0.085 ±0.003°C ($p<0.001$); **15 minutes after heater-off transition, T_{scap} increased 0.085 ±0.006°C ($p<0.001$).

Heater-on step transition with passively and actively cooled subjects

The data were subdivided into passively and actively cooled subjects to examine changes resulting from a step change response. Responses from passively and actively cooled subjects exposed to a heater-on transition were

similar to the combined data shown in Figure 8.1 (A).

Heater-off step transition with passively and actively cooled subjects

Passively cooled (anapyrexic) subjects that had a heater-off transition showed a different response to actively cooled subjects. A plot of each is shown in Figure 8.2 with a magnified temperature scale.

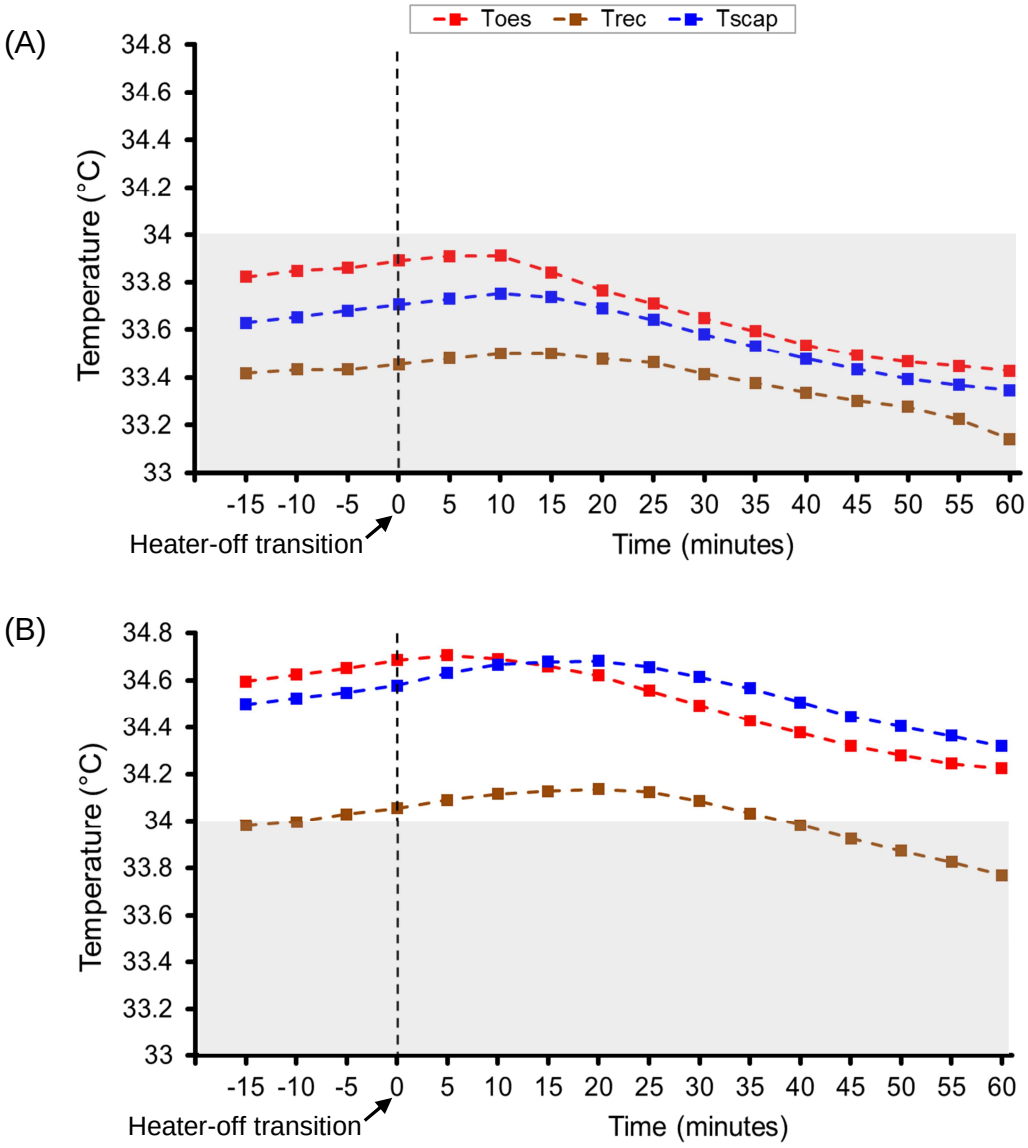


Figure 8.2 Heater-off response for passive and actively cooled subjects

Observed mean temperatures from the lower oesophagus(T_{oes}), rectum (T_{rec}), inter-scapular skin (T_{scap}) from 9 subjects showing the response to a heater-off step down transition for: (A) passively cooled (anapyrexic) subjects (n=2); (B) actively cooled subjects (n=7). SE was less than 0.05°C for all temperatures and therefore not shown. The shaded area represents the targeted range of T_{rec} (33-34°C). The overhead radiant warmer was manually adjusted by the bed-side nurse when T_{rec} approached, or was outside the target range.

Heater-off step transition (continued)

Compared to the group of actively cooled subjects at the time of the heater-off step transition shown in Figure 8.2, passively cooled subjects showed a lower T_{oes} (-0.79 ±0.07°C, p<0.001) and a lower T_{rec} (-0.61 ±0.03°C, p=0.09). The T_{oes}-T_{rec} difference from active to passive reduced to 0.19 ±0.04°C (p<0.001).

For both passively cooled and actively cooled subjects during the lead up to the heater-off transition, T_{scap} was relatively steady and in between T_{rec} and T_{oes}. After the heater-off transition, passively cooled subjects showed a transient increase in T_{scap} of 0.05 ±0.005°C (p<0.001) 10 minutes after the heater-off transition while T_{scap} remained in between T_{rec} and T_{oes}.

A different response was observed with actively cooled subjects. After the heater-off transition, T_{scap} transiently increased 0.10 ±0.01°C (p<0.001) twenty minutes after the heater-off transition while T_{scap} remained warmer than T_{oes} for the following 45 minutes.

Infra-Red temperatures following a heater-off transition

Temperatures of the neck nape from nine subjects were measured from thermal images taken shortly after the radiant heater was turned off. Temperatures were averaged at approximately 5 minute intervals from 1 to 60 minutes after the radiant heater was turned off (Table 8.1).

When the radiant heater was turned off, temperatures showed a general decrease over the 60 minute period. T_{nape} showed an initial non-statistically significant decrease in mean ±SE temperature of 0.22 ±0.12°C (p=0.09) twenty minutes after the heater-off transition. From this minimum, T_{nape} then maximally increased 0.13 ±0.06°C (p=0.05) 30 minutes after the heater-off transition. The response of T_{nape} is shown on a combined plot with T_{oes} and T_{scap} in Figure 8.3.

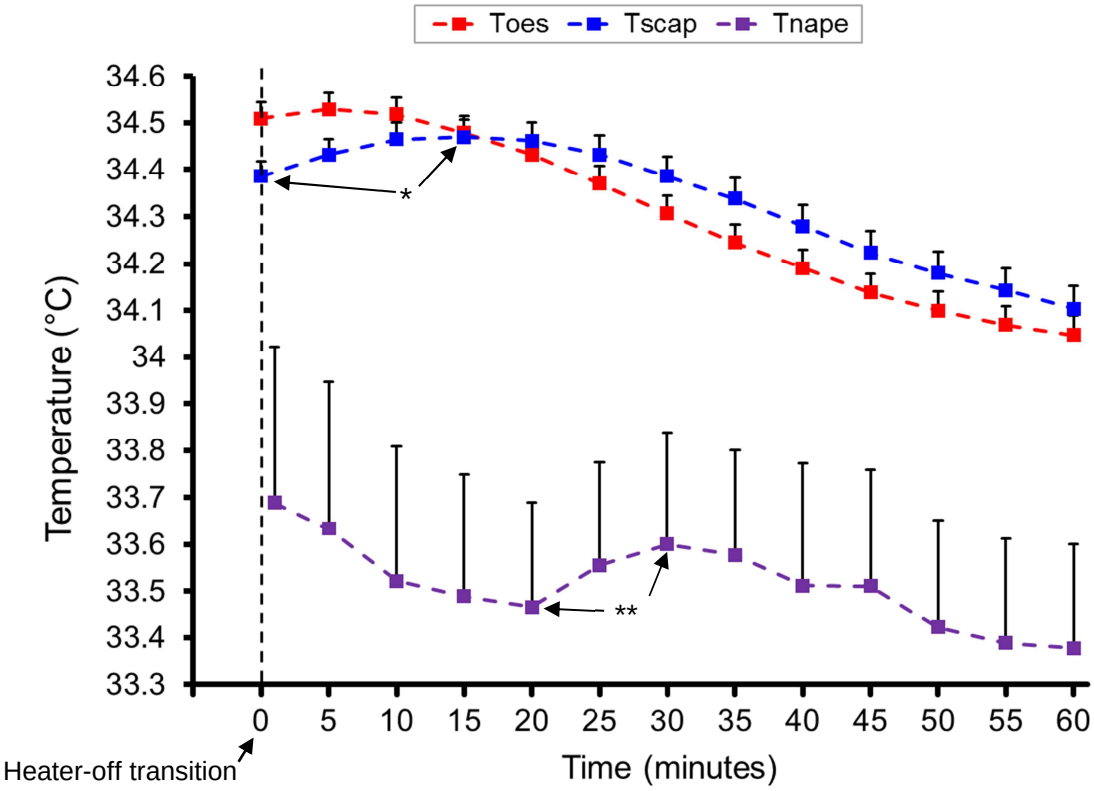


Figure 8.3 Contact and non-contact temperatures after heater-off

Mean (+SE) temperatures of the lower oesophagus (T_{oes}), inter-scapular skin (T_{scap}) and nape of neck (T_{nape}) showing the response to a heater-off transition. T_{oes} and T_{scap} sensors were in contact with body tissue (n=9), whereas T_{nape} was measured using infra-red thermography (non-contact) from the average of a 1 cm line positioned over the neck nape (n=9). The overhead radiant warmer was manually turned off by the bed-side nurse when T_{rec} approached or was above the upper target limit of 34°C. Note: *15 minutes after heater-off transition, T_{scap} increased 0.085 ±0.006°C ($p<0.001$); **from 20 to 30 minutes after heater-off, T_{nape} increased 0.13 ±0.06°C ($p=0.05$).

Time (min.)	T_{nape} (°C)	T_{heart} (°C)	$T_{up\text{-}arm}$ (°C)	$T_{lo\text{-}arm}$ (°C)	T_{thigh} (°C)	Tlo-leg (°C)	$T_{mat\text{-}H}$ (°C)	$T_{mat\text{-}F}$ (°C)
1	33.69	32.71	31.70	30.67	31.34	30.33	27.66	27.33
5	33.63	32.68	31.57	30.56	31.22	30.28	27.38	27.39
10	33.52	32.60	31.34	30.21	31.09	30.06	27.20	27.29
15	33.49	32.71	31.22	30.11	30.96	29.90	27.14	27.08
20	33.47	32.68	31.09	30.01	30.74	29.91	27.11	27.03
25	33.56	32.63	31.08	29.77	30.79	29.84	27.21	27.03
30	33.60	32.57	31.14	29.60	30.73	29.77	27.23	27.01
35	33.58	32.50	30.93	29.56	30.67	29.64	27.09	26.99
40	33.51	32.43	30.79	29.43	30.63	29.64	26.95	26.98
45	33.51	32.46	30.88	29.57	30.43	29.46	27.04	27.00
50	33.42	32.18	31.02	29.67	30.46	29.36	27.00	26.98
55	33.39	32.33	30.87	29.33	30.42	29.38	26.96	26.96
60	33.38	32.31	30.82	29.51	30.31	29.21	27.02	27.01

Table 8.1 Mean IR temperatures after a heater-off transition

Mean IR temperatures at eight sites for 60 minutes from 9 subjects following a heater-off step down transition. Sites of temperature measurement: nape of the neck (T_{nape}), skin above the heart (T_{heart}), upper arm ($T_{up\text{-}arm}$), lower arm ($T_{lo\text{-}arm}$), thigh (T_{thigh}), lower leg ($T_{lo\text{-}leg}$), mattress surface near the subject's head ($T_{mat\text{-}H}$), and mattress surface near the subject's feet ($T_{mat\text{-}F}$). Where there was more than one 60 minute heater-off period, the period with the longest preceding heater-on period was chosen for analysis. There was a general trend of decreasing temperature over the 60 minute period, except for T_{nape} which transiently increased 0.13 ±0.06°C (p=0.05, borderline significance) from 20 to 30 minutes after the heater-off transition.

Extubation during whole body hypothermia

Of the 21 neonates, 14 were ventilated with heated humidification of inspired gases (37°C). Three of these subjects were extubated and therefore commenced spontaneous breathing of room air during the period of whole body hypothermia. T_{oes}, T_{rec} and T_{scap} were analysed for 15 minutes before and 30 minutes after extubation (Figure 8.4). From the time leading to extubation to 5 minutes after extubation, T_{oes} and T_{rec} decreased by 0.22 ±0.02°C ($p<0.001$) and T_{rec} by 0.13 ±0.03°C ($p<0.001$) respectively.

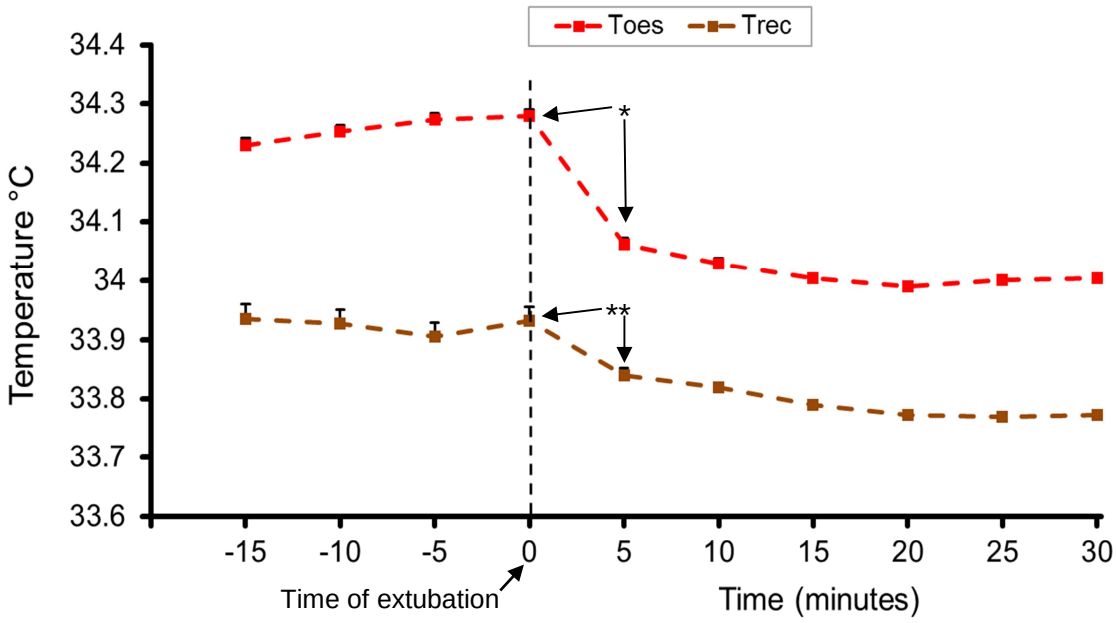


Figure 8.4 Endotracheal extubation during cooling

Observed mean (+SE) temperatures of the lower oesophagus (T_{oes}) and rectum (T_{rec}) from 3 actively cooled subjects. The response to the sudden removal of heated inspiratory gas from the ventilator and heated humidifier by removal of the endotracheal tube to allow spontaneous breathing of room air is plotted. Note that after extubation: * T_{oes} decreased by 0.22 ±0.02°C ($p<0.001$); ** T_{rec} decreased by 0.13 ±0.03°C ($p<0.001$).

Steady state temperature during cooling

Of the 21 neonates, 8 contributed both heater-on and heater-off steady state periods and all 21 subjects contributed one heater-off steady state period. Figure 8.5 displays observed mean temperatures for T_{oes}, T_{scap} and T_{rec} during heater-on and heater-off steady thermal states. Table 8.2 shows the mean and mean differences in temperature during thermal steady state at each site relative to T_{oes} (reference) in each heater on-off state. Although 3 neonates were extubated during the 72 hours of cooling, no extubations occurred during analysed steady state periods.

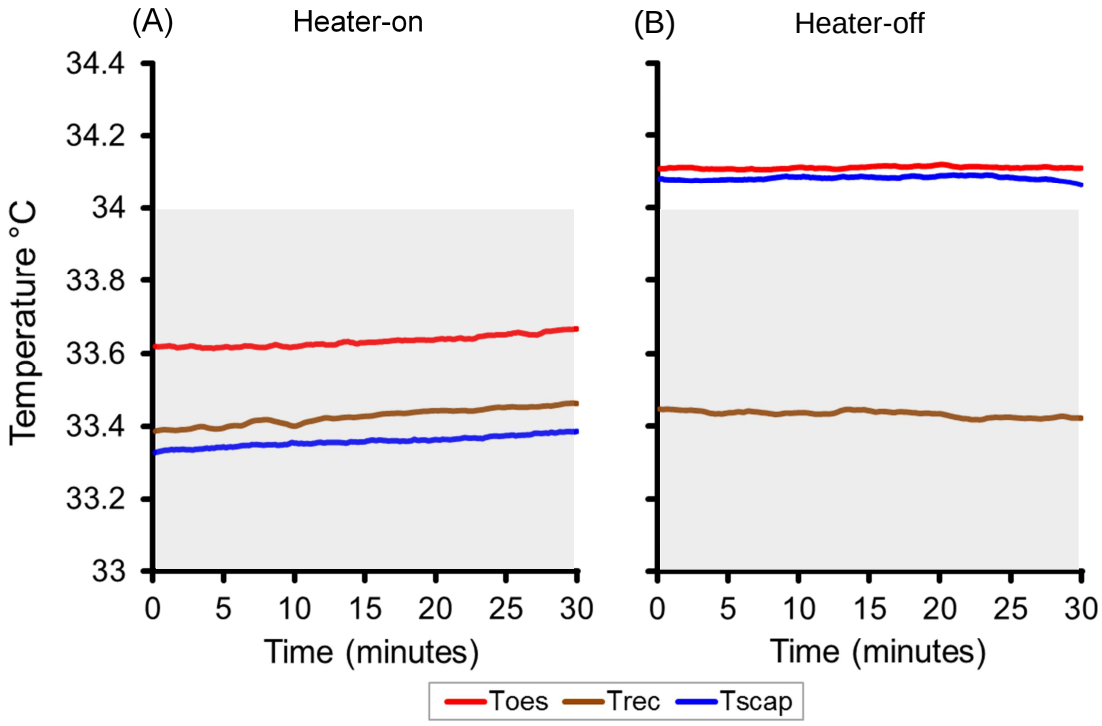


Figure 8.5 Temperatures during steady state heater-on and heater-off periods for the whole study group

Observed mean temperatures of the lower oesophagus (T_{oes}), rectum (T_{rec}) and inter-scapular skin (T_{scap}) from 21 cooled subjects during continuous 30 minute steady state periods showing: (A) thermal equilibrium with radiant heater-on (n=8); (B) thermal equilibrium with radiant heater-off (n=21). The shaded area shows the target T_{rec} range (33-34°C).

Site	Heater state	Observed mean temperature	Temperature difference from T_{oes} (reference)	Change in relative temperature difference heater-on to heater-off
T_{oes}	On	33.63 (0.001)		
T_{rec}	On	33.43 (0.002)	0.21 (0.002)*****	
T_{scap}	On	33.36 (0.001)	0.28 (0.002)*****	
T_{oes}	Off	34.11 (0.001)		
T_{rec}	Off	33.43 (0.001)	0.68 (0.001)*****	-0.47 (0.002)******
T_{scap}	Off	34.08 (0.001)	0.03 (0.001)*****	0.25 (0.002)******

*****p≤ 0.001 versus T_{oes} **p≤ 0.001 versus heater on

Table 8.2 Overall temperatures and differences during steady state heater on and off periods

Observed mean (±SE) temperatures (°C) of the lower oesophagus (T_{oes}), rectum (T_{rec}) and inter-scapular skin (T_{scap}) and temperature differences from 21 cooled subjects during 30 minute steady state periods with radiant heater-on (n=8) and with radiant heater-off (n=21).

T_{rec} was within the target range of 33-34°C in both steady heater states. T_{oes} was warmer than T_{rec} under both steady state conditions (p<0.001, Table 8.2). During heater-on steady state T_{oes} was 0.28 ±0.002°C higher than T_{scap} (p<0.001) and this difference reduced to 0.03 ±0.001°C (p<0.001) during heater-off steady state. This was a change in temperature difference from heater-on compared to heater-off of 0.25 ±0.002°C (p<0.001).

In contrast, the difference between T_{oes} and T_{rec} was 0.21 ±0.002°C (p<0.001) during heater-on steady state and this difference increased to 0.68 ±0.001°C (p<0.001) during heater-off steady state. This was a change in temperature difference from heater-on compared to heater-off of -0.47 ±0.002°C, (p<0.001). During heater-on steady state, T_{rec} was higher than T_{scap} (T_{rec} – T_{scap} was 0.07 ±0.002°C, p<0.001) whereas during heater-off, T_{rec} was lower than T_{scap} (T_{rec} – T_{scap} was -0.65 ±0.001°C, p<0.001).

Subgroup analysis of steady state periods from passively cooled (anapyrexic) neonates

Of the 21 neonates, 6 anapyrexic subjects (2 intubated) received passive cooling (heater-off) to lower or keep T_{rec} between 33-34°C. Four subjects contributed one heater-on steady state period and all 6 subjects contributed one heater-off steady state period. Figure 8.6 shows observed mean temperatures of T_{oes}, T_{rec} and T_{scap} during heater-on and passive heater-off steady thermal states. Table 8.3 shows overall mean and mean differences in temperature during thermal steady state at each site relative to T_{oes} (reference) in each heater on-off state.

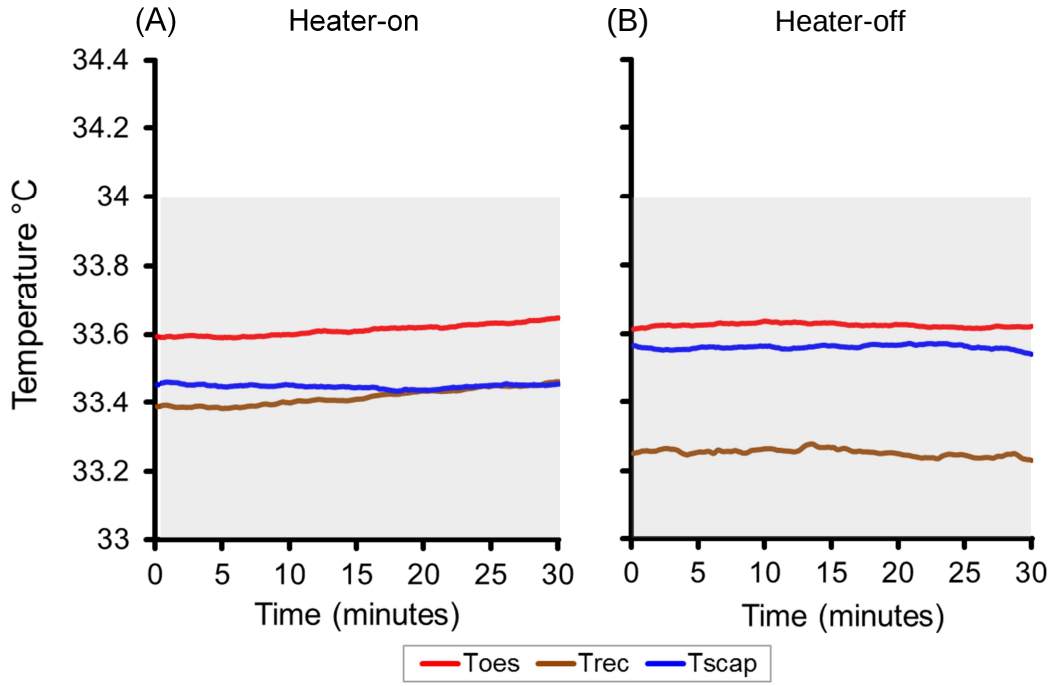


Figure 8.6 Steady state temperatures from passively cooled subjects

Observed mean temperatures of the lower oesophagus (T_{oes}), rectum (T_{rec}) and inter-scapular skin (T_{scap}) from 6 passively cooled (anapyrexic) subjects during continuous 30 minute steady state periods showing: (A) thermal equilibrium with radiant heater-on (n=4); (B) thermal equilibrium with radiant heater-off (n=6). The shaded area shows the target T_{rec} range (33-34°C).

Both T_{rec} and T_{oes} were within the target range of 33-34°C in both steady heater-on and heater-off states, with T_{oes} warmer than T_{rec} under both steady state conditions ($p<0.001$). During heater-on steady state, T_{oes} was 0.17 ±0.002°C higher than T_{scap} ($p<0.001$) and this difference reduced to 0.06 ±0.001°C ($p<0.001$) during heater-off. This was a change in temperature difference from heater-on steady state compared to passive heater-off steady state of 0.10 ±0.002°C ($p<0.001$).

During heater-on steady state, the difference between T_{oes} and T_{rec} was 0.20 ±0.002°C ($p<0.001$) and this difference increased to 0.37 ±0.001°C ($p<0.001$) during heater-off. This was a change in temperature difference from warming compared to cooling of -0.18 ±0.002°C ($p<0.001$). With warming, T_{rec} was slightly cooler than T_{scap} ($T_{rec} – T_{scap}$ was -0.03 ±0.002°C, $p<0.001$) whereas during heater-off T_{rec} was considerably lower than T_{scap} ($T_{rec} – T_{scap}$ was -0.31 ±0.001°C, $p<0.001$).

Site	Heater state	Observed mean temperature	Temperature difference from T_{oes} (reference)	Change in relative temperature difference heater-on to heater-off
T_{oes}	on	33.61 (0.001)		
T_{rec}	on	33.42 (0.002)	0.20 (0.002)*****	
T_{scap}	on	33.45 (0.001)	0.17 (0.002)*****	
T_{oes}	off	33.62 (0.001)		
T_{rec}	off	33.25 (0.001)	0.37 (0.001)*****	-0.18 (0.002)******
T_{scap}	off	33.56 (0.001)	0.06 (0.001)*****	0.10 (0.002)******

*****p≤ 0.001 versus T_{oes} **p≤ 0.001 versus heater on

Table 8.3 Temperatures and differences during steady state heater-on and heater-off periods (passively cooled neonates)

Observed mean (±SE) temperatures (°C) of the lower oesophagus (T_{oes}), rectum (T_{rec}) and inter-scapular skin (T_{scap}) and temperature differences from 6 anapyrexic subjects during 30 minute steady state periods with radiant heater-on (n=4) and with radiant heater-off (n=6).

Steady state temperatures from actively cooled subjects

Of the 21 neonates, 15 subjects (10 intubated) received active cooling measures. These measures were fan-on or application of cool packs to lower or maintain T_{rec} to be within the target range of 33-34°C. Four subjects contributed one heater-on steady state period and all 15 actively cooled subjects contributed one heater-off steady state period.

Figure 8.6 displays observed mean temperatures of T_{oes}, T_{scap} and T_{rec} during heater-on and heater-off steady thermal states from 15 subjects who received active cooling. Table 8.4 shows mean and mean differences in temperature during thermal steady state at each site relative to T_{oes} (reference) in each heater on-off state.

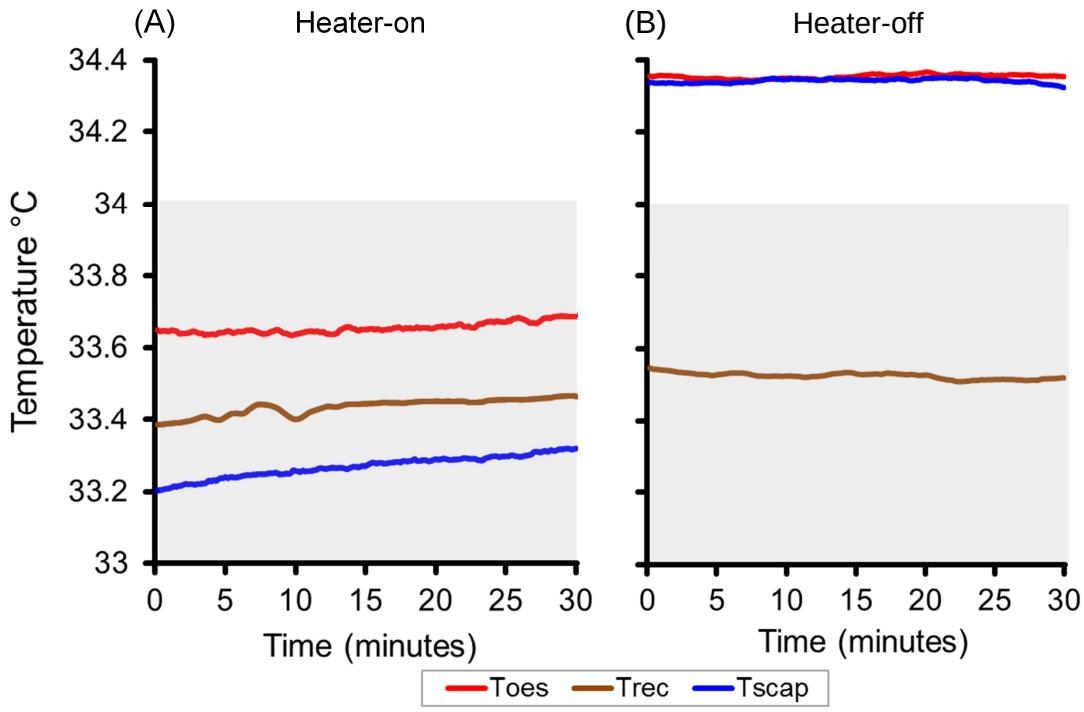


Figure 8.7 Steady state temperatures from actively cooled subjects

Observed mean temperatures from 15 actively cooled subjects of the lower oesophagus (T_{oes}), rectum (T_{rec}) and inter-scapular skin (T_{scap}) during hypothermia in continuous 30 minute steady state periods showing: (A) thermal equilibrium with radiant heater-on (n=4); (B) thermal equilibrium with radiant heater-off (n=15). The shaded area shows the target T_{rec} range (33-34°C).

T_{rec} was within the target range of 33-34°C in both steady heater-on and heater-off states while T_{oes} was warmer than T_{rec} under both steady state conditions ($p<0.001$, Table 8.4). T_{oes} was within the target range of 33-34°C during heater-on steady state conditions and during heater-off steady state conditions, T_{oes} was above 34°C. During heater-on steady state, T_{oes} was 0.39 ±0.002°C higher than T_{scap} ($p<0.001$) and during heater-off this difference reduced to 0.01 ±0.001°C ($p<0.001$). This was a change in temperature difference from warming compared to passive heater-off cooling 0.38 ±0.002°C ($p<0.001$).

During heater-on steady state, the difference between T_{oes} and T_{rec} was 0.22 ±0.002°C ($p<0.001$) and during heater-off, this difference increased to 0.83 ±0.001°C ($p<0.001$). This was a change in temperature difference from warming compared to cooling of -0.61 ±0.002°C ($p<0.001$). During heater-on steady state, T_{rec} was warmer than T_{scap} ($T_{rec} – T_{scap}$ was 0.17 ±0.003°C, $p<0.001$) whereas during heater-off, T_{rec} was cooler than T_{scap} ($T_{rec} – T_{scap}$ was -0.82 ±0.001°C, $p<0.001$).

Site	Heater state	Observed mean temperature	Temperature difference from T_{oes} (reference)	Change in relative temperature difference heater-on to heater-off
T_{oes}	On	33.66 (0.001)		
T_{rec}	On	33.43 (0.002)	0.22 (0.002)*****	
T_{scap}	On	33.27 (0.002)	0.39 (0.002)*****	
T_{oes}	Off	34.35 (0.001)		
T_{rec}	Off	33.52 (0.001)	0.83 (0.001)*****	-0.61 (0.002)******
T_{scap}	Off	34.34 (0.001)	0.01 (0.001)*****	0.38 (0.002)******

*****p≤ 0.001 versus T_{oes} **p≤ 0.001 versus heater on

Table 8.4 Temperatures and differences during steady state heater-on and heater-off periods (actively cooled neonates)

Observed mean (±SE) temperatures (°C) of the lower oesophagus (T_{oes}), rectum (T_{rec}) and inter-scapular skin (T_{scap}) and temperature differences from 15 hypothermic subjects during 30 minute steady state periods with radiant heater-on (n=4) and with radiant heater-off (n=15).

Steady state (normothermic) temperature after rewarming

Temperature monitoring continued with 16 cooled subjects for a short period after rewarming. Twelve of these subjects contributed one heater-on steady state period (two subjects remained intubated) of which 7 subjects had been actively cooled (Figure 8.8). Eight subjects contributed one heater-off steady state period (one subject remained intubated) of which six had been actively cooled. Table 8.5 shows the mean and mean differences in temperature during thermal steady state at each site relative to Toes (reference) in each heater on and off state.

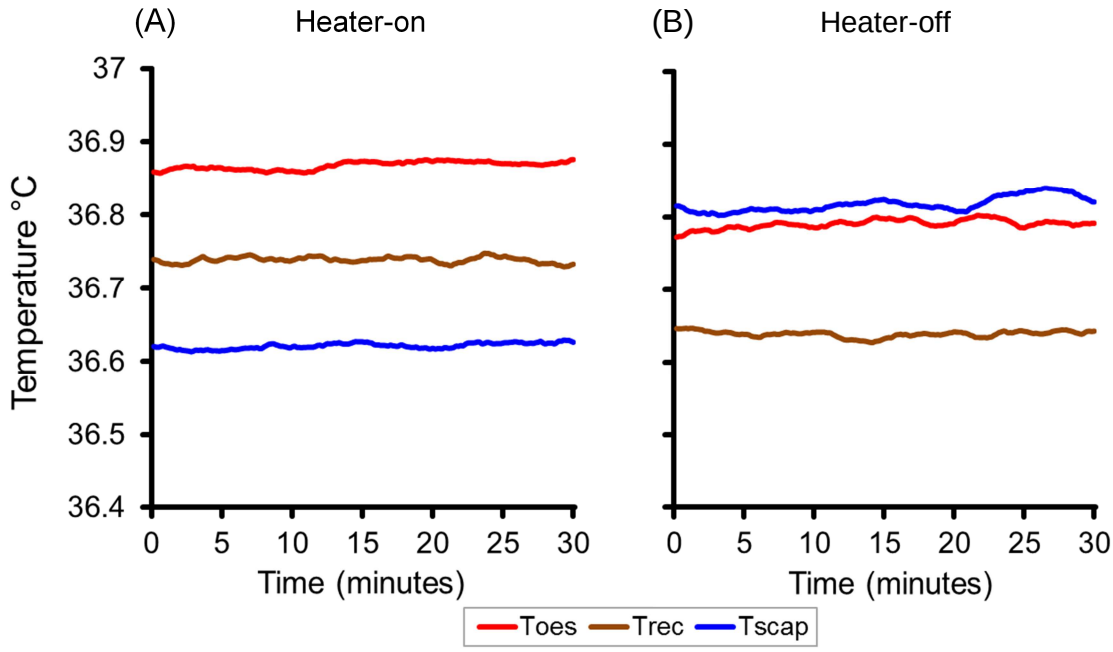


Figure 8.8 Normothermic temperatures during steady state heater-on and heater-off periods after rewarming

Observed mean temperatures after rewarming of 12 actively cooled subjects of the lower oesophagus (T_{oes}), rectum (T_{rec}) and inter-scapular skin (T_{scap}) during continuous 30 minute steady state periods showing: (A) thermal equilibrium with radiant heater-on (n=12); (B) thermal equilibrium with radiant heater-off (n=8). The shaded area shows the target T_{rec} range (33-34°C). Of the 12 neonates with heater-on, 7 had received active cooling. Of the 8 neonates with heater-off, 6 had received active cooling.

Site	Heater state	Observed mean temperature	Temperature difference from T_{oes} (reference)	Change in relative temperature difference heater-on to heater-off
T_{oes}	On	36.87 (0.001)		
T_{rec}	On	36.74 (0.001)	0.13 (0.001)*	
T_{scap}	On	36.62 (0.001)	0.25 (0.001)*	
T_{oes}	Off	36.79 (0.001)		
T_{rec}	Off	36.64 (0.001)	0.15 (0.001)*	-0.02 (0.002)**
T_{scap}	Off	36.82 (0.002)	-0.03 (0.002)*	0.27 (0.002)**

*p≤ 0.001 versus T_{oes} **p≤ 0.001 versus heater on

Table 8.5 Overall temperatures and differences after rewarming during steady state heater on and off periods

Observed mean (±SE) temperatures (°C) of the lower oesophagus (T_{oes}), rectum (T_{rec}) and inter-scapular skin (T_{scap}) and temperature differences after rewarming from 12 post cooled (normothermic) subjects during 30 minute steady state periods with radiant heater-on (n=12) and with radiant heater-off (n=8).

Both T_{rec} and T_{oes} were within the normothermic range of 36.5-37.5°C in both steady heater states, with T_{oes} warmer than T_{rec} under both steady state conditions ($p<0.001$, Table 8.5). With heater-on, T_{oes} was 0.25 ±0.001°C higher than T_{scap} ($p<0.001$). However, T_{scap} became warmer than T_{oes} by 0.03 ±0.002°C ($p<0.001$) during heater-off. This was a change in temperature difference from heater-on steady state compared to heater-off of 0.27 ±0.002°C ($p<0.001$).

In contrast and with heater-on steady state, the difference between T_{oes} and T_{rec} was 0.13 ±0.001°C ($p<0.001$) and this difference slightly increased to 0.15 ±0.001°C ($p<0.001$) during heater-off. This was a change in temperature difference from heater-on compared to heater-off of -0.02 ±0.002°C, ($p<0.001$). During heater-on steady state, T_{rec} was higher than T_{scap} ($T_{rec} – T_{scap}$ was 0.12 ±0.001°C, $p<0.001$) whereas during heater-off, T_{rec} was cooler than T_{scap} ($T_{rec} – T_{scap}$ was -0.18 ±0.002°C, $p<0.001$).

Normothermic neonates: post-cooled versus non-cooled

Heater-on and heater-off steady state temperatures occurring with post-cooled subjects were compared to heater-on and heater-off steady state temperatures from a cohort of normothermic neonates (Chapter 6) who had not been exposed to therapeutic cooling. Figure 6.3 in Chapter 6 shows steady state heater-on and heater-off periods for intubated critically ill normothermic neonates who had not been exposed to therapeutic cooling. Although both cohorts had T_{rec} temperatures in the normothermic range (36.5-37.5°C), absolute temperatures were slightly different between the two cohorts. Therefore temperature differences within each cohort were used for comparison between the two cohorts.

Temperature difference sites	Heater state	Post-cooled (n=12) temperature difference	Non-cooled (n=12) temperature difference	Post cooled – non cooled temperature difference
T_{oes}-T_{rec}	On	0.13 (0.001)*****	0.13 (0.001)*	0.00 (0.001)
T_{oes}-T_{scap}	On	0.25 (0.001)*****	0.21 (0.001)*	0.04 (0.002)**
T_{rec}-T_{scap}	On	0.12 (0.001)*	0.08 (0.001)*	0.04 (0.002)**
Temperature difference sites	Heater state	Post-cooled (n=8) temperature difference	Non-cooled (n=9) temperature difference	Post cooled – non cooled temperature difference
T_{oes}-T_{rec}	Off	0.15 (0.001)*****	0.32 (0.002)*	-0.17 (0.002)**
T_{oes}-T_{scap}	Off	-0.03 (0.002)*****	0.11 (0.002)*	-0.14 (0.002)**
T_{rec}-T_{scap}	Off	-0.18 (0.002)*	-0.21 (0.002)*	0.03 (0.002)**

*****p≤ 0.001 ******p≤ 0.001 post-cooled versus non-cooled

Table 8.6 Temperature differences during normothermic steady state heater on and off periods between post-cooled and non-cooled neonates

Observed mean (±SE) temperature differences (°C) between the lower oesophagus (T_{oes}), rectum (T_{rec}) and inter-scapular skin (T_{scap}) during 30 minute normothermic steady state heater-on and heater-off periods, for post-cooled (n=12) and non-cooled neonates (n=13).

The major quantitative difference in the two groups was the difference between T_{oes}-T_{rec} and T_{oes}-T_{scap}. During thermal steady state with heater-off, the T_{oes}-T_{rec} difference (-0.17 ±0.002°C, $p<0.001$) was less in the post-cooled group than with the non-cooled group. Also, during thermal steady state with heater-off, the T_{oes}-T_{scap} difference was inverted in the post-cooled group compared to the non-cooled group such that T_{scap} was warmer than T_{oes} in the post-cooled group by 0.03 ±0.002°C ($p<0.001$).

8.4 DISCUSSION

Temperatures during non-steady state

Most of the cooling period was occupied with non-steady state core and skin temperatures where manual on/off adjustment of the radiant heater (passive) or application of cooling fan or cool packs (active) led to step change transitions. The response at different body sites varied for each particular type of step transition.

After a step-up (heater-on) transition, T_{scap} transiently decreased maximally 20 minutes after the transition. The converse occurred prior to a step down (heater-off) transition where T_{scap} transiently increased maximally 15 minutes after the transition. Although the magnitude of change is small (0.085 ±0.003°C, $p<0.001$), the accuracy of the method of temperature measurement suggests the change is real. These results are similar to, but of greater magnitude to observations in Chapter 6 with normothermic neonates. The fact that the change is in the opposite direction to environmental heating or cooling is suggestive of thermogenesis beneath inter-scapular skin switching off/on respectively. The hypothesis that BAT is active during cooling is also supported by the observation that babies requiring active cooling have a greater magnitude of increase in T_{SCAP} after a step down transition when compared to passively cooled babies who do not mount a defense of core temperature. However, the small number of step-down transitions in passively cooled babies (N=2) prevents a firm conclusion in this regard. The changes in T_{scap} are transient and this is likely to reflect the influence of heat transmission to and from the adjacent core tissues as the core temperature changes.

T_{nape} was compared to T_{scap} during heater-off step-down transitions and T_{nape} transiently increased maximally 30 minutes after the heater was turned off whereas T_{scap} transiently increased 15 minutes after the heater was turned off. This supports the assertion made in Chapter 6 that a sudden decrease with skin temperature seemed to first stimulate thermogenesis in the inter-scapular region. A possibility is that returning warmed blood to the central circulation acts to defend brain temperature. The later transient increase in T_{nape} may suggest an increase in warm venous return from the brain just beneath the skin in the neck. However this assertion cannot be made with confidence, as equally plausible explanations include transmission of heat from the back or a delay in neck BAT thermogenesis.

The heater-off step-down transition with normothermic neonates (Chapter 6) showed T_{scap} and T_{nape} transiently increasing maximally at 10 and 20 minutes respectively. In the present study, the heater-off step down transition with hypothermic neonates showed T_{scap} and T_{nape} increasing maximally at 15 and 30 minutes respectively. These data may suggest that hypothermia slowed down the response time of thermogenesis. However, a more likely explanation is that the detection of temperature change by cutaneous sensors may be impeded by vasoconstriction during therapeutic hypothermia.

Thermal steady state

The steady state data show that the magnitude of difference between T_{oes} and T_{scap} changes depending on whether the skin is warmed or cooled, and whether the neonate is difficult to cool (active defence of core temperature) or easy to cool (passively cooled without a strong defence of normal core temperature i.e. anapyrexia). These changes are similar to those observed in normothermic neonates. As noted in Chapter 6, T_{scap} should always follow T_{oes} in true steady state according to the principle of zero heat flux, and the observation that T_{scap} changes in absolute terms and relative to T_{oes} suggests local thermogenesis. The direction of change (increase in T_{scap} which is closer to T_{oes} with cooling, and decrease in T_{scap} becoming further from T_{oes} during warming) supports BAT thermogenesis defending central temperature with cooling.

The fall in T_{scap} relative to T_{oes} with skin warming is also consistent with a lowering of the threshold for BAT activation. Skin cooling or warming has been shown to influence the thresholds for shivering in adult humans.[199] Cheng et al lowered core temperature using intravenous 3°C fluid to determine vasoconstriction and shivering thresholds in adult males and females, while maintaining different and fixed skin temperatures between 31 and 37°C. Core temperature response thresholds were lowered if mean skin temperature was warmer with a greater contribution to the control of vasoconstriction and shivering from the skin of the chest, neck and face.[199]

In addition, those neonates showing a more vigorous defence of core temperature (requiring active cooling measures) showed greater change both in T_{scap}, and the difference between T_{oes} and T_{scap} with heater on and off states. This suggests that BAT responsiveness is dampened below normal in neonates requiring passive cooling, or that BAT is stronger than normal in neonates who actively defend core temperature. A dampening of BAT activity in passively cooled neonates is consistent with a reduction in core temperature set-point as would be expected with anapyrexia.

Active cooling provides a more potent cold signal to cutaneous receptors that are integrated centrally, and in response, may increase the activity of BAT. Rate of change in skin temperature has also been determined to influence sweating thermoregulatory responses in adults.[200] However, active cooling was initiated in response to the neonate's thermoregulatory defence and this favours a primary central difference in BAT activity between passively and actively cooled neonates.

The data also suggest that T_{oes} is influenced by T_{scap}. For example, T_{oes} moved in the same direction as T_{scap} in all heater states with both active and passive cooling, and when T_{oes} was raised, T_{scap} became closer to T_{oes}. These observations are consistent with BAT warming central venous blood returning to the heart. One important source of central venous blood is cerebral venous return and therefore the warmer T_{oes} may reflect a warmer brain temperature.

Changes in T_{scap} may be influenced by the mattress temperature in contact with the back. However, in Chapter 5 and 6 data were presented to show that

mattress surface temperature as measured by IR imaging along with under-mattress temperature did not explain similar changes in T_{scap} during normothermia. Furthermore, in Chapter 5 validation studies using a 3 kg saline bag showed that the mattress beneath a neonate does not retain significant heat as the body cools.

This study extends the observations made in Chapter 7 that T_{oes} is warmer than T_{rec} during therapeutic hypothermia when T_{rec} is targeted with manual controlled cooling, by demonstrating the potential influence of BAT on T_{oes}. In Chapter 7 the slower response time of T_{rec} to environmental temperature change contributed to overshoot in the more rapidly responsive T_{oes}. In the current study, steady state T_{oes} is also shown to be warmer than T_{rec} in steady state, revealing that the difference in lag times between sites is not the only explanation for the warmer T_{oes}. While cool skin blood returning from the legs can lower T_{rec}, as shown in Table 8.1 and Chapters 5 and 6, T_{rec} was controlled in the studied neonates because it was the target temperature. Consequently, T_{rec} did not vary appreciably in heater-on and heater-off states and the difference between T_{oes} and T_{rec} is only partially explained by lowering of T_{rec}.

This study also included post warming steady state observations which were compared to steady state observations made in Chapter 6 during normothermia in critically ill neonates who had not been exposed to therapeutic cooling. The post-cooled neonates in the current study showed a remarkable difference in that T_{scap} was warmer than T_{oes} during heater-off steady state. This novel observation suggests recruitment of thermogenic capacity after exposure to cold. While most post-cooled neonates were extubated and the normothermic comparison group were all intubated, an effect of intubation on temperature recordings is unlikely because tracheal temperatures should be the same in both groups. Postnatal exposure to cold is necessary in the newborn rat pup to recruit BAT and adapt to the environment after birth, with the recruitment of BAT inhibited by maintaining a thermoneutral environment.[201] BAT recruitment has not been previously demonstrated in human neonates. However, BAT recruitment has been demonstrated in adult humans as part of cold acclimation using 18

fluorodeoxyglucose positron-emission tomography (PET).[142] Chronic nor-adrenergic stimulation of BAT via sympathetic nervous system activation is considered the basis for recruitment of BAT in response to cold.[39]

Endotracheal extubation during cooling

Activity of BAT is not the only factor resulting in a higher T_{oes} during hypothermia when T_{rec} is being monitored. Intubation and warmed inspired gas increases T_{oes} by approximately 0.3°C as shown in the three subjects where temperature monitoring occurred during extubation events. When intubated, pulmonary capillary blood perfusing ventilated alveoli will be warmed to inspired alveolar gas temperature (approximating 37°C) and pulmonary venous return will warm blood in the left heart and aorta. The extent of warming will depend on pulmonary blood flow and the degree of intra-pulmonary shunt. In contrast, inspired gases at an ambient temperature of approximately 25°C in non-intubated neonates will be warmed in the airway and result in alveolar gas temperatures and pulmonary venous return with at best, a temperature of 33-34°C.

Limitations

As with all studies where skin temperature is measured, this study was limited because it did not directly measure temperature in BAT. The activity of BAT could also have been explored by measurement of oxygen consumption. Unfortunately the equipment used in Chapter 5 was developed for oxygen consumption measurement in healthy spontaneously breathing neonates and time and technical constraints prevented the measurement of oxygen consumption in ventilated neonates. A further limitation was that all the neonates studied received medications that are known to influence thermoregulation.[106]

8.5 CONCLUSION

The data support the concept of BAT thermogenesis contributing to warming the central core (T_{oes}). Intubation and the use of warmed inspired gas also increases T_{oes}. The clinical implication of this finding is that brain temperature may be inadvertently warmer than 34°C when T_{rec} is targeted. Active BAT may heat the brain by warming venous return in the neck and reducing convective heat loss, and warming aortic blood that perfuses deep brain tissue. In addition, BAT activation is a metabolic stress that may adversely affect the brain with HIE. BAT activation may therefore be potentially detrimental to neonates with HIE. However, the role of BAT in this clinical context has not been studied before and further data are required. An important observation is that differences in BAT activation were noted between neonates who were easy to cool passively compared to those who were difficult to cool and required active cooling measures. While the clinical importance of this finding is uncertain, an active BAT response to cold stress may indicate less central injury rather than diminishing the efficacy of hypothermia in neuro-protection.

The next Chapter explores MRI findings in neonates who received active and passive cooling in an attempt to determine the clinical relevance of a poor versus a brisk BAT response to therapeutic hypothermia.

CHAPTER NINE

9 HYPOTHERMIC BAT AND MRI FINDINGS

9.1 INTRODUCTION

In Chapters 7 and 8, neonates receiving hypothermia treatment were classified into two groups based on whether passive or active manual cooling was required to lower T_{rec} to 33-34°C. Neonates receiving passive cooling (anapyrexic) appeared to have impaired thermoregulatory defences, whereas neonates requiring active cooling appeared to mount a vigorous defence of core temperature. In Chapter 8 the observation was made that the absolute T_{scap} and the difference between T_{scap} and T_{oes} during steady state changed during hypothermia with skin warming or cooling. With cooling of the skin (radiant heater-off) T_{scap} increased and the difference between T_{scap} and T_{oes} decreased, while the opposite occurred when the skin was gently warmed (radiant heater-on). These observations were consistent with BAT thermogenesis responsive to changes in skin temperature. Differences were found between neonates requiring passive and active cooling in the reactivity of BAT. There were considerably larger changes in T_{scap} and T_{scap}-T_{oes} in heater-on and heater-off states noted in those neonates that received active cooling. This suggests an active defence of core temperature due to BAT activity in neonates who are difficult to cool.

There are no published data as to whether an active thermoregulatory defence is beneficial or detrimental to the brain in the context of HIE. However, the physiological role of BAT is to heat the brain. Therefore on first principles, active BAT is likely to act against therapeutic hypothermia. Data from the current series of experiments have shown that neonates requiring active cooling, where T_{rec} is monitored, have a larger mean difference between T_{oes} and T_{rec}. Here, T_{oes} is greater than the upper targeted temperature limit of 34°C for longer periods (Chapter 7). As T_{oes} reflects aortic blood temperature,[120] these data suggest that the temperature of aortic blood that perfuses deep brain is higher in neonates mounting a defence of core temperature. Therefore the hypothesis was formulated that active BAT thermogenesis is detrimental to neonates with HIE by acting against protective

hypothermia.

The aim of this Chapter is to test this hypothesis by examining the association between passive and active cooling of HIE neonates with MRI evidence of brain injury. In the context of this PhD, MRI evidence was chosen as the endpoint due to timeframe constraints.

9.2 METHODS

This was a cross-sectional comparison study based on the same cohort of HIE neonates described in Chapters 7 and 8. Institutional ethics approval was granted by the Southern Adelaide Clinical Human Research Ethics Committee and the Children Youth and Women's Health Service Human Research Ethics Committee and informed consent was obtained from parents.

Neonates received therapeutic cooling based on the NICU protocol that required moderate to severe encephalopathy, ≥35 weeks gestation, and a minimum of two of the following criteria: Apgar score of <6 at 10 minutes; continued need for airway positive pressure at 10 minutes; a cord arterial pH <7.0; base excess -12 or lower from the umbilical cord or neonate at <60 minutes of age; a history or evidence of an acute perinatal event that may result in HIE. Consideration was given to therapeutic cooling if criteria were met and the neonate was less than 6 hours of age.

Assessment of encephalopathy

The assessment of encephalopathy typically occurred over the first 6 hours of life based on clinical criteria of Shankaran et al[9] and aEEG monitoring when this was available. Encephalopathy was classified according to modified Sarnat criteria into stage 1, 2 or 3 which is based on the evolution of the encephalopathy over the first days of life.[202]

Methods of cooling and temperature recording are the same as documented in Chapters 7 and 8. The aEEG information was not available for all subjects and so not included in the present study.

MRI method

An MRI was planned in all cooled neonates between 5 to 7 days of age. MR images were included if they were taken within the first 4 weeks after birth - the optimum period in which to image brain lesions that occur around the time of birth.[203] The images were from routine neonate brain scan series using conventional T1-weighted and T2-weighted sequences at 1.5 Tesla (T). A 1.5T scanner was used to avoid potential heating of the neonate and to improve T1-weighted contrast.

The routine MRI protocol for HIE neonates is as follows: Sagittal T1 spin echo (SE), Axial T1 SE, Axial T2 Turbo Spine Echo (TSE), Axial diffusion, Axial T2* gradient echo (GRE), Coronal T2 TSE. Slice thickness is usually 3mm to obtain high resolution images. This is followed by 2 single voxel spectroscopies (30ms) and intermediate (135ms) echo times at the basal ganglia and the contralateral parietal lobe white matter were routinely obtained.

While radiologists who reported on the MR image were unaware of the active cooling or passive cooling status of neonates, other routine clinical notes including the Sarnat grading were recorded on MRI request forms. All scans were reported by paediatric radiologists. The presence of any abnormality in the basal ganglia, thalamus, brainstem, cortex, posterior limb of the internal capsule, or white matter was obtained from the radiology reports. Grading of the severity of the abnormalities was not performed in the routine reporting.[63] Non-HIE abnormalities including subdural haematomas were also recorded.

Statistical methods

Data were analysed using IBM SPSS version 22 (IBM Corp. NY, USA).
Due to the small sample size, clinical characteristics between groups were compared by Mann-Whitney U Test (2-tailed) for continuous data or Fisher's exact test (2-tailed) for categorical data.

Calculation of an odds ratio (OR) and adjusted OR with 95% confidence intervals (CI) were performed to assess the effect of the type of cooling received (active v passive) on cerebral lesions seen on MRI. Adjusted OR used age at scan and gender as confounding factors. A Chi squared test was used to determine statistical significance for OR and adjusted OR.

The alpha level for statistical significance was chosen as $p<0.05$.

For OR calculation, where an input variable contained a zero, 0.5 was added to all four input variables in a 2x2 table to allow an approximation using the following formula:

$$OR = \frac{a/b^*}{c/d^{**}}$$

	Brain abnormality	
	Yes (abnormal)	No (normal)
Actively cooled	a	b
Passively cooled	c	d

* Odds of brain abnormality with actively cooled = a/b

** Odds of brain abnormality with passively cooled = c/d

±95% confidence intervals of OR using the natural log of OR:

$$= \exp\left[\ln(OR) \pm 1.96\sqrt{\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}}\right]$$

Manual p value calculation: $\frac{(a+b)!\,(c+d)!\,(a+c)!\,(b+d)!}{n!\,a!\,b!\,c!\,d!}$

9.3 RESULTS

Clinical details of 21 neonates who received either active or passive manual cooling methods are summarised in Table 9.1.

Clinical characteristics:	Actively cooled (n=15)	Passively cooled (n=6)	p-value for comparison
Gestational age (weeks)	40 (39-40)	39.5 (38.25-40.25)	0.57[#]
Birthweight (g)	3470 (3240-3790)	2950 (2653-3423)	0.08[#]
Apgar score at 10 min	5 (5-6)[$]	6 (4.5-7.5)	0.35[#]
Age at MRI scan (days)	7 (6-8)	7 (6-9)	0.79[#]
Male:female	11:4	4:2	1.00
Clinical seizures	4 (27%)	3 (50%)[†]	0.29
HIE stage 1	6 (40%)	0	0.12
HIE stage 2	8 (53%)	5 (83%)	0.34
HIE stage 3	1 (7%)	1 (17%)	0.50
Drugs administered:			
Phenobarbitone	8 (53%)	4 (67%)	0.66
Opioid	11 (73%)	2 (33%)	0.15
Midazolam	10 (67%)	4 (67%)	1.00
Inotrope	7 (47%)	3 (50%)	1.00

[#] Mann-Whitney U Test. [$] Apgar score at 5 min was used in two subjects because the 10 min Apgar score was not recorded. [†] Result unknown with one subject.

Table 9.1 Clinical characteristics of actively cooled and passively cooled neonates

Data for age, birthweight and Apgar score are median (inter quartile range). Remaining data are n(%).

Cerebral abnormalities seen on MRI for the 21 neonates who received either active or passive manual cooling methods are summarised in Table 9.2.

Compared to the actively cooled group, neonates in the passively cooled group were between 1.4 to 100 times more likely to have any HIE related brain injury on MRI, 2.3 to 100 times more likely to have an abnormality in the posterior limb of the internal capsule and 1.2 to 100 times more likely to have a brainstem abnormality.

MRI results:	Actively cooled (n=15)	Passively cooled (n=6)	Adjusted OR* (±95% CI)	p	Unadjusted OR (±95% CI)	p
Any brain abnormality	2 (13%)	4 (67%)	0.06 (0.01-0.75)	0.03	0.08 (0.01-0.74)	0.02
Basal ganglia and thalami	2 (13%)	3 (50%)	0.14 (0.01-1.41)	0.10	0.15 (0.02-1.37)	0.08
Brainstem	0 (0%)	3 (50%)	na		0.03# (0.01-0.82)	0.02#
Cortex	1 (7%)	2 (33%)	0.08 (0.01-2.02)	0.12	0.14 (0.01-2.01)	0.12
Posterior limb of internal capsule	0 (0%)	4 (67%)	na		0.02# (0.01-0.43)	0.00#
White matter	1 (7%)	2 (33%)	0.12 (0.01-2.30)	0.16	0.14 (0.01-2.01)	0.12
Subdural haematoma	7 (47%)	2 (33%)	1.68 (0.19-14.48)	0.66	1.75 (0.24-12.64)	0.58

* Adjusted for age and gender. # Manual calculation due to zero actively cooled subjects.

Table 9.2 Cerebral lesions seen on MRI in actively cooled and passively cooled
Data are n(%) and odds ratio (±95% CI) for presence or absence of MRI abnormalities in actively cooled and passively cooled neonates.

9.4 DISCUSSION

Neonates in the passively cooled group were more likely to have MRI abnormalities in the brainstem and posterior limb of the internal capsule. Abnormalities in other areas of the brain were similar between the two groups. Of the 2 neonates that were categorised as HIE stage 3, one received active cooling measures and had no reported brain abnormality, the other neonate was passively cooled and had reported widespread brain abnormalities except in white matter. The severe HIE neonate who received active cooling suffered shoulder dystocia, was reportedly asystolic for 12 minutes and subsequently developed seizures. Although the clinical grading ranked this neonate with severe encephalopathy, active cooling was required to keep T_{rec} below 34°C. Closer examination of steady state temperature recordings showed a brisk BAT response with this subject. As thermogenesis in BAT requires an intact central command centre and sympathetic output from the brain, the BAT response suggests normal brain functioning. This is consistent with the MRI report.

The higher incidence of anapyrexia in neonates with MRI abnormalities suggests that the core temperature response is related to injury in central control centres. The pre-optic nucleus of the anterior hypothalamus (POAH) is the central integrating centre containing populations of warm sensitive, cold sensitive and temperature insensitive neurons that generate a core temperature set-point.[204] Input to the POAH from peripheral and visceral thermo-receptors, and from the limbic system influences the autonomic output from the central nervous system which modulates BAT activity.[204]

The MRI results do not suggest that direct damage to the POAH is related to the thermoregulatory response. Rather, the input or effector systems especially those traversing the brainstem are more likely to be affected. The results of this study do not support the hypothesis that an active BAT response is detrimental to the brain during HIE. Data from previous Chapters have suggested that BAT activation may result in a warmer brain based on the finding of a warmer than expected T_{oes} when T_{rec} is targeted during therapeutic hypothermia. While a warmer brain may antagonise the neuro-protective

effects of hypothermia, the data presented in this Chapter however suggest that an active BAT response reflects intact central command centres and is a favourable prognostic sign.

Limitations

The study has significant limitations and any conclusions can only be made with caution. The main limitation is the small numbers of subjects studied and the low frequency of adverse MRI findings. This suggests that the cohort contained relatively low grade HIE neonates. Therefore larger numbers are required to confirm this study's preliminary findings. Furthermore, the radiologists were not asked to systematically grade the severity of abnormalities on MRI, and a structured review of the MRI scans is required. A normal MRI in the first 4 weeks after HIE is highly predictive of a normal outcome with a negative predictive value of approximately 90%.[63] However, long term developmental follow-up of the cohort is required.

9.5 CONCLUSION

The neonates who received passive cooling to maintain hypothermia therapy (anapyrexia) were more likely to have a brain abnormality, particularly in the posterior limb of the internal capsule or brainstem, compared to neonates who received active cooling. The neonates who received active cooling to maintain hypothermia therapy were able to mount a strong thermoregulatory defence to cooling and this appears to be a favourable prognostic sign.

CHAPTER TEN

10 THESIS CONCLUSION

Brain injury caused by the combination of inadequate blood flow and oxygen delivery to the brain before and during birth is called neonatal HIE. Whole body hypothermia has been shown by randomised controlled trials to reduce brain injury due to hypoxic-ischaemic insults and is an accepted treatment for HIE. However, HIE still results in considerable morbidity with 40% or more of cooled neonates dying or suffering moderate or severe long-term impairment.

The aim of whole body hypothermia is to lower brain temperature to 33-34°C within 6 hours of birth and to continue this for 72 hours before rewarming to normothermia. However, direct measurement of brain temperature is not generally possible in the human neonate during whole body hypothermia. The assumption with whole body hypothermia is that systemic core temperature is similar to brain temperature and T_{rec} has been the site widely used to direct the depth of whole body cooling. However, the use of the rectum as a monitoring site has not been adequately validated by comparison with temperature at other core sites such as the lower oesophagus during whole body hypothermia in human neonates.

The inability of hypothermia to prevent all brain injury following HIE is likely to relate to a complex interplay of many factors including the timing of injury, the application of cooling and inadequate knowledge of the ideal depth and duration of cooling. Furthermore, hypothermia is energetically costly when thermoregulation is activated to defend a core temperature set-point, and induces a hormonal stress response which may be harmful.[80, 145]

The literature suggests that stress has an adverse effect on neuronal recovery from HIE. Restraint in rats induces a catecholamine response increasing cerebral oxygen consumption.[146] Glucocorticoids released during stress reduce neuronal glucose uptake and increase brain glutamate levels.[147] Thoresen et al performed 24 hours of therapeutic hypothermia of HIE piglets without sedation which failed to reduce neuropathologic damage or seizure activity, in contrast to the finding of neuro-protection with hypothermia and

anaesthesia.[148] The neo.nEURO.network RCT used systemic therapeutic hypothermia combined with regular sedation. The authors speculated that hypothermia with morphine as a co-treatment contributed to the apparently greater benefit of cooling in their RCT compared to other cooling RCTs.[10]

The human neonate defends core temperature when exposed to hypothermia via the activation of BAT. This is different to the predominant thermoregulatory mechanisms in adult humans, and in some of the animal models of hypothermia which underpin the theoretical basis of therapeutic hypothermia in neonatal HIE.[3, 13, 17, 19, 22, 101, 104] BAT has been shown in animal models to have a primary function of warming the brain.[54, 144] The anatomical distribution of BAT in the inter-scapular area and around the great veins in the neck is ideal for heating central venous blood that then perfuses the brain and minimises convective heat loss from the brain.[39]

The activity of BAT during hypothermia treatment for HIE has not been examined in the human neonate. Nonetheless this aspect of neonatal physiology might be important because BAT thermogenesis might counteract neuro-protection afforded by hypothermia. Potential mechanisms of such an effect are via a metabolic stress response and/or warming of the brain.

An important concept emphasised in this thesis is that induced hypothermia is quite different to anapyrexia. Anapyrexia is the adaptive response to hypoxia and highly conserved in evolution where the core set point is lowered which reduces oxygen consumption and stress responses.[205] Survival is increased if animals are allowed to reduce core body temperature during exposure to hypoxia.[206] In contrast, hypothermia implies that the core set point is normal and defended by thermoregulatory mechanisms.

This thesis focused on the basic physiology of temperature control during whole body hypothermia in human neonates with an emphasis on BAT and temperature gradients between core body sites. The following hypotheses were tested: (i) thermogenesis in BAT is active during therapeutic hypothermia for HIE; (ii) BAT activity influences T_{oes} more than T_{rec}; (iii) T_{rec} does not accurately reflect T_{oes}; (iv) BAT activity is associated with severity of brain injury.

Manually controlled hypothermia using T_{rec} as the target temperature site was studied because this is the standard practice in South Australian tertiary NICUs. Furthermore, servo-controlled cooling blankets (by cooling the skin of the back) preclude the assessment of changes in inter-scapular skin temperature that may be associated with BAT thermogenesis.

Experiments and observational studies to test the four hypotheses

A series of experiments and observational studies measured two core temperature monitoring sites (T_{rec} and T_{oes}) and surface inter-scapular skin temperature (T_{scap}) using standard temperature probes. Additionally, exposed surface temperatures were measured using IR imaging in healthy neonates and in critically ill normothermic and hypothermic neonates nursed supine with the back in contact with an insulating mattress.

First, the validation of T_{scap} as a reflection of BAT activity was considered in normothermic neonates. T_{scap} has been measured previously in term neonates with the back exposed,[133, 134] but not with the back in contact with the mattress in a supine posture. Experiments with healthy term neonates in the postnatal ward measured T_{rec}, T_{oes} and T_{scap} over a 30 minute period of warming, followed by 30 minutes of mild cooling and then 30 minutes of rewarming along with concurrent measurement of oxygen consumption. Here, T_{scap} transiently increased at the commencement of cooling in contrast to other sites and this finding was not explained by mattress temperature in contact with the back. The increase in T_{scap} was associated with an increase in oxygen consumption. This result suggested that T_{scap} may reflect BAT activity. The magnitude of change in T_{scap} was noted to be small and the effect transient. This was interpreted to indicate a confounding effect of heat transfer to the core, indicating a limitation of the use of T_{scap} measurements after step changes in environmental temperature.

Secondly, T_{oes}, T_{rec} and T_{scap} were observed in ventilated, supine normothermic term neonates in intensive care during steady state thermal conditions. This showed that in steady thermal state the difference between T_{oes} and T_{scap} changed, depending on whether exposed skin was warmed or cooled. The T_{oes}-T_{scap} difference reduced with cooling and increased with

warming. These steady state observations were also supportive of the T_{oes}-T_{scap} difference as indicative of BAT thermogenesis. T_{oes} was also noted to be consistently warmer than T_{rec}, and T_{rec} also varied with skin warming or cooling. The T_{oes}-T_{rec} difference increased with cooling and reduced with warming, indicating that T_{rec} was disproportionately influenced by temperature of the lower body and legs when compared to T_{oes}. IR imaging confirmed that the legs cooled to a greater extent than the arms when radiant heating was turned off.

Thirdly, neonates were studied during therapeutic hypothermia. The relationship between T_{rec} and T_{oes} during therapeutic cooling showed that T_{oes} was higher than T_{rec} and fluctuated considerably when compared to T_{rec} due to the relatively slow response of T_{rec} to changes in environmental temperature. Neonates receiving ventilator support during hypothermia appeared to increase T_{oes} when compared to spontaneous breathing. These observations indicate the potential for the brain to be warmer than anticipated when T_{rec} is manually controlled at 33-34°C. This monitoring issue is poorly appreciated in clinical practice. Additionally, two groups of neonates were identified: hypothermic neonates that required active cooling (cool packs, wet cloths and fans), and neonates who allowed a passive fall in core temperature and required intermittent radiant heating to prevent a fall in core temperature below 33°C. The latter group were interpreted as having an anapyrexic response to asphyxia.

During thermal steady state with hypothermia, there were large changes in the T_{oes}-T_{scap} difference with radiant heating and cooling of the exposed skin in the same direction as noted during normothermia. During skin cooling, T_{oes}-T_{scap} difference was reduced and T_{scap} was warmer. During skin warming, T_{oes}-T_{scap} difference was greater and T_{scap} was cooler. Furthermore, the reactivity of T_{scap} in relation to T_{oes} was greater in neonates who demonstrated a vigorous defence of core temperature (hypothermic neonates) when compared to neonates who readily allowed core temperature to fall (anapyrexic neonates). After 72 hours of hypothermia when the neonates were re-warmed and in normothermic steady state, T_{oes}-T_{scap} differences were also markedly more reactive to skin warming and cooling when compared to ventilated

normothermic neonates of a similar age.

These findings suggest that BAT is active in many neonates with HIE during hypothermia. The effect of skin temperature on reducing putative BAT activity in the HIE neonate is consistent with adult human studies which show that warming the skin reduces the shivering threshold.[199] This means that shivering thermogenesis occurs at a lower core temperature when the skin is maintained at a warm temperature. The greater reactivity of T_{scap} post re-warming is also suggestive of adaptation to cooling by recruitment of BAT, a phenomenon that is well documented in rat pups.[201] Neonates that mount a vigorous thermoregulatory defence to cold appear to have a greater capacity to activate BAT when compared to anapyrexic neonates who appear not to defend core temperature at least at a T_{rec} of 33-34°C.

These three findings support the first three hypotheses: (1) that BAT thermogenesis is active during therapeutic hypothermia for HIE, (2) that BAT activity influences T_{oes} more than T_{rec}, and (3) that T_{rec} does not accurately reflect T_{oes}.

Fourthly, MRI findings in hypothermic neonates with high BAT reactivity who received active cooling measures were compared to neonates who had low BAT reactivity and allowed core temperature to fall spontaneously. MRI abnormalities were more frequent in neonates without BAT reactivity suggesting that brain injury interferes with normal thermoregulatory responses. While these findings support the fourth hypothesis that BAT activity is associated with severity of brain injury, the findings do not support the assertion that BAT activation accentuates brain injury by counteracting neuro-protection from hypothermia.

Limitations

The main limitation of the thesis is that BAT itself has not been measured by an invasive temperature probe, but rather BAT thermogenesis is inferred from T_{scap}. However the fluctuation in T_{oes}-T_{scap} differences with skin warming and cooling, the greater T_{scap} reactivity in neonates who are difficult to cool and the apparent post-cooling augmentation of T_{scap} reactivity are all consistent with

known physiological principles, and support the assertion that T_{scap} is reflecting BAT thermogenesis.

Measurement of oxygen consumption during steady state hypothermia was not possible in this thesis but would be desirable to confirm the observations. Low subject numbers and a lack of long term neurodevelopmental follow-up are limitations to determining the significance of BAT thermogenesis to the brain.

Future research

Future research directions could include consideration of a randomised controlled trial to determine if T_{oes} monitoring compared to T_{rec} monitoring improves clinical outcome with whole body hypothermia. However, such a study is unlikely to occur with manual cooling as servo-controlled blankets are increasingly common. More data are therefore required between T_{oes} and T_{rec} differences using servo-controlled cooling blankets.

Neonates that showed a brisk/poor BAT response to cooling were more/less likely to have a normal brain. Therefore the influence of BAT thermogenesis on the recovery of the brain during hypothermia requires further study with a larger study cohort and long term follow-up.

Concluding remarks

There are two main conclusions to the thesis. First, rectum is an inappropriate site to monitor as a surrogate of brain temperature and that the lower oesophagus is preferred over rectum to monitor and regulate core temperature during hypothermia. There are a number of factors supporting this statement: (i) there is a longer lag time to change after a change in environmental temperature that promotes temperature fluctuations in more rapidly responding sites such as T_{oes}; (ii) T_{oes} is increased by intubation and the use of warmed humidified gases is an effect that is not reflected by T_{rec}; (iii) T_{rec} is lowered by leg temperature; (iv) BAT is active in many neonates during whole body hypothermia and thermogenesis is more closely aligned to T_{oes} than T_{rec}. Each of these points results in an underestimate of T_{oes} if T_{rec} is targeted. T_{oes} is therefore more likely to provide a more stable and relevant measure of brain

temperature and should be used in clinical practice.

Secondly, the finding that BAT appears to be active during therapeutic hypothermia raises important questions about cooling strategies with HIE neonates. Active BAT thermogenesis appears to indicate intact central thermoregulation based on the MRI findings in this thesis. This may indicate less damage in such babies, but a beneficial effect of BAT activity on brain recovery can't be excluded. However, the adverse effects of metabolic stress on neurological recovery in animal models, and the possible contribution of BAT activity to warming the brain during hypothermia suggest that further studies on the effects of induction of anapyrexia following asphyxial injury may be clinically important.

APPENDICES

APPENDIX A. SENSOR CONNECTOR BOX

The sensor connector box was used to minimise interference to nursing staff and is pictured with all patient leads attached in Figure A1. The sensor box was wiped over with an alcohol based cleansing wipe between studies.

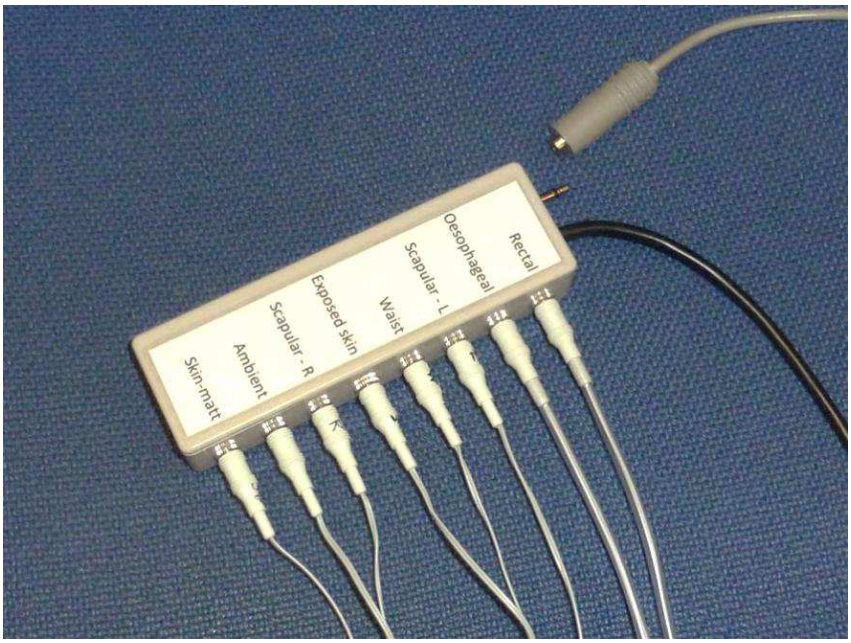


Figure A1. Sensor connector box used for experiments and studies

(A) Temperature sensors plugged into a sensor connector box that used a single cable to connect an eight input datalogger. The monitor output from the sensor connector box connected to the bedside patient monitor to display rectal temperature for nursing staff.

(B) Inside a sensor connector box showing internal circuitry incorporated gain, offset and impedance buffering that allowed the datalogger to record a voltage in relation to temperature without interfering with the bedside patient monitor display of rectal temperature.

(C) The sensor connector box showing the location of the on-off switch and gain and zero adjustments that allowed use with several brands of patient monitor.

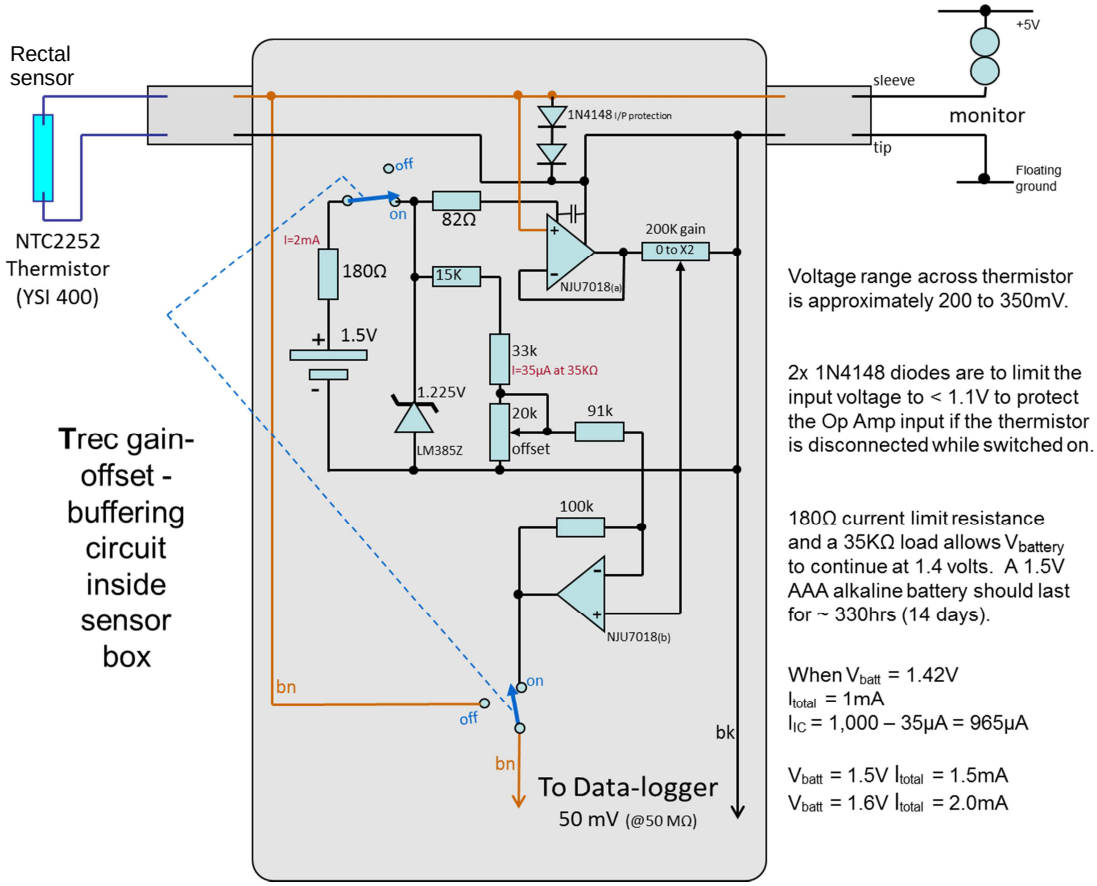
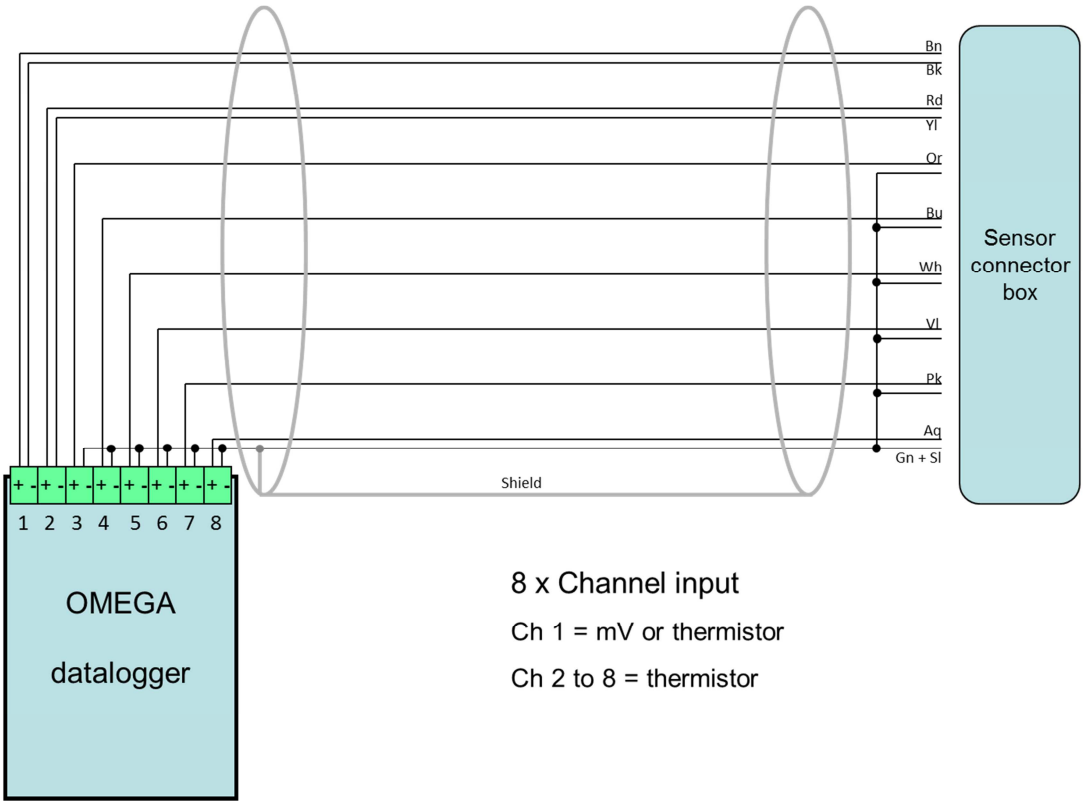


Figure A2. Rectal sensor interface circuit inside the sensor connector box

(A) While the patient monitor measured T_{rec}, the electronic interface circuit allowed the datalogger to simultaneously record small voltages that varied in relation to the T_{rec}. The interface circuit incorporated an impedance buffering amplifier to avoid interfering with the patient monitor as well as an operational amplifier to provide gain and offset to optimise use with the datalogger.



(B) System interconnection wiring using a 12 core shielded cable from sensor connector box to the eight datalogger inputs.

APPENDIX B. EXTENSION BATTERY PACK

To avoid a connection to mains power, two rechargeable external extension battery packs were constructed to keep the Omega Datalogger fully charged for 96 hours (48 hours each) during long running studies.

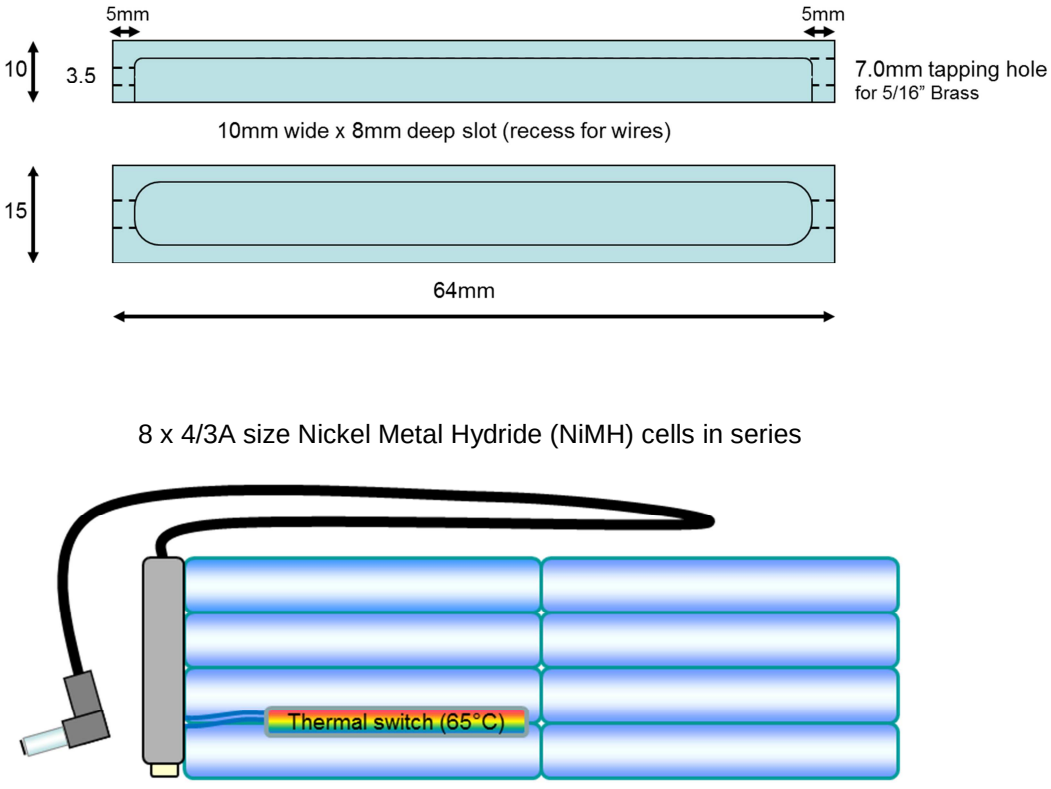


Figure B1. Extension battery pack for datalogger

A PVC end block was machined to contain electrical connections and was secured on the end of a pack of eight 4/3A size rechargeable Nickel Metal Hydride cells.

The battery supplied 9.6 Volts (V) with 3.5 amp-hours (Ahr) capacity. A right-angled 2.1 mm DC connecting plug on a short flexible lead attached at one end of the PVC block and a 2.1 mm DC power socket was fitted to the other end. The DC socket was used to charge the battery while the DC plug connected to the datalogger. To avoid thermal overload, a 65°C thermal cut-out switch was attached in series with to the battery. The physical layout of the battery is shown.

The extension battery ensured that the datalogger was fully charged for at least 48 hours. A second extension battery was used for longer studies. A charging connector on the extension battery allowed the battery and datalogger (if connected) to be recharged before a study.

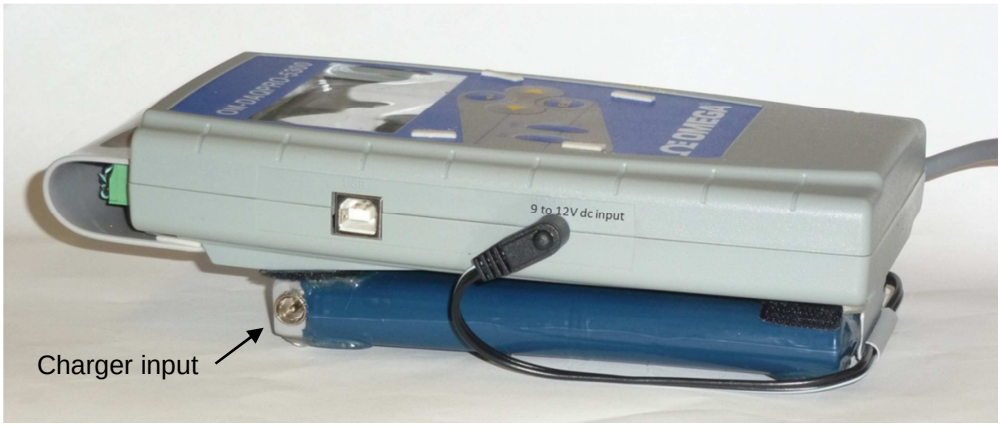


Figure B2. An extension battery in situ beneath the datalogger

An Omega datalogger is pictured above an external extension battery.

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