Selective Cerebral Hypothermia For Term Infants Following Hypoxic Ischaemic Injury

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Abstract

Perinatal hypoxic ischaemic injury is an important cause of both neonatal death and long-term disability. The sequence of resuscitation followed by a latent phase then a secondary cascade of injury is well documented. This thesis covers key steps toward the utilization of selective hypothermia as an intervention during the latent phase to ameliorate the secondary injury and improve subsequent outcome.

The technique was shown to be both feasible and well tolerated. Specifically, a rectal temperature of 35 °C and 34.5 °C, in term infants with neonatal encephalopathy, was not associated with an excessive requirement for cardio-respiratory support. Although a decrease in heart rate occurred during cooling, this was expected and there was no significant change in blood pressure during either the cooling or rewarming phase. Additional reassuring findings were that neither major electrolyte disturbance; hypoglycaemia or haematological changes, including excessive haemorrhage, were observed during hypothermia.

The study of neurodevelopmental outcome established that selective cerebral hypothermia was not associated with late adverse effects and, in infants with moderate to severe encephalopathy, the combined cooled groups demonstrated a trend towards better outcome. These data confirmed the potential for selective cerebral hypothermia to provide neuroprotection following perinatal asphyxia. In further chapters cerebral CT scan was confirmed as a helpful adjunct to clinical staging in predicting neurodevelopmental outcome and important clinical experience was reported including rebound seizures following rewarming; sclerema neonatorum associated with hypothermia; and abnormal flow in the superior sagittal sinus, associated with perinatal asphyxia.

Lastly a review of infants assessed but not recruited to the CoolCap trial based on aEEG criteria was performed. As these aEEG criteria could be applied to future clinical use it was considered important to ensure large numbers of infants

with potential to benefit were not excluded from intervention. Neurodevelopmental status for those infants excluded by the aEEG criteria was largely favourable but a small number had adverse outcome and the majority manifested short term morbidity.

In conclusion, the work presented in this thesis suggests that intervention with selective hypothermia offers the potential to change disease progression and improve subsequent outcome following perinatal asphyxia at term.

Preface

There can be no greater motivating force than the sad experience of witnessing the evolving consequences of severe perinatal neurological injury; the newborn infant starts perfectly formed but by some tragic event, or events, is left devastated with cerebral palsy, seizures and microcephaly. Such misfortune is indeed a heavy loss for them, their families and society.

I came to New Zealand in 1997 to work as a neonatologist at National Women's Hospital and very quickly became involved in the work on selective hypothermia as an intervention following perinatal asphyxia. The chance to offer some hope to families of affected babies was compelling in itself but the added opportunity to work with local and international collaborators and to be the site investigator for a large multicentre randomized controlled trial of selective cerebral hypothermia was truly an enlightening and rewarding experience. However, the true measure of the work will be in the transfer of the technique to routine clinical practice and hopefully the subsequent improvement in outcome for future infants.

Dedication

To my family: Paula, Georgia, Lydia and Toby who have missed me while I have been writing.

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List of Abbreviated Terms

Alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxazole Propionate (AMPA)

Amplitude Integrated EEG Recording (aEEG)

Anti Diuretic Hormone (ADH)

Beats per minute (BPM)

Brain Specific Proteins (BSPS)

Brainstem Auditory Evoked Responses (BAER)

Cerebral Blood Flow (CBF)

Cerebral Blood Flow Velocity (CBFV)

Cerebral Function Monitoring (CFM)

Cerebrospinal Fluid (CSF)

Computer Tomography (CT)

Concentration Ratio Of Phosphocreatine To Inorganic Phosphate ([Pcr]/[Pi])

Corrected QT (QTc)

Disseminated Intravascular Coagulation (DIC)

Electroencephalography (EEG)

Gamma Amino Butyric Acid (GABA)

Glial Fibrillary Acidic Protein (GFAP)

Glycine-Prolint-Glutamate (GPE)

Grams (g)

Hypoxic Ischaemic Encephalopathy (HIE)

Hypoxic-Ischaemic (HI)

Insulin-Like Growth Factor-L (IGF-I)

International Normalised Ration (INR)

Intracranial Pressure (ICP)

Lower Segment Caesarean Section (LSCS)

Magnetic Resonance Imaging (MRI)

Magnetic Resonance Spectroscopy (MRS)

Mean Arterial Blood Pressure (MAP)

Negative Predictive Value (NPV)

Neonatal Encephalopathy (NE)

Neuronal Nitric Oxide Synthetase (Nnos)

Neuron Specific Enolase (NSE)

Partial Pressure of Oxygen (Pao2)

Persistent Pulmonary Hypertension (PPHN)

Poly(ADP-Ribose) Polymerase (PARP)

Positron Emission Tomography (PET)

Posterior Limb of the Internal Capsule (PLIC)

Proton (1H)

Resistive Index (RI)

Sclerema neonatorum (SN)

Somatosensory Evoked Potentials (SSEPS)

Standard Deviation (SD)

Subcutaneous fat necrosis of the newborn (SCFN)

Superior Sagittal Sinus (SSS)

Troponin T (TnT),

Visual Evoked Potentials (VEPS)

Weeks (Wks)

Years (Yrs)

1 Introduction

1.1 Selective cerebral hypothermia for term infants following hypoxic ischaemic injury

Hypoxic ischaemic (HI) insult is a major cause of acute perinatal neurological injury in the full term infant (Hill 1999; Legido A 2000; Levene 2001). Affected infants suffer a range of adverse clinical outcomes including mortality and long-term neurodevelopmental morbidity. Regrettably, this fact continues to be true, with no substantial improvement in the rates of impairment in affected infants, despite the many advances in obstetric and neonatal care that have occurred in the last 25 years (Jarvis et al. 1985; Miller et al. 2004; Pharoah et al. 1998; Pierrat V et al. 2005; Stanley and Blair 1991). This thesis will explore the use of a novel technique, selective cerebral hypothermia, to improve the clinical outcome in term infants following HI insult.

This literature review will initially outline the normal process of transition at birth and fetal responses to normal labour. Then define the relevant important terms and discuss important clinical concepts such as: identification of at risk infants; antecedents and associations of neonatal encephalopathy; temporal changes in the incidence of perinatal asphyxia; clinical manifestations neurological injury; and outcome following perinatal asphyxia, including problems with prediction. The next section will describe the pathways of

neurological injury with specific mention of endogenous protective mechanisms, neonatal management including resuscitation and potential interventions for neonatal encephalopathy. Included in this will be an introduction to hypothermia that discusses the side effects, mechanism of neuroprotection and potential for clinical use.

1.1.1 Transition at birth

The fact is, that there was considerable difficulty in inducing Oliver to take upon himself the office of respiration - a troublesome practice, but one which custom has rendered necessary to our existence; and for some time he lay gasping.....rather unequally balanced between this world and the next: the balance being decidedly in the favour of the latter.

Oliver Twist by Charles Dickens, 1837

For some infants, such as Oliver in the above quotation, the process of labour and delivery is not well tolerated and birth results in an infant who has difficulty making the transition to an ex-utero existence. It is vital that within minutes of delivery the newborn infant makes the transition from a dependence on the placenta for delivery of oxygen and elimination of carbon dioxide to an extra-uterine existence with independent pulmonary gas exchange. For transition to happen smoothly several interdependent physiological changes should occur including major alterations in circulation, pulmonary mechanics, gas exchange and respiratory control. In addition there are a number of non-cardiorespiratory adaptations that occur (Table 1).

Table 1. Non-cardiorespiratory changes occurring as part of birth transition.

System	Fetal	Neonatal
Renal	No excretory function and large volumes of dilute urine	Fully functional for excretion and fluid conservation
Gastro-intestinal	Minimal digestion	Fully functional and able to cope with enteral nutrition
Endocrine	Relatively inactive system	Fully functional system capable of homeostasis

Although breathing movements may be observed in pregnancy (Boddy and Mantell 1973; Boddy and Mantell 1972; Dawes et al. 1970; Dawes et al. 1972) the fetal lung does not perform gas exchange. The fetus thus depends on maternal oxygenation, placental perfusion and the gradient in oxygen binding between adult and fetal haemoglobin to supply the needs of aerobic metabolism. The fetal partial pressure of oxygen (PaO2) is lower than the adult and varies from approximately 30 mmHg in the umbilical vein to 25 mmHg in the ascending aorta and 18-20 mmHg in the umbilical arteries (Bhutani 1997). However, the fetus normally has a surplus of oxygen for its requirements. Furthermore, the fetal circulation is adapted to supply maximal oxygenated blood to the cerebral circulation. The pulmonary circulation is in a low flow, high resistance state receiving only 10 % of the combined cardiac output (Fineman et al. 1995). There are also fetal shunts that ensure the comparatively oxygen rich blood from the umbilical vein is preferentially supplied to the cephalic circulation. Specifically, the portal circulation is bypassed by the ductus venousus and the pulmonary circulation is bypassed via the foramen ovale and the ductus arteriosus.

1.1.2 Fetal responses and normal labour

In normal labour the blood supply to the placenta is interrupted during uterine contractions (Janbu and Nesheim 1987), which causes a temporary fall in fetal blood oxygen levels that recovers once flow resumes. The fetus has a number of adaptive mechanisms that normally allow it to cope with this relative decrease in oxygen supply. Fetal blood has a relatively increased oxygen carrying capacity due to a combination of higher haemoglobin concentration and the greater oxygen affinity of fetal haemoglobin. For a PO2 of 30 mm HG maternal blood would be about 50% saturated in contrast to about 80% for fetal blood (Carter AM 1999). Furthermore, the cardiac output in proportion to body weight is much higher in the fetus than in the adult (Cohn et al. 1974; Peeters et al. 1979). Also the placental supply includes some reserve in terms of meeting the normal oxygen requirement; in the fetal lamb the supply is about a double the basal requirement (Carter AM 1999). Finally, fetal tissues can adapt by increasing oxygen extraction from the blood. Experimentally, the process of increased oxygen extraction will maintain the fetal requirements until it drops by more than 50 % to approximately 0.5mmol/min/Kg (Carter AM 1999; Richardson 1989).

Fetal hypoxia causes an increase in both autonomic activity (Parer 1983) and catecholamine levels (Jones and Robinson 1975; Lagercrantz and Slotkin 1986; Phillippe 1983). The catecholamines have both cardiovascular and metabolic effects and if blocked there is an impaired fetal ability to survive hypoxia (Dagbjartsson et al. 1985).

1.1.2.1 Fetal Cardiovascular Responses

There are two main changes that occur in response to hypoxia. Firstly, the tissues may extract an increased fraction of the oxygen available (Richardson 1989). Secondly, blood flow may be redirected to the brain, heart and adrenal glands and away from the skin, gut and kidneys (Cohn et al. 1974). The release of catecholamines paradoxically causes the heart to work harder hence it is unable to reduce its oxygen requirement and is vulnerable to HI insult.

1.1.2.2 Fetal Neurological and Behavioural Responses

The fetus decreases activity levels so decreasing metabolic requirements in response to hypoxia; however this is a relatively late response to hypoxia (Richardson et al. 1992). In addition to the behavioural responses there is also an ability to respond to severe hypoxia with cerebral vasodilatation and following this redistribution of the blood flow to the brain stem (Ashwal et al. 1981) (Lou et al. 1985).

Although a healthy fetus can compensate for a decrease in oxygen supply during labour ultimately the supply needs to match requirement if fetal compromise is to be avoided. In situations of chronic placental deficiency when reserve is already used up the fetus may poorly tolerate the extra stress of labour. Similarly if the decrease in supply during labour is extreme or prolonged then even a previously healthy fetus may be compromised.

1.2 Definition of the terms

Hypoxia may be defined in physiological terms as a partial or complete deficiency of oxygen in one or more tissues of the body including the blood stream, when the term hypoxaemia may be used. *Asphyxia* is a state of disrupted gas exchange. In the perinatal period this may be due to interruption of either pulmonary or placental function. The clinical picture is characterised by hypoxaemia accompanied by a mixed respiratory and metabolic acidosis. Respiratory acidosis occurs as a direct consequence of sub-optimal pulmonary or placental gas exchange, while metabolic acidosis is caused by accumulation of lactic acid in the tissues from anaerobic glycolysis (Towell 1966). Finally *ischaemia* is a reduction in or cessation of blood flow. It can derive from a variety of causes that include decreased cardiac output, systemic hypotension or occlusive vascular disease.

The term birth asphyxia has been used in the past to describe both the exposure and the resulting neurological abnormality. For this reason it is no longer considered to be appropriate (Blair 1993). Encephalopathy is a general term used to describe a composite of clinical neurologic signs. It does not imply a particular cause or specific time course. However, the term hypoxic ischaemic encephalopathy (HIE) refers to the clinical picture of disturbed brain function following hypoxia ischaemia (Sarnat and Sarnat 1976) and may be staged one, two and three or graded as mild, moderate and severe. The clinical features of this grading are described in section 1.4.2 (Clinical neurological manifestations of HI injury). One problem with the term hypoxic ischaemic encephalopathy is that it implies the aetiology is verified but there

may not always be evidence to support a clear aetiology (Edwards and Nelson 1998). Use of the term Neonatal Encephalopathy (NE) "a clinically defined syndrome of disturbed neurological function in the earliest days of life in the term infant, manifested by difficulty with initiating and maintaining respiration, depression of tone and reflexes, sub normal level of consciousness and often seizures" has therefore been promulgated (Nelson and Leviton 1991). This thesis will use the preferred nomenclature of NE although at times this may be at variance with the term used in the original literature.

1.3 Infants at risk of adverse sequelae following perinatal asphyxia

Although the lay perception is of an all-or-nothing phenomenon, in fact, asphyxia is a continuous process that may occur to a variable degree. Moreover there is no discrete threshold or defined degree of insult required before adverse long-term outcome ensues. The lack of a primary measure of insult that would identify infants at risk of adverse outcome has led to researchers using a variety of secondary markers in this role.

Early studies used single clinical markers including time to first breath (James LS 1958; MacDonald et al. 1980; Mir et al. 1989). Subsequently Apgar scores (Apgar V 1953; Casey et al. 2001; Ergander et al. 1983; Palme-Kilander 1992), encephalopathy (Finer et al. 1981; Levene et al. 1985) or neonatal seizures (Gunn and Gunn 1997) have been utilised. More recently there has been an appreciation that combinations of markers may be more useful (Ekert et al. 1997; Perlman and Risser 1996). Thus some caution is needed in the

interpretation of the perinatal asphyxia literature as both the incidence and the strength of association with adverse neurological outcome may vary depending on the criteria used to select the study group.

1.3.1 Studies using ventilation requirement as inclusion criteria

MacDonald et al. (MacDonald et al. 1980) prospectively studied 38,405 consecutive deliveries and used the requirement of greater than one minute of positive pressure ventilation to identify infants suffering from asphyxia at birth. The study population included a mixture of term and preterm infants. Of note, the latter group were more common with 62.3% of infants less than 27 weeks' gestation affected compared to only 0.4% in infants greater than 38 weeks' gestation. Similar findings were reported by Mir (Mir et al. 1989) who reported that 2.8% of newborns required more than one minute of positive pressure ventilation at birth but the data was obtained across a mixture of gestational ages and birth weights, so it over-estimates the rate for term infants. Specifically, 68% of infants of less than 1,000 g compared to only 1.2% of infants between 3 and 4 Kg birth weight received ventilation. Thus gestational age and birth weight are major confounders that make initial ventilation requirements, used as a single item, a poor criteria for identification of infants at risk of adverse sequelae from perinatal asphyxia.

1.3.2 Studies using Apgar scores as inclusion criteria

Apgar score(Apgar V 1953; Casey et al. 2001) is an alternative approach that has been used to identify infants at risk of adverse outcome following perinatal asphyxia. Ergander et al defined asphyxia as an Apgar score equal to or below three at five minutes (Ergander et al. 1983) and calculated a rate of

2.9/1000 live births in a single hospital centre over a six-year period. Again the study population was mixed for gestation with 40 preterm and 76 full-term infants affected. Palme-Kilander calculated a lower rate of 1.7% based on 1633 cases of low Apgar score from all 97,648 live births in Sweden during a 12 month period. However, a different definition of an Apgar score of three or less at one minute or six or less at five minutes was used (Palme-Kilander 1992).

There are several problems with the use of simple Apgar scores, in this way, to identify infants at risk of neurodevelopmental impairment. The first problem, as highlighted above, is that even in two studies from the same country using Apgar scores there is a lack of consistency in the exact criteria used. The second problem is the limited availability, in New Zealand, of accurate and reliable centrally collated Apgar score data (Pattison and Teele 2001). In the Report on Maternity 2000 & 2001 (Ministry of Health 2003) data on five minute Apgar score is not available for 3540 babies (8.9 %) in 2000 and 1797 (4.8 %) in 2001. Also the number of babies with an Apgar score of zero greatly exceeds the death rate and thus is likely to be erroneous. The third problem is that studies using Apgar scores alone, as with those using ventilation criteria, frequently include a mixture of term and preterm infants. Although preterm infants have a high incidence of adverse neurological outcome, the mechanism of neurological insult is different from that in the term infant (Inder and Volpe 2000) and the management of neurologic injury in the term newborn is the focus of this thesis. The fourth problem is that Apgar scores may be influenced by other factors such as drugs or anomalies. Although

useful as a screening tool, they do not establish the presence of perinatal asphyxia and are insufficient to predict either encephalopathy or subsequent adverse outcome (Groenendaal and de Vries 2000). Thus even if the other problems were solved the method would remain sub-optimal and alternative approaches should be explored.

1.3.3 Studies using Neonatal Encephalopathy as inclusion criteria

An alternative approach is to define the study population by the presence of encephalopathy early in neonatal period, usually within the first 7-days. Finer et al. (Finer et al. 1981) prospectively reviewed 20,155 term infants and identified 95 with evidence of neonatal encephalopathy following perinatal asphyxia. A rate of 4.7 cases per 1000 live births was calculated. Of the affected infants 39 were moderate and 12 were judged as severe, giving a combined rate for moderate or severe encephalopathy of 2.5 per 1000 live births.

Levene et al (Levene et al. 1985) assessed the incidence of clinically significant perinatal asphyxia in 20,975 live births over a four year period in a single teaching hospital. One hundred and twenty six infants developed NE giving an overall incidence of 6.0 per 1000 live-born deliveries of whom 2.1 per 1000 showed severely abnormal features including seizures or coma. A subsequent study (Levene et al. 1986) examined the sensitivity and specificity of six different grades of Apgar score depression and the presence or absence of encephalopathy to predict outcome. Although a 10 minute Apgar score less than or equal to five was the most sensitive method of predicting

adverse outcome (sensitivity 43%, specificity 95%) this was much less sensitive than the presence of moderate or severe encephalopathy in predicting death or severe handicap (sensitivity 96%, specificity 77%). The presence of NE is thus potentially a very useful method to identify those infants at highest risk of a subsequent adverse outcome.

1.3.4 Studies using seizures as inclusion criteria

An alternative approach that has been validated is to identify intrapartum asphyxia at or after term by using early onset seizure data (Grant 1993). Gunn prospectively identified term infants with seizures and evidence of perinatal asphyxia in Auckland over two time periods: 1978-1981 and 1991 (Gunn and Gunn 1997). Infants with multiple congenital abnormalities, hypocalcaemia or infection were excluded. The incidence for the two cohorts was 1.90 and 1.78 per 1,000 live births respectively.

1.3.5 Population versus single centre studies

Studies of a defined geographical region or population are superior to single centre studies for the purposes of calculating incidence and prevalence data. Specifically, better definition of both the numerator and the denominator is possible due to more complete case identification and limited movement in or out of the study region for treatment purposes.

A combination of Apgar scores and other neonatal morbidity was used in a population study performed in Sweden; birth data from all births in Goteborg 1985-91 were reviewed (Thornberg et al. 1995b). From the 42,203 live born

infants it was calculated that 6.9/1000 had an Apgar score below seven at five minutes, 5.4/1000 had birth asphyxia defined as neonatal unit admission with that diagnosis and 1.8 /1000 developed encephalopathy subsequent to that diagnosis. This data aptly illustrates that the number of babies with low Apgar scores is vastly greater than the number of infants developing neonatal encephalopathy, requiring intensive care or indeed subsequently manifesting adverse neurological outcome.

Another important population based report is the case control study performed for term infants with neonatal encephalopathy in Western Australia (Badawi et al. 1998a; Badawi et al. 1998b). Inclusion criteria for cases with moderate or severe newborn encephalopathy were either seizures alone or two of the following for greater than 24 hours: Abnormal consciousness, difficulty maintaining respiration presumed central, difficulty feeding presumed central in origin, abnormal tone and reflexes. Severe encephalopathy was defined as: ventilation for more than 24 hours, two or more anticonvulsants, comatose or stuporous, death in the neonatal period. In this unmatched case control study of 164 infants with encephalopathy and 400 controls the birth prevalence of moderate to severe encephalopathy was calculated as 3.8 / 1000 live births. Fifteen cases and no controls died giving a case fatality of 9.1%.

Although national data is not available for New Zealand, data has been collated on moderate and severe NE cases from National Women's Hospital Auckland, which has a catchment of approximately 12,000 deliveries per year. For the period January 1997 to December 2000 there were 64 term infants,

including three from outside the region, identified with moderate (50) or severe (14) NE giving an approximate rate for moderate to severe NE of 1.7/1000 live births (West C 2005a; West C 2005b). Considering the aforementioned difficulties with both definition and different time periods sampled this data is comparable to that published elsewhere. It also provides one estimate of the number of local infants that could in the future utilise neuroprotection, if intervention was proven to be safe and effective.

1.3.6 Antecedents and associations of Neonatal Encephalopathy at term

The incidence of antenatal and intrapartum asphyxial insult is higher in complicated pregnancies (Table 2), particularly those with diminished placental reserve including hypertensive disease of pregnancy or pre-eclampsia, intrauterine growth restriction, placental abruption, fetal anaemia and postmaturity.

Table 2. Conditions that predispose to fetal and newborn asphyxia adapted from Vannucci (Vannucci and Vannucci 1997).

Maternal	Obstetric	Labour & Delivery	Neonatal
Pre-eclampsia	Placental abruption	Abnormal presentation	Prematurity
Diabetes mellitus	Cord prolapse	Precipitate delivery	Respiratory distress
Vasoactive drugs e.g. Cocaine	Placenta previa	Prolonged labour	Septic shock
Isoimmunization		IUGR	Haemolytic disease
Collagen vascular disease		Difficult instrumental delivery	Cardiopulmonary anomalies
		Uterine rupture	
		Post term	

IUGR = intrauterine growth restriction

1.3.6.1 Prediction of risk prior to birth

The prediction and diagnosis of intrapartum fetal asphyxia is based on three main strategies: clinical risk scoring, electronic fetal monitoring and assessment of fetal blood gas for acid-base status. The ideal is that risk scoring identifies those pregnancies at risk then monitoring detects the fetus exposed to asphyxia, which in turn is confirmed to be causing significant biochemical perturbation by acid-base measurement.

Antenatal markers such as reduced liquor volume (Chauhan et al. 1999), intrauterine growth restriction (Heinonen and Saarikoski 2001; Leijon 1992) and meconium staining of the liquor (Milsom et al. 2002) are recognised to be associated with a low Apgar score and neonatal compromise. In addition, conditions such as maternal fever in labour (Impey et al. 2001a) and pre-eclampsia (Impey et al. 2001b) are also associated with an increased risk of NE. However, these markers are not ideal (Greenwood et al. 2003) and despite the known associations of NE clinical risk scoring is recognised to have a limited sensitivity (Low et al. 2001; Low et al. 1995).

Intrapartum electronic fetal monitoring has been shown to decrease the rate of neonatal seizures (MacDonald et al. 1985) but not to have a significant effect on long-term outcome (Grant et al. 1989) and concerns have been raised about the high rate of false positives leading to an increase in operative deliveries (Nelson et al. 1996).

1.3.6.2 Outcome with respect to clinical care

The relative contribution of antenatal hypoxia, sentinel event and sub-optimal clinical care to adverse outcome as determined retrospectively by case review has been reported from both New Zealand (Westgate et al. 1999) and the UK (Draper et al. 2002; Gaffney et al. 1994; Westgate et al. 1999).

Westgate et al (Westgate et al. 1999) reported that 23% of cases had preceding evidence of antenatal hypoxia and a further 23% experienced obstetric catastrophes beyond the control of the clinician, in their study of 22 infants presenting with moderate to severe encephalopathy. A recent audit of antecedent causes for moderate to severe NE in infants cared for at National Women's Hospital found evidence of antenatal hypoxia in 15%, a sentinel event in 25% and suboptimal fetal monitoring practice in at least 42% of cases (West C 2005a). In contrast, independent review of cases from the Trent Health Region, UK reported significant or major episodes of sub-optimal care for 64% of cases of encephalopathy with 75% of deaths thought due to intrapartum asphyxia (Draper et al. 2002). Furthermore there was a trend towards an increase in the severity of outcome with the number of suboptimal care episodes, with a mean of 2.8 and 2.5 episodes of sub-optimal care identified for death and encephalopathy respectively. The link between intrapartum care and cerebral palsy or neonatal death has been examined further in two case control studies. Niswander (Niswander et al. 1984) studied 34 children with CP and failed to establish a link with sub-optimal care, but the study could be criticised for small numbers and the fact that the group included preterm infants who probably developed CP by a different

mechanism. Gaffney (Gaffney et al. 1994) examined a larger group of term singleton babies including 141 with cerebral palsy and 62 intrapartum or neonatal deaths. Failure to respond to signs of severe fetal distress was more common in cases with cerebral palsy (odds ratio 4.5) and in those who died (odds ratio 26.1). In a further study of 210 singleton children with cerebral palsy, 35 (17%) were identified as a likely intrapartum cause and 26 (12%) had evidence of sub-optimal care (Gaffney et al. 1995). Notwithstanding this data, it should be stressed that the majority of cerebral palsy is not associated with sub-optimal care.

1.3.6.3 Home Birth

Planned home birth is an option that some women in New Zealand choose (Gulbransen et al. 1997). However, the role of home birth in the root cause of adverse outcome following delivery is contentious. Differences between provision of care during pregnancy and labour exist between different health care systems and the risk may depend more on the ability to detect and act on a non reassuring fetal condition than on the physical location of the birth. Some studies report that healthy low risk women are probably at no increased risk of harm to either themselves or to their babies (Ackermann-Liebrich et al. 1996; Gulbransen et al. 1997) (Mehl-Madrona and Madrona 1997) (Anonymous 1996). In contrast one population based study from Australia (Bastian et al. 1998) reported a higher rate of adverse outcome with home birth compared with that in hospital. A perinatal death rate, for infants weighing more than 2500 g, of 5.7 compared with 3.6 per 1000 (relative risk 1.6) was reported. Similarly an intrapartum death (not due to malformations or

immaturity) rate of 2.7 compared with 0.9 per 1000 (relative risk 3.0) was also reported. Moreover 52% of deaths were associated with intrapartum asphyxia. Underestimation of risk associated with post-term birth, twin pregnancy and breech presentation, and a lack of response to fetal distress were identified as major factors (Bastian et al. 1998). Finally, two more recent large studies from North America (Janssen et al. 2002; Johnson and Daviss 2005) have reported no increase in adverse maternal or neonatal outcomes for planned low risk home birth supervised by a certified professional midwife.

1.3.6.4 Developing world

In the developing world poor access to antenatal care and differing standards of intrapartum care may be compounded by impaired maternal growth and nutrition status, thus the incidence of encephalopathy may be higher and the outcome expected to be worse. Ellis et al (Ellis et al. 1999) reported a neonatal encephalopathy rate of 6.1 / 1000 live born term infants with 63% moderate or severe encephalopathy and death in 31% for Kathmandu, Nepal. Similar numbers are reported from Papua New Guinea (Oswyn et al. 2000) with a rate of 5.5 / 1000 and 31% mortality. An even higher rate has been reported from a referral hospital in Nigeria (Airede 1991) with an incidence of 26.5/1000 live births, over a 3-year period, of whom 12.1/1000 were severely abnormal. Moreover, in a survey from South Africa the perinatal mortality rate for intrapartum related birth asphyxia was 4.8/1,000 births. (Buchmann et al. 2002). The most frequently reported avoidable factors were delay by mothers in seeking attention during labour (36.6%), incorrectly interpreted signs of fetal

distress (24.9%), inadequate fetal monitoring (18.0%) and no response to poor progress in labour (7.0%) (Buchmann et al. 2002).

1.3.7 Temporal changes in incidence of perinatal asphyxia

The literature is somewhat contradictory regarding changes in incidence of neonatal encephalopathy over time. Levene's study from the 1980s reported that the incidence of this condition has not changed over a 10-year period (Levene et al. 1985). Similarly Rivkin reported no change in incidence over 40 years (Rivkin and Volpe 1993). A small increase in incidence was reported from Sweden for the years 1985 to 1991 (Thornberg et al. 1995b). In contrast, a falling incidence of NE, as identified by all clinical grades, has been reported from Derby, UK with a rate of 7.7 /1000 live births for 1976-80 versus 4.6/1000 for 1984-8 and 1.9/1000 for 1992-6 (Hull and Dodd 1992; Smith et al. 2000). If the mild cases are excluded and only moderate or severe cases are considered the change between the first two epochs is no longer significant, (2.6 per 1000 compared with 1.8 per 1000 live births) but the change between first and latest epoch remains significant (2.6 versus 1.2 per 1000). This data from a single centre should be interpreted with caution. Although the identification of severe cases may be assumed to be constant the management of mild cases may vary and incidence is prone to common cause variation over time. Similarly the recently reported apparent decrease in numbers of infants in California diagnosed with "birth asphyxia" using coding information may be influenced by changes in definition and may not apply to other populations (Wu et al. 2004).

Local data from Auckland New Zealand suggests that the rate of moderate to severe encephalopathy identified by hypoxic-ischaemic seizures or clinical grading, in term infants, was relatively constant between the periods 1978-1981 and 1991 and 1997-2000. The rates were 1.9 per 1,000, 1.78 per 1,000 (Gunn and Gunn 1997) and approximately 1.8 per 1000 live births per year (West C 2005a; West C 2005b).

1.3.8 Temporal Trends in Cerebral Palsy

A possible alternative approach to identifying trends in term asphyxia is to examine cerebral palsy data for specified groups and time periods. In a series of papers examining the changing panorama of cerebral palsy in Sweden, (Hagberg et al. 2001; Hagberg et al. 1984; Hagberg et al. 1993a; Hagberg et al. 1996; Hagberg et al. 1989; Hagberg and Olow 1975) Hagberg reported a prevalence of 1.3 cases per 1000 for children born at term in the early 1990s compared with 1.4 per 1000 for the period 1987-90 and 1.56 per 1000 for 1983-86. Similar data from the UK registers of cerebral palsy give a cerebral palsy prevalence of 1.1 per 1000 neonatal survivors in infants weighing above 2500 g at birth and suggest no significant trends over time in any of the birth weight groups (Pharoah et al. 1998). However, there are two major problems with such an approach. Firstly, the retrospective designation of the cause of cerebral palsy. Secondly, in the latest cohort from Sweden, perinatal asphyxia was noted to be the likely cause of cerebral palsy in only 28 % of affected term births. The potentially complex relationship between NE and cerebral palsy is explored further in the following section. A third potential problem with interpretation of cerebral palsy rates is that increased use of neuro-imaging

over time may identify lesions such as migration abnormalities and middle cerebral infarction in cases previously assigned to perinatal asphyxia. Although this practice improves the accuracy of classification it may produce an artefactual decrease in the rate of cases attributed to perinatal asphyxia and so hamper direct comparison between differing time periods. Notwithstanding these issues the data does not suggest a significant decrease in the incidence of cerebral palsy associated with altered rates of HI insult in the term infant.

1.3.9 Relationship between NE and Cerebral Palsy

Since the original work of William Little (Little 1862) linked abnormal parturition, difficult labour, prematurity and asphyxia with subsequent cerebral palsy there has been much debate about the purported connection between NE and cerebral palsy. Three main pathways may link NE in a term infant and cerebral palsy (Badawi et al. 1998a; Badawi et al. 1998b; Evans K 2001). Firstly, an otherwise normal healthy fetus is affected by a major catastrophic event leading to an encephalopathy followed by adverse long-term outcome including cerebral palsy. Secondly, the encephalopathy results from a causal pathway that started during the pregnancy and resulted in cerebral palsy but did not manifest with fetal distress during labour and delivery. Thirdly, a major antepartum insult, rather than intrapartum, results in intrapartum fetal distress, encephalopathy and adverse long-term outcome with cerebral palsy. Important studies from Stanley and others have shown that the origins of many cases of cerebral palsy are in fact antenatal in origin (Adamson et al. 1995; Blair and Stanley 1988). However there remain a significant number of

cases that are likely to be due to an intrapartum cause. The reported figure varies between about 8% and 25% (Blair and Stanley 1988; Freeman and Nelson 1988; Grant et al. 1989; Naeye et al. 1989; Nelson and Ellenberg 1986; Torfs et al. 1990). However, this range may in part be due to the non specific measures of asphyxia used in earlier studies. In contrast, a recent MRI study found that 80% of infants presenting with neonatal encephalopathy had evidence of acute insult and less than 1% had signs of established injury (Cowan et al. 2003). Although it is possible that referral patterns biased the patient population, the data does question the established idea outlined above and supports a significant perinatal contribution to neurodevelopmental morbidity.

Recently the relationship between neonatal encephalopathy and cerebral palsy has been examined further in a cohort study that investigated the evidence for hypoxic ischaemic injury as the cause of neonatal encephalopathy associated CP in term infants. In 57,159 consecutive births there were 150 cases with NE of whom 92 had at least one seizure and 58 had no seizures. This gave an overall incidence of NE of 2.62 per 1000 births and of NE with fits of 1.61 per 1000 births (Evans K 2001). The infants with NE were followed-up and 13 cases of four-limb cerebral palsy and three with hemiplegia were identified in the survivors. Twelve of the 13 cases of four-limb CP had clinical factors including low Apgar scores and an early evolving encephalopathy that suggested an acute intrapartum event as the cause. An obstetric event likely to cause acute hypoxic injury was identified in one-third of the cases of CP.

1.3.9.1 Consensus Statements

Consensus statements have been important in promulgating criteria for defining asphyxia that may then be linked to cerebral palsy or other subsequent adverse outcome. The statement made jointly in 1992 by the American Academy of Pediatrics and the American College of Obstetricians and Gynaecologists (American College of Obstetricians and Gynecologists 1992) regarded four clinical features to be essential: profound metabolic acidosis (pH < 7.00); persistence of an Apgar score of 0-3 beyond three minutes; clinical neurological sequel in the immediate neonatal period that include seizures, hypotonia, coma, neonatal encephalopathy; evidence of multiorgan dysfunction in the neonatal period.

Meanwhile, the International Cerebral Palsy Task Force consensus statement on CP and birth hypoxia (MacLennan 1999) stated that essential criteria to define an acute hypoxic event as the cause of cerebral palsy were : evidence of metabolic acidosis (pH < 7) and early onset of severe or moderate neonatal encephalopathy. Other useful criteria that were supportive but not essential were: a sentinel event, e.g. cord prolapse, uterine rupture or placental abruption; sudden, rapid and sustained deterioration in fetal heart rate; an Apgar score below six after five minutes; early evidence of multisystem involvement, renal failure, cardiac or respiratory complications; and early evidence of acute cerebral abnormality such as acute oedema of imaging.

These two statements and the latest joint American statement (American College of Obstetricians and Gynecologists 2002) include similar core items. Specifically they use easily available clinical markers and are particular in considering an encephalopathy to be a cardinal feature. Although it has been suggested that these statements should be used to define groups for future research, acceptance has not been universal and there has been opposition to some of the criteria used. Chiefly there were concerns around the evidence of documented fetal acidosis when the data is not always available (Dear and Newell 2000; Rosenbloom and Rennie 2000) or sodium bicarbonate has been used in the resuscitation making blood gas interpretation difficult (Dear and Newell 2000).

1.4 Clinical and pathological aspects of HI in the term infant

1.4.1 Distribution of brain injury

The occurrence, severity and distribution of brain injury depend on several factors including the severity and nature of the insult, gestation of the fetus or infant and presence of systemic stress or fever. In term newborn infants some regions of the brain are at particular risk. These include areas of the cortex, the hippocampus, basal ganglia and thalamus plus subcortical and periventricular white matter (Inder and Volpe 2000).

In term infants the watershed areas of the major cerebral arteries are vulnerable to hypoxic ischaemic brain injury and selective neuronal necrosis is the most common type of resulting neuronal injury. The depths of the sulci and posterior cerebrum are particularly vulnerable with the latter representing

the vascular border zone of all three major cerebral arteries. Injury in the watershed region of the parasagittal cortex and subcortical white matter is characteristically bilateral and symmetrical in distribution. More severe insults result in cortical cystic degeneration, with cysts extending into deeper cortical layers and into deep sulci of the brain. Profound damage to the thalamus, basal ganglia and brain stem may also occur. During the last trimester of gestation, the thalamus and other diencephalic structures have the highest rate of vascularisation and oxygen consumption thus making them particularly vulnerable to asphyxial insult at this time. The term *Status Marmoratus* describes an uncommon pattern of injury, affecting predominantly the basal ganglia and thalamus with neuronal loss, gliosis and hypermyelination. Haemorrhage or haemorrhagic infarction of the basal ganglia associated with a more extensive pattern of injury outside the basal ganglia is more commonly observed. Further injury types include single or multiple infarction subsequent to interruption of blood flow through single or multiple vessels and injury characteristically seen in the preterm infant including periventricular leukomalacia, periventricular haemorrhage and post haemorrhagic hydrocephalus.

1.4.2 Clinical neurological manifestations of HI injury

Newborn infants who experience intrapartum asphyxia that is serious enough to cause long term sequelae invariably exhibit abnormal neurologic features in the first week of life (Freeman and Nelson 1988). However, if the insult occurred before the intrapratum period there may not be an overt

encephalopathy during the neonatal period despite the insult being severe enough to cause long term adverse effects (Hill and Volpe 1989).

The clinical manifestations of hypoxic ischaemic encephalopathy reflect the underlying pathology. The findings are predictable in pattern but vary in severity. The description of a clinical and electrophysiological staging system for encephalopathy by Sarnat and Sarnat in 1976 should be considered a landmark in the study of term brain injury. Subsequently several other authors (Amiel-Tison 1978; Amiel-Tison and Ellison 1986; Fenichel 1983; Finer et al. 1981; Levene et al. 1985) have modified the system to give a clinical emphasis rather than the original a mixture of clinical and electroencephalography (EEG) features. Also the nomenclature that is now used most commonly is mild, moderate and severe encephalopathy rather than the original stage one, two and three.

1.4.2.1 Mild encephalopathy

Mild encephalopathy is characterised by an infant who is hyper alert, jittery, irritable, and over-sensitive to stimulation but shows no alteration in the level of consciousness. The infants appear to spend more than an appropriate time awake. On examination, passive tone is normal but there is an increase in active tone. There is moderate head lag but extension is stronger than flexion when sitting. Limb reflexes are normal or slightly increased but sustained clonus and an exaggerated Moro may be elicited. There is evidence of sympathetic over-stimulation with tachycardia, dilated pupils and jitteriness. Sucking may be weak and infants need encouragement to complete feeds.

There are no seizures and the EEG is normal. There is wide variation in the length of time different authors allow for recovery from a few hours (Sarnat and Sarnat 1976) to seven days (Amiel-Tison and Ellison 1986).

1.4.2.2 Moderate encephalopathy

Moderate encephalopathy is characterised by the infant displaying lethargy, hypotonia and a reduction in spontaneous movements. Posture is often hypotonic with abducted arms and legs. Affected infants are slower to react to stimuli, a higher threshold may be needed and reaction may not be complete. There is parasympathetic over-stimulation with low resting heart rate, small pupils, and copious secretions. The sucking response is poor and naso-gastric tube required. The EEG is abnormal and up to 70% of infants will have overt seizures.

Seizures most commonly manifest after 12 hours but may occur earlier(Gluckman et al. 2005). They are often subtle and are usually controlled easily, with one drug. Most infants are improving by the end of first week but complete recovery, if it occurs, may take longer. Sarnat reported the mean duration of abnormal behaviour to be 4.7 days (Sarnat and Sarnat 1976).

1.4.2.3 Severe encephalopathy

Severe encephalopathy is characterised by a flaccid stuporous infant. Respiratory support may be required from birth. Both tendon and primitive reflexes are absent and there is a lack of spontaneous movement. The infants have no suck reflex but may have abnormal suck-like episodes. Pupils are

frequently fixed and dilated or have only a sluggish response to light. The infant may have seizures and has an abnormal EEG with decreased background activity and/or voltage suppression. Seizures are frequent, occur early and are often prolonged. Episodes may be difficult to control often needing more than one anticonvulsant drug. The most severely affected have an isoelectric EEG pattern.

1.4.2.3.1 Clinical progression of the encephalopathy

The neurological signs in both moderate and severe encephalopathy follow a recognisable progression. In the first hours the infant will breathe spontaneously and show increasing tone and movements. Moderate and severe HI is the most common cause of seizures in the term newborn infant. Although not characteristic in the first few hours the onset may be as early as 30 minutes after birth or delayed until up to 24 hours of age (Ekert et al. 1997; Gluckman et al. 2005). The seizure type includes subtle, focal clonic, multifocal clonic or tonic in nature. Seizures may occur either alone or in combination and may be single or repetitive and, in some cases, refractory to treatment. In the severe cases seizures are often initially subtle but then become more overt and longer lasting. There is a suggestion that earlier onset seizures are linked with more severe insult and more adverse long-term outcome (Phelan et al. 1998). However in many clinical situations the exact time of insult is not known and it is possible that the injury preceded delivery by a considerable time.

In the severely affected infant the initial stupor may be followed by an apparent improvement in the level of consciousness. At this stage there is

often hypotonia. Some infants go on to improve and survive with hypertonicity (Ahn et al. 1998) but in the more severely affected other aspects of the neurological examination deteriorate resulting in deep coma between 24 and 72 hours of age. Periodic breathing may be seen and is due to bilateral hemisphere dysfunction. Apnoea may also occur probably due to impaired brain stem function. The most severe injury may manifest as respiratory arrest.

Other aspects of brain stem function including pupillary reactivity, extraocular movements and bulbar function mirror the global neurological status and progressive deterioration may be seen during the first 72 hours. These changes accompany the evolving encephalopathy. Thus pupil responses and extraoccular movements that are initially intact may, not uncommonly, evolve to dilated and fixed pupils with absent oculocephalic responses. Impaired bulbar and pseudobulbar function may manifest as impaired sucking swallowing and gag reflexes causing poor feeding or aspiration (Hill and Volpe 1989). Disproportionate injury to the brain stem structures and thalamus with sparing of the cortex and sub cortical white matter may occasionally occur (Koide 1985; Leech and Alvord 1977; Leech and Brumback 1988; Pasternak et al. 1991; Roland et al. 1988; Schneider et al. 1975). This distribution of injury reflects the high metabolic demand of these tissues and may occur following severe, acute disruption of blood and oxygen supply (Pasternak et al. 1991). This is in contrast to the usual cerebral hemisphere injury produced by a more prolonged but less severe insult that allows shunting of blood to protect the thalamus and brainstem. The encephalopathy in these cases is

characteristically moderate to severe often with prominent signs of brainstem dysfunction. This pattern has been demonstrated in primate models following acute total asphyxia (Myers 1975). Although there may be sparing of cerebral cortex and white matter in some cases, other infants with severe basal ganglia and thalamic lesions manifest poor head growth and on MRI demonstrate lesions in the cerebral white matter that were not evident in the neonatal period (Mercuri et al. 2000). It has been suggested that white matter atrophy in such infants is secondary, to either disruption of thalamo-cortical connections or lack of white matter growth (Mercuri et al. 2000). Clinical features of generalised hypotonia and paucity of spontaneous movements are common in severe hypoxic ischaemic encephalopathy. Examination may reveal shoulder girdle and upper arm weakness associated with typical parasagittal injury. Clinically recognisable rises in intracranial pressure are not common and only occur in the severely affected infant. Moreover, it is likely that this is the result of severe injury rather that the cause of ongoing insult (Lupton et al. 1988).

Impaired cerebral autoregulation may contribute to brain injury in the sick newborn infant. Cerebral autoregulation has been found to be absent in high-risk term and preterm infants but present in term, neurologically healthy infants undergoing intensive care (Boylan et al. 2000). The passive response seen in high-risk infants may reflect the severity of the underlying neurological disease.

1.4.3 Multisystem effects of HI insult

The newborn infant brain is relatively resistant to hypoxic ischaemic insult. Hence if an insult is severe enough to result in an encephalopathy then systemic problems affecting other organ systems are likely and should be recognised. In the case series reporting the frequency and spectrum of severity of multisystem problems in term infants with perinatal asphyxia (Martin-Ancel et al. 1995) (Perlman et al. 1989; Shankaran et al. 1991) multi-system involvement was common and included renal impairment, pulmonary hypertension, hypotension and abnormal findings on echocardiograph. This illustrates the importance of recognition of the extent and distribution of organ injury in the "asphyxiated" infant. As slightly different criteria are used to define organ involvement in each study (Martin-Ancel et al. 1995; Perlman et al. 1989; Shankaran et al. 1991; West C 2005b) it is helpful to summarise the data before looking at each system in turn (Table 3).

Table 3. Incidence of major organ problems in reported series (see text for references).

System	Martin-Ancel n=72	Shankran n=28	Perlman n=35	West n=64
Renal	42 %,	54 %	50 %	77 %
Pulmonary	26 %,	86 %	25 %	83 %
Cardiac	29 %	50 %	25 %	31 %
Gastrointestinal	29 %	-	Rare	-
Haematological	-	36 %	-	20 %
CNS	72 %	79 %	37 %	100 %

The low rate of CNS problems in the Perlman series may reflect selection by requirement for intubation not encephalopathy. In a further study (Barnett et al. 1997) of 58 selected infants who died from hypoxic ischaemic injury, major

pathology was detected in the heart in 63%, lungs in 33%, kidneys in 28%, liver in 22% and gut in 6%.

1.4.3.1 Renal dysfunction

Renal impairment is a well recognised clinical outcome following perinatal asphyxia (Anand 1982; Anand et al. 1978; Barnett et al. 1997) particularly as one cerebroprotective mechanism associated with intrauterine hypoxia is redistribution of cardiac output to the brain, heart and adrenals at the expense of perfusion of the kidneys, gut and skin. (Cohn et al. 1974; Peeters et al. 1979; Perlman et al. 1989; Sheldon et al. 1979)

Renal impairment may manifest as oliguria and/or elevated creatinine levels with non-oliguric failure (Karlowicz and Adelman 1995). Significant electrolyte disturbance is potentially associated with both the acute and recovery stages. Clinical problems particularly arise in the former when an elevated plasma potassium concentration may predispose to abnormal cardiac arrhythmias, although serious events of that kind are rare. In one series of 98 asphyxiated infants there were no cases of rhythm disturbance (Lackmann et al. 1991). If the infant survives then renal impairment is usually transient, (Drago et al. 1977) resolving by 10-14 days of age, with no residual long term consequences. However, fluid and electrolyte disturbance secondary to renal impairment has the potential to increase the adverse outcome.

There have been some attempts to correlate the degree of renal impairment with both the degree of perinatal asphyxia (Willis et al. 1997) and neurological

injury. In a study of 120 asphyxiated infants, (Perlman and Tack 1988) oliguria persisting for more than 36 hours was significantly associated with clinical signs of NE, including seizures, and long-term neurological deficits. In another study, of 31 infants, the relationship between the degree of oliguria following severe birth asphyxia and developmental outcome at 12 months was examined. However, using a stepwise regression model encephalopathy was found to be more powerful predictor of poor outcome than duration of oliguria (Bourchier 1991).

1.4.3.2 Liver dysfunction

In addition to renal impairment perinatal asphyxia may cause biochemical and/or clinical abnormalities in liver function. In a study of 31 asphyxiated infants liver function was deranged in nearly two thirds and a gradient in elevation was observed between survivors and non-survivors (Saili et al. 1990). In a further study increased levels of enzymes were reported to persist for up to 72 hours postpartum (Lackmann et al. 1993). Asphyxia has also been implicated as a potential causal factor in term and preterm asphyxiated newborns who develop early idiopathic neonatal cholestatsis (Vajro et al. 1997; Vajro et al. 1999). The clinical course for this was benign and somewhat similar to sporadic "idiopathic" neonatal hepatitis with abnormal liver function resolving within 12 months of birth in most cases (Vajro et al. 1997). In severe perinatal asphyxia overt bleeding due to disseminated intravascular coagulation may result from a combination of liver insult and excessive consumption of coagulation factors.

1.4.3.3 Pulmonary dysfunction

Respiratory complications of perinatal asphyxia, particularly meconium aspiration syndrome, pulmonary haemorrhage and pulmonary hypertension are common (Martin-Ancel et al. 1995; Perlman 1989; Shankaran et al. 1991; Thibeault et al. 1984). Transient respiratory insufficiency, with improvement in the first 24 hours of life, may also occur in infants with normal lungs after asphyxia (Thibeault et al. 1984).

The interplay of several factors that may reinforce each other probably influences the clinical course, and impact on survival, for affected infants. Specifically the combination of acidosis, meconium aspiration, hypoxia, and in some cases, sepsis may contribute to the development of respiratory comprornise. Although the exact role and timing of meconium aspiration has been questioned (Ghidini and Spong 2001) it is recognised that perinatal asphyxia and relative vulnerability of the fetus are important factors (Coughtrey et al. 1991). Excellent reviews have been published on the diagnosis and management of Meconium Aspiration Syndrome (Cleary and Wiswell 1998; Wiswell 2001a; Wiswell 2001b) and persistent pulmonary hypertension (Abman 1999).

1.4.3.4 Cardiac dysfunction

Perinatal asphyxia may be associated with cardiac impairment although the fetal and neonatal myocardium is somewhat resistant to hypoxia (Fisher 1984). Transient myocardial ischaemia is well recognised (Farru et al. 1986; Finley et al. 1979; Flores-Nava et al. 1990; Oh et al. 1985; Rowe and Hoffman 1972) and is associated with a significant mortality (Flores-Nava et al. 1990).

Dysfunction may be manifest as cardiomegaly (Flores-Nava et al. 1990; Oh et al. 1985) or on echocardiography with an abnormal fractional shortening, peak aortic velocity and mean aortic acceleration. Echocardiography may also rule out pathology such as hypoplastic left heart or critical aortic stenosis that may also be associated with poor cardiac output (Ranjit 2000). Major ischaemic ECG changes and increased levels of the cardiac enzymes may be observed (Barberi et al. 1999; Ranjit 2000). A response to treatment with inotropic support has been documented in observational studies (Walther et al. 1985). A link between reduced cardiac output associated with severe asphyxia and additional ischaemic or hemorrhagic cerebral damage because of lack of autoregulation has been suggested (Van Bel and Walther 1990; Walther et al. 1985). Transient tricuspid valve dysfunction is a recognised complication of asphyxia that in part may be linked with a reversible papillary muscle dysfunction (Bucciarelli et al. 1977). In survivors the clinical features including the tricuspid regurgitation murmur and ischaemic ECG changes spontaneously resolve. However, ischaemic papillary muscle necrosis is reported as a postmortem finding. In one autopsy study this finding occurred in 38% of infants and correlated well with both atrioventricular valve insufficiency and signs of congestive heart failure (Donnelly et al. 1980). Transient mitral regurgitation associated with myocardial ischaemia and reduced left ventricular function may also occur. An ECG obtained after injury may show ischaemic changes (Barberi et al. 1999) or abnormal rhythms, the latter if present suggest a poor prognosis.

There has recently been interest in the use of cardiac troponins as markers for

cardiac insult in newborn infants (Clark et al. 2001; Fleming et al. 2001; Moller et al. 1998; Trevisanuto et al. 1998). Troponin T (TnT), a regulatory contractile protein, is a sensitive marker for ischaemic myocardial cell injury in both the adult and paediatric population. However, very little is known about the relationship with fetal or neonatal cardiac status. In one study of infants presenting with bradycardia at birth the median concentrations were statistically comparable to the laboratory reference values for healthy term newborns although these were higher than healthy adult TnT levels (Trevisanuto et al. 1998). In another study there was no significant difference observed in cardiac troponin I levels between infants with significant fetal heart rate abnormalities and those without. Umbilical artery serum N-terminal pro-BNP is another potential marker which has been found to be elevated in association with fetal heart rate abnormality in the late stage of labour (Fleming et al. 2001). Finally asphyxia may also be associated with cardiac arrhythmia due to either hyperkalaemia or disruption of conduction and automaticity.

1.4.3.5 Haematological dysfunction

Asphyxia has a number of effects on the coagulation system (Castle et al. 1986; Castle et al. 1988; Chessells and Wigglesworth 1971). Levels of many coagulation factors are low in the healthy infant and even lower in the asphyxiated or premature infant. In the lamb model asphyxia resulted in thrombin generation and brisk consumption of coagulation factors (Andrew et al. 1988). Severe hypoxia has also been reported to significantly shorten the

survival of platelets (Castle et al. 1988) and to be an important risk factor for transient destructive thrombocytopenia (Castle et al. 1986).

1.4.3.6 Other metabolic and endocrine dysfunction

Metabolic and endocrine changes also occur in the asphyxiated infant (Khare 1977; Procianoy et al. 1988). Hypoglycaemia may be ascribed to depletion of glycogen stores. However, it is well recognised that in some cases a transient hyperinsulinism develops (Clark and O'Donovan 2001; Collins and Leonard 1984; Giroux et al. 1997; Sann 1999; Schultz and Soltesz 1991). This is characterised by glucose requirements of greater than 5-8 mg/kg/min and inappropriate elevation of insulin levels. The combination of hyperinsulinism with other hormonal disturbances and sometimes fluid restriction may predispose to persisting hypoglycaemia. This abnormal milieu has been associated with adverse outcome (Davis et al. 1999).

In summary, any infant who presents as clinically sick following perinatal asphyxia should unquestionably be considered as having a multisystem insult. It is important to look for signs of any systemic complications and consider the impact of these on both the outcome and any potential treatment options.

1.4.4 Outcome Following Perinatal Asphyxia

1.4.4.1 Problems with published data on outcome following perinatal asphyxia

The two most commonly used measures of outcome following perinatal asphyxia are postnatal survival and the presence of impairment. However, it is important to recognise that stillbirth is an important third adverse outcome. Which of the three outcomes eventuate is dependent on a combination of

insult timing, severity of neurological and systemic injury, site of neurological injury, vulnerability of the fetus and, to some extent the level of clinical intervention or treatment. Although some publications (Smith et al. 2000) include stillbirth data many do not and this outcome is both poorly measured and easily overlooked.

Much of the published data on outcome following perinatal asphyxia comes from follow-up of inception cohorts with all infants assessed at given time points (Levene et al. 1986; Robertson and Finer 1985; Robertson and Finer 1988; Robertson and Finer 1993; Robertson et al. 1989). This type of prospective study is very useful but considerable effort is required to ensure that a high follow-up rate is attained and a delay is inevitable before neurodevelopmental outcome can be assessed. Furthermore, there is considerable potential for bias in such cohorts, as not all term infants exposed to perinatal asphyxia will be referred to the tertiary centre (Saigal S 1991). This is particularly true for very severe cases, including stillbirths or deaths in labour ward, or mild cases that may be cared for in the primary centre. In contrast, there has been much effort to ensure that follow-up cohorts of premature infants are both regional and all inclusive.

Unfortunately there are other major problems with the published data on outcome following perinatal asphyxia. In fact many of the criticisms regarding the data on the incidence of HI insult are also pertinent to the literature regarding outcome subsequent to such insult. Specifically there is inconsistency in criteria used to select the study population; small study populations, with inadequate details on severity of encephalopathy; and lack

of detail on the relative contribution of term versus preterm infants to the study population. There is also much variation in the age at follow-up and methods of assessment.

1.4.4.2 Survival data from studies using a single indicator of perinatal insult

The outcome for perinatal asphyxia depends upon the criteria used to make the diagnosis. A significant delay in spontaneous breathing has been strongly associated with death as an outcome. Peliowski and Finer 1992 (Peliowski and Finer 1992) reviewed the literature on 25 infants who did not breath spontaneously by 30 minutes and found that 80% died or were handicapped. Similarly Ergander (Ergander et al. 1983) reported that only 25% of babies not spontaneously breathing by 20 minutes survived without significant handicap. In contrast the outcome for an infant with very poor condition at birth but a good response to resuscitation is less grim. Rapid and effective resuscitation of babies born apparently dead may be associated with subsequently normal neurodevelopmental outcome (Jain et al. 1991; Scott 1976). In a recent paper, (Casalaz et al. 1998) Casalaz reported 26 term babies with an 1 minute Apgar score of zero who were resuscitated effectively. On review 46% survived and were described as normal, 23% were disabled and 31% had died before discharge. Indeed, the relationship between depression of Apgar score and adverse outcome is not particularly clear in the term infant. An Apgar score below three at five minutes is poorly related to adverse outcome, with most infants surviving without cerebral palsy (Nelson and Ellenberg 1981; Thomson et al. 1977). Nelson and Ellenberg reviewed data from over 40,000 births and reported infants with severely depressed Apgar score up to 20 minutes. The

rates of death and cerebral palsy for infants above 2500g birth weight with an Apgar score of 0-3 at intervals are given below (Table 4). It is not until the Apgar score remains below three for 20 minutes that there is a relatively high risk of cerebral palsy (Nelson and Ellenberg 1981).

Table 4. Risk of cerebral palsy with Apgar scores 0-3 at varying time from birth in infants greater than 2500g - data from Nelson and Ellenberg (Nelson and Ellenberg 1981)

Age (min)	Death in first year (%)	Cerebral palsy (%)
1	3	0.7
5	8	0.7
10	18	5
15	48	9
20	59	57

1.4.4.3 Neonatal encephalopathy and outcome

There is a strong relationship between outcome and severity of neonatal encephalopathy (Peliowski and Finer 1992). The results of several studies (Finer et al. 1981; Levene et al. 1986; Low JA et al. 1984; Robertson and Finer 1985; Sarnat and Sarnat 1976) looking at this relationship are given below (Table 5). The rate of death with mild, moderate and severe encephalopathy was <1%, 20% and 70% respectively (Peliowski and Finer 1992). It is also notable that no infant with mild encephalopathy is reported to have developed subsequent serious impairment solely as a result of perinatal asphyxia. Although one mild case was reported to have an abnormal outcome this was due to a congenital myopathy. Several reports combine infants who have died with those manifesting marked neurological impairment to give a single outcome of death / severe impairment. The median rate of death / severe impairment from five follow-up studies in full-term infants with

moderate encephalopathy was 25% (range 15-27%) and for severe encephalopathy 92% (range 75-100%) (Levene 2001).

Table 5. Summary of studies (see text for references) reporting outcome based on severity of NE (% severe/dead).

Study	N	Mild	Mod.	Severe	Follow-up
Sarnat	21	-	25 %	100 %	1 yr
Finer	89	0	15 %	92 %	3.5 yrs
Robertson	200	0	27 %	100 %	3.5 yrs
Low	42	-	27 %	50 %	1
Levene	122	1	25 %	75 %	2.5 yrs

1.4.4.4 Relationship of impairment with site of injury

The relationship between the site of injury and the pattern of impairment may not be as predictable in children as in adults due to the plasticity of the developing brain.

Mental retardation may occur with parasagittal cortex damage (Volpe 2001) and is common in survivors of severe perinatal asphyxia. Impaired cognitive function is particularly likely to occur if there is damage to the thalamus even if damage to the parasagittal cortex is limited (Malamud 1950). Language and visuo-spatial problems are commonly associated with damage to the parieto-temporal-occipital regions, where auditory and visual functions are associated. Indeed asphyxiated infants with visual impairments may also have moderate to severe cognitive impairment, learning disabilities and language disorders due to associated problems with other posteriorly located functions. However, the characteristic adverse neurological outcome following term perinatal asphyxia is of motor impairment coming under the rubric of cerebral palsy. The clinical manifestations and distribution of injury are variable and are

described in more detail in the next section. Severe cases are commonly associated with a variety of other adverse outcomes including cognitive impairment, cortical visual loss, sensory neural hearing impairment and seizure disorders (Robertson and Finer 1993).

1.4.4.5 Types of Cerebral palsy following NE

There are two characteristic types of cerebral palsy that occur following term perinatal asphyxia (Rosenbloom 1995). The most common form is spastic cerebral palsy. This is mostly a spastic quadriplegia but also occur in a hemiplegic distribution. These forms are usually disabling and may also have dystonic or athetoid features. Affected children frequently have a protracted clinical course often with major feeding problems. In addition to the impairment affected infants also may have a shortened life expectancy (Strauss et al. 1998).

In infants subsequently affected by spastic cerebral palsy, abnormalities on neurological examination may be present at the time of discharge, most commonly manifest as either increased or decreased tone, particularly hypotonia of the shoulder girdle (Robertson and Finer 1993). In contrast to preterm infants, tone abnormalities in term infants at this stage are usually signs of evolving permanent motor impairment.

The second form of cerebral palsy that is characteristic following HI insult in a term infant is the Athetoid or Dystonic form (Rosenbloom 1994). This is less common and particularly reflects damage to the basal ganglia. Clinically there

is often an initial hypotonic phase with the subsequent development of extrapyramidal movements over the first year of life. Cognitive impairment may be frequently associated but this is not necessarily so and in some cases cognition is entirely preserved (Rosenbloom 1994).

1.4.4.5.1 Assessment for adverse outcome

The presence or absence of cerebral palsy is the most commonly reported neurodevelopmental outcome following HI insult in the term infant. Assessment is mostly performed at pre school age, usually 1-2 years and a latent period is required before neurodevelopmental outcome, especially higher cognition, can be assessed with any certainty. During this period the manifestations of any injury are modulated by the increasing maturity of the central nervous system thus impairment may not be evident before about 12 months. An example of this is that basal ganglia damage may cause involuntary movements that become manifest after a delay sometimes beyond one year of age. (Saint Hilaire et al. 1991)

1.4.4.6 Cognitive impairment following neonatal encephalopathy

Classic HI injury in the term infant results in CP with or without cognitive impairment. Cognitive impairment alone defined by an IQ below 70 without other neurological abnormality is not a characteristic manifestation of perinatal asphyxia (Freeman and Nelson 1988). Furthermore, a normal neurodevelopmental examination at 1-2 years is unlikely to be followed by a major abnormality or developmental delay at five years of age (Shankaran et al. 1991). However, subtle but significant effects on IQ are reported following asphyxia. These may not be evident in the individual but represent a shift in

the population range and may be graduated with severity of insult; in a cohort of children with moderate encephalopathy the median Stanford Binnet score was 92.3 at 3.5 years compared with 101.5 in unaffected children (Robertson and Finer 1985).

In addition to the specific effects on IQ, term infants with moderate encephalopathy but no major motor handicap on pre-school assessment have been shown to have considerable delays of more than a year in skills including reading, maths or spelling (Robertson et al. 1989). In a separate study of 34 asphyxiated infants who were considered normal at two years of age, eight had minor neurological dysfunction and/or perceptual-motor difficulties, one had only cognitive impairment and 25 were normal when assessed at school age. The minor neurological dysfunction and/or perceptual-motor difficulties could largely be predicted by the pattern of injury on MR imaging. Most of those with a normal outcome had no injury or minimal white matter lesions but 80% of those with minor neurological dysfunction and/or perceptual-motor difficulties had basal ganglia or more marked white matter lesions (Barnett et al. 2002).

Long-term sequelae after perinatal asphyxia have generally been assessed at preschool or school age, but little is known about possible late cognitive consequences and it is possible to miss impairment of cognitive and memory functions that does not become apparent until education and adult life imposes extra demands. Three long term studies have explored this.

In the first study 59 infants identified by a history of resuscitation at birth for perinatal asphyxia but considered healthy at 18 months were reviewed in adult life by questionnaire. There were no differences in coping with school, the rate of university entry or living conditions between the two groups suggesting no adverse effect on educational achievement and social adjustment had occurred in these individuals (Kjellmer, Beijer etal.2002). In the second study in young adults who had experienced either mild or moderate asphyxia there were no major differences between the two groups and normal controls in any aspects of cognitive function or intelligence. Although all groups performed within the normal range in all tests there was tendency to minor deficits in verbal ability in the mild group compared to the controls and one subject had a clear, defined memory deficit (Viggedal et al. 2002). These two studies reassure somewhat that long-term adverse sequalae are uncommon in infants following perinatal asphyxia but apparently intact on pre school assessment. However this is limited by the small size of the cohorts in both studies and the selection by requirement for neonatal resuscitation rather than encephalopathy in the first study. The third study (Marlow N 2005), investigated neurocognitive and behavioural outcomes in 65 children with neonatal encephalopathy, identified using the Trent Neonatal Survey database for 1992-1994. At age of seven years they were examined at school by a paediatrician and a psychologist. Fifteen children had cerebral palsy and eight were in special school with severe cognitive irnpairment. Disability was present in 6% of the moderate and 42% of the severe encephalopathy group. In the 50 children without motor disability there was a mean IQ difference from their peers of -11.3 points in those who had severe

NE and -1.7 points in those with moderate NE. Neuropsychological testing showed that memory and attention/executive functions were impaired in the severe group.

A further report (Gadian et al. 2000) has suggested that a mild HI insult may produce selective damage to particularly vulnerable regions of the brain, such as the hippocampi, without causing more severe neurological and cognitive deficits. Clinically this may manifest as impairments in episodic memory that are difficult to recognisein early childhood but may have debilitating consequences later in life.

1.4.4.7 Special senses

1.4.4.7.1 Vision

Visual impairment may result from perinatal asphyxia damaging the visual cortex which is located in the watershed region of the posterior and middle cerebral arteries (Roland et al. 1986). Infarction in this region is commonly seen following term insult (Roland et al. 1986) and atrophy occurs in nearly two thirds of infants with term asphyxia (Simon 1999).

Visual impairments are difficult to detect in children with multisystem neuodevelopmental problems. However, early detection is important for optimal intervention with visual impaired programs as visual cues play a major role in achieving postural and motor milestones. Hence formal visual assessment is an important part of follow-up for an affected child.

1.4.4.7.2 Hearing

Perinatal hypoxia ischaemia is a recognised cause of hearing impairment. However only a small proportion of neonatal hearing loss is caused by perinatal factors and the hearing, speech, and language in survivors of perinatal asphyxia are mostly favourable (D'Souza et al. 1981). When language and hearing impairment is reported from large cohorts (Robertson and Finer 1985) or epidemiological studies (Meyer et al. 1999) (Fortnum and Davis 1997) asphyxia is an uncommon cause of hearing impairment in term infants. Indeed, in this way, there are similarities between hearing loss and cerebral palsy due to perinatal insult.

There is a critical period of sensitivity to HI insult during development of the auditory system when adverse outcome may occur. Prematurity in combination with asphyxia has a higher risk of hearing impairment but children older than about three months who survive severe or prolonged asphyxia with residual neurodevelopmental deficit do not have evidence of permanent hearing loss (Jiang 1995). The mechanism of impairment is likely multifactorial and may involve CNS damage or direct damage to the organ of Corti. In addition to the known neurological effects, asphyxia may cause haemorrhages in the inner ear and damage to the auditory pathway in the brainstem (Simon 1999) the cochlear nuclei are also damaged. Experimentally low pH in the blood perfusing the cochlea has been shown to be important in causation (Galambos and Despland 1980). Clinically factors

such as prolonged artificial ventilation, seizures, other organ damage and IUGR (Mencher and Mencher 1999) or the total number of risk factors increase the probability of a sensorineural hearing loss (Borg 1997). The occurrence of brainstem auditory evoked responses (BAER) abnormalities has been related to the duration as well as the degree of asphyxia and found to occur more frequently in those children with neurodevelopmental deficits following asphyxia than those without (Jiang 1995).

1.4.4.8 Changes in outcome over time

There is some evidence that the outcomes for infants with diagnosed NE improved in the 1970s and early 1980s (Finer et al. 1983) (Svenningsen et al. 1982). Finer reported an improved outcome in the last two years (1977-1978) compared to the first two years (1975-1976) of the studied cohort and suggested that improved recognition and neonatal management may be responsible for the decrease in significant sequelae. (Finer et al. 1981). In a Swedish study comparing survivors of NE during two time periods, 1973-6 and 1976-9, 50% of infants in the first epoch had adverse sequalae compared with 17% in the latter. It was suggested that the improvements in outcome might be related to brain-orientated intensive care (Svenningsen et al. 1982). The regimen included phenobarbitone administration, ventilation and other measures aimed to control postasphyxial cerebral oedema and avert abrupt changes in blood pressure and oxygenation. There is no evidence of a further improvement in outcome over the subsequent twenty years.

1.5 Prediction of outcome

1.5.1 Reasons for prediction

There are two main reasons to attempt to predict outcome for infants following perinatal asphyxia. The first is to counsel parents and, in some cases, make decisions about discontinuation of resuscitation or withdrawal of support. The second is to select infants who may potentially benefit from intervention with neuroprotective agents either in a clinical trial or future clinical practice. Although similar investigations may be performed for either of these two purposes the requirements and timing of investigation may vary considerably.

In order to accurately counsel families, it is desirable to have as much information as possible available including clinical data. This may entail a wait of several days in order to document the most severe stage of the encephalopathy and observe any resolution. Also some investigations such as EEG or imaging with MRI may be more easily performed or be more informative in the convalescent stage at about 5-10 days (Cowan 2000). The exception to this is when an early withdrawal of intensive care is contemplated in the first few days of life.

In contrast, selection for trials of neuroprotective agents needs to be completed as early as possible after an insult. Animal data suggests that for intervention, using hypothermia, this window of opportunity extends for a maximum of six hours after the insult (Thoresen 2000) (Vannucci and Perlman 1997). Accordingly, any method used to augment selection needs to not only provide reliable data before six hours of age in a sick infant receiving neonatal

intensive care but also needs to be quickly and easily interpreted. However, when considering study recruitment, rather than withdrawal of care, it may be reasonable for entry criteria to be set at a lower threshold, such as *likely to* have a moderate or severe encephalopathy rather than certainty of adverse long term outcome.

1.5.2 Problems with prediction

There are several potential issues in using the published literature to predict outcome following perinatal HI insult. Firstly, in many reports, it is not specified and probably not known if the insult has been a single occurrence or repeated episodes. Secondly, the exact timing and / or aetiology of the insult are seldom clear. Thirdly, follow-up is typically 12-18 months which may be considered to be quite short term and miss impairment arising during school age. Finally it is not easy to assess the presence of bias with cases being ascertained retrospectively and selected to support the use of the reported methodology.

1.5.3 Methods of prediction

1.5.3.1 Clinical evaluation

Clinical evaluation is one of the primary factors used to predict outcome and inevitably is the most important factor in any early decision to cease cardiopulmonary resuscitation. If a newly born infant does not respond to resuscitation or requires a prolonged resuscitation, over 20 minutes, then efforts will likely be discontinued. Asystole at 10 minutes despite adequate resuscitation, including adrenaline, is highly predictive of death or severe

neurological abnormality (Jain et al. 1991; Nelson and Ellenberg 1986; Patel and Edwards 1997). However, if there is a good response to the resuscitation then the initial condition and requirement for resuscitation is poorly predictive of outcome (as described in section 1.3.1). Similarly, the relationship between Apgar score and outcome is weak (as reviewed in section 1.3.2).

Clinical evaluation using neurobehavioral assessments, such as Prechtl, may be useful in prognostication (Majnemer and Mazer 1998; Prechtl et al. 1993) but has limitations. A strong relationship exists between normal neonatal examination and normal outcome but the prognostic value is limited by the high rate of false positives i.e. abnormal findings on examination but normal outcome. The assessments also take time to perform and the assessor requires significant training. This limits the scope for neurobehavioral assessment to be used in selection of infants for acute intervention although it may provide useful information with which to counsel parents.

1.5.3.1.1 Cord blood gas

Cord arterial blood gas may be used to document acidosis, hypoxia and hypercarbia at birth but has limitations in predicting outcome due to low specificity. Goldaber reported that the incidence of neonatal death and neonatal seizures did not increase until the umbilical artery pH was below 7.05 (Goldaber et al. 1991). Even for a pH below 7.0 approximately 80% of cases result in normal outcome (Goodwin et al. 1992). The respiratory component of the blood gas was initially considered to be less important than the metabolic. However, one study reported that a difference of more than 25

torr between the cord arterial and venous PCO_2 was a highly sensitive and specific parameter in identifying asphyxiated infants with seizures, hypoxic-ischemic encephalopathy, and abnormal development (Belai Y et al. 1998). Furthermore, another two studies reporting just umbilical arterial blood gas analysis reported higher initial PCO_2 values, despite mechanical ventilation, in infants with HIE including seizures (Engle et al. 1999; van den Berg PP et al. 1996).

1.5.3.1.2 Assessment of encephalopathy

Formal assessment of encephalopathy staging has been the staple source of outcome prediction. The details of outcome for each stage have been previously described (section 1.4.2). In addition to the proven link between severity of encephalopathy and outcome (Finer et al. 1983; Finer et al. 1981; Robertson and Finer 1985; Thornberg et al. 1995b) there is a link between duration of encephalopathy and outcome (Robertson and Finer 1985) (Sarnat and Sarnat 1976). For infants with an encephalopathy, persistence of abnormal signs beyond six days is associated with a subsequent poor outcome (Sarnat and Sarnat 1976). This is very important and useful for counselling purposes but, as maximal staging is not reached until after the first 24 hours and can only be ascribed in retrospect, caution is needed in using staging of encephalopathy for selection of candidates for neuroprotective agents.

1.5.3.1.3 Serial Head Circumference

Microcephaly following neonatal encephalopathy has a major prognostic significance although it may not be manifest until more than 12 months of age.

Early measurement of head circumference has been studied and a decrease in head circumference ratio of greater than 3.1% has a 90% sensitivity for eventual development of microcephaly (Cordes et al. 1994). This may be present as early as four months of life. However, some infants with initially abnormal head growth will not ultimately develop microcephaly and the specificity is only 85% (Cordes et al. 1994). Although serial head circumference measurement has a reported sensitivity of 58% and specificity 90% for prediction of adverse outcome the utility is limited by time lapse required so it can not be used for selection of infants for intervention studies. Notwithstanding this a single abnormal head circumference measurement at birth may indicate a more long-standing problem such as previous injury, malformation, hydrocephalus or growth restriction. This information may be useful in counselling parents.

1.5.3.2 Use of neurophysiologic techniques to predict encephalopathy and outcome

1.5.3.2.1 Electroencephalography

Conventional 12 lead EEG may be performed acutely in the neonatal intensive care unit or, more commonly, in the convalescent period when the infant is more stable and can be transferred. Several characteristics have been examined for correlation with outcome. These include abnormal patterns of activity such as burst suppression, the presence of seizures and the overall level of background activity.

Burst suppression is a poor prognostic feature (Aso et al. 1989; Grigg-Damberger et al. 1989; Pezzani et al. 1986) particularly if demonstrated

outside the first 24 hours (Rose and Lombroso 1970). However, this pattern is only seen in a small number of infants so has a low sensitivity hence other measures of discontinuity have been used (Biagioni et al. 1999; Pezzani et al. 1986). Menache reported, from a retrospective series, that an interburst interval of more than 30 seconds was found in two thirds of infants with a poor outcome following neonatal encephalopathy presumed to be of hypoxic origin. The presence of seizures has also been correlated with outcome following NE (McBride et al. 2000). However, perhaps the most useful variable is the interictal background activity. This is of greater prognostic significance than the presence EEG seizures (Holmes et al. 1982) (Holmes and Lombroso 1993; van Lieshout et al. 1995) although the latter are commonly demonstrated. Meta-analysis of four studies reports the pooled risk of death or handicap to be 3.2% for a normal or mildly abnormal EEG characterised by early mild depression or complete recovery of EEG background by day seven (Peliowski and Finer 1992). In contrast persistence of depression after day 12 is associated with a poor outcome. Furthermore for a moderately abnormal EEG, characterised by slow wave activity and for severely abnormal EEG characterised by burst suppression, low voltage or an isoelectric pattern the risk for an adverse outcome was 64% and 95% respectively. In cases where the initial EEG is obtained before 36 hours of age a second EEG at seven to nine days may improve the prognostic value of the investigation (Zeinstra et al. 2001).

Although EEG may provide data that is useful in predicting outcome following perinatal asphyxia this data comes in the form of lengthy recordings and

requires expert interpretation. Moreover, a significant interobserver variability is reported for EEG interpretation (Williams et al. 1985). These difficulties limit the speed at which useful data may be obtained. Furthermore studies may be better performed in the convalescent stage away from the electrical interference of neonatal intensive care equipment. While this limits the utility for early selection such issues are not so much of a problem for counselling parents, for which EEG has a clear role.

In view of the difficulties with EEG interpretation attempts have been made to use objective measures of EEG or simplified techniques. Power spectral analysis (Bell et al. 1990) has been reported for pilot data only but amplitude integrated EEG (aEEG) has become popular in clinical use.

1.5.3.2.2 Amplitude Integrated EEG

Single channel aEEG is a technique that may be used as an alternative or to supplement 12 lead EEG data and is otherwise known as cerebral function monitoring (CFM). It may be used to detect either a depressed background, which is characteristic early in the course, or seizures. A good correlation has been demonstrated between data obtained by this technique and that obtained by simultaneous 12 lead EEG (Toet et al. 2002). Although some concerns exist about the detection of short or focal seizures using this technique (Rennie et al. 2004) recognition of the overall pattern remains a useful assessment. The results of a number of studies that have examined the use of aEEG to predict outcome (Eken et al. 1995) are summarised below (Table 6).

Table 6. Prediction of adverse neurological outcome using bedside EEG tools.

Study	Number of subjects	Age at study	Sensitivity (%)	Specificity (%)
Eken	34	6 hrs	94	79
Toet	68	3 hrs	85	77
		6 hrs	91	86
Hellstrom-Westats	47	6 hrs	95	89
al Naqeeb	24	<12 hrs	100	82
Azzopardi	16	< 4 hrs	100	71
Azzopardi (Azzopardi et al. 1999)	22	< 12 hrs	100	94

The fact that the technique is simple to both perform and interpret makes it potentially valuable for both counselling parents and trial selection purposes. Moreover, there is a significant volume of published data demonstrating this utility (Table 6) including the relative sensitivity and specificity. In particular the data obtained before six hours (Eken et al. 1995) demonstrated that major abnormality was associated with a poor prognosis, which makes it ideal for selection of infants for intervention trials. In a prospective assessment an abnormal aEEG was more specific (89% vs 78%), had a greater positive predictive value (73% vs 58%), and had similar sensitivity (79% vs 78%) and negative predictive value (90% vs 91%) when compared with an abnormal early neurological examination. Furthermore, the combination of the aEEG and the neurological examination shortly after birth enhanced the ability to identify high-risk infants and limited the number of false positives identified with either evaluation used alone (Shalak et al. 2003).

1.5.3.2.3 Other continuous EEG monitors

Continuous EEG may also be obtained using other systems such as the Oxford Medilog recorder and used to predict outcome (Azzopardi et al. 1999; Wertheim et al. 1994). However, these systems do not provide continuous

display and still require expert analysis. Newer devices such as BrainZ monitor do provide continuous display and allow automated analysis, so offer promise, but have not yet been rigorously assessed in the clinical arena with regard to predicting outcome.

1.5.3.2.4 Visual and somatosensory evoked potentials

Visual evoked potentials (VEPs) are altered or abolished by hypoxia and may be obtained in the neonatal intensive care unit using a flash stimulus (Whyte 1993). A delayed or absent VEP within six hours of birth has been reported to predict death or adverse neurological outcome with a sensitivity of 94.7%, specificity of 73.3%, and a positive predictive value of 81.8% (Eken et al. 1995). The technique may also be used at a later stage to predict both visual and general neurodevelopmental outcome. In one series of 36 infants, a normal VEP in the first week of life was recorded in all 14 who survived without neurological deficits but 20 of the 22 who developed neurological deficits had an abnormal VEP that persisted beyond seven days.(Muttitt et al. 1991). The technique may be particularly of help in infants classified as moderate encephalopathy when outcome prediction based on clinical staging is difficult. In this group a sensitivity and specificity of 100% was observed (Muttitt et al. 1991). Although the technique may be useful for prognostication it is not suited for selecting infants for intervention trial due to equipment and expertise required.

1.5.3.2.5 Somatosensory evoked potentials

Somatosensory evoked potentials (SSEPs) may be obtained in the neonatal unit. In a similar manner to VEPs they interrogate the nervous system at a number of levels and may be used to assist with predicting outcome. In a study of 20 survivors of asphyxia at term all 13 infants with normal outcome had normal SSEP by four days of age and seven infants with subsequent deficits had an abnormal response beyond day four (Gibson et al. 1992).

Abnormal SSEP predicted death or later impairment with a sensitivity of 89.5%, specificity of 66.7%, and a positive predictive value 77.3% (Eken et al. 1995). Other authors have consistently reported normal SSEPs obtained before 14 days of age to be reassuring (Harbord and Weston 1995) (De Vries et al. 1991). Notwithstanding the Eken data (Eken et al. 1995), the small numbers of infants studied by this technique and the variety of time points used limit recommendations about utility. However, it is likely that if it has a role this would be prognostication rather than early selection for trials.

A combination of somatosensory and visual evoked potentials has been recommended (Taylor et al. 1992) but there is little data to show any benefit for either selection or counselling purposes.

1.5.3.3 Use of neuro-imaging techniques to predict encephalopathy or outcome

The choice of imaging modality may vary with condition of the infant, timing of injury and specific question to be asked (Barkovich 1997; Ment et al. 2002). Imaging with both computer tomography (CT) and magnetic resonance imaging (MRI) has been used to obtain prognostic information. However, both

techniques require major handling and transport of the infants; therefore may be problematic to obtain early in the clinical course. In addition, there may be difficulties with reliability and interpretation of images as some abnormalities may not be apparent in the first 24 hours. Although diffusion weighted imaging on MRI may demonstrate lesions early (Bydder et al. 2001) changes evolve and become more obvious after 24 hours (Robertson et al. 1999).

1.5.3.3.1 Cranial ultrasonography

Cranial ultrasonography may be performed at the cotside in sick infants. It is useful to detect haemorrhage and confirm normal gross brain development. Parenchymal lesions (Babcock and Ball 1983) and cysts in the basal ganglia and thalamus (Siegel et al. 1984) may particularly be demonstrated but routine ultrasound imaging tends to underestimate cortical damage. Use of a 10 mHz ultrasound probe has been reported to predict periventricular or sub-cortical white matter abnormalities and cortical damage, in infants who die, with a sensitivity of 100%, and specificity of 83% (Eken et al. 1994). However, this has not been translated into predicting clinical outcome for survivors. Thus ultrasound is considered to have a limited role in predicting outcome in term infants with NE (Hill and Volpe 1989).

1.5.3.3.2 Cerebral hemodynamics

A reactive increase in cerebral blood flow is well recognised to occur from approximately 10 hours after resuscitation from a HI insult (Hossmann and Kleihues 1973). This may persist for several days. In cases of severe injury this finding is accompanied by loss of reactivity to carbon dioxide tension and

autoregulation of blood pressure (Pryds and Edwards 1996). These findings may be detected using a variety of techniques and used to predict outcome.

The simplest and most accessible method of detecting changes in cerebral blood flow at the bedside is by cerebral Doppler, interrogating either the middle or anterior cerebral artery. These studies may be performed by clinicians and require minimal training. Quantification using cerebral blood flow velocity (CBFV) and the Pourcelot resistance index (peak systolic velocity minus end diastolic velocity divided by peak systolic velocity) provides useful prognostic information (Archer et al. 1986) (Levene et al. 1989) (Gray et al. 1993; Jongeling et al. 2002). Specifically an abnormally low Pourcelot resistance index less than 0.55 or a high mean flow velocity above three SD from the mean has a positive predictive value for adverse outcome of up to 94% (Eken et al. 1995; Levene et al. 1989). Some of these studies may be criticised for small numbers or follow-up to only 12 or 18 months (Gray et al. 1993) (Archer et al. 1986). However, in a large study infants with an abnormal resistive index following perinatal asphyxia were found to be 8.8 times more likely to have either died or demonstrate cerebral palsy at three years (Jongeling et al. 2002). Of practical note abnormal results are reported to occur before 62 hours (Archer et al. 1986) but may not be present by 24 hours (Levene et al. 1989) hence repeated measurements must be performed. Although this excludes it as a method of selection for intervention trials the technique may still have a role in diagnosis and prognostication.

1.5.3.3.3 Magnetic resonance imaging (MRI)

The MRI findings following HI injury are well described (Barkovich et al. 1995; Martin and Barkovich 1995; Rutherford et al. 1996; Rutherford et al. 1998)

with several patterns of abnormality that are clearly linked with adverse outcomes (Mercuri et al. 1999; Rutherford et al. 1996). T1 shortening occurs in the injured area two to three days after injury and is followed by T2 shortening six to seven days after injury. This is postulated to be due to breakdown of lipid components or mineralization (Barkovich et al. 1995).

Brain swelling is not always present particularly if the scan is performed in the first 24 hours or the damage is limited to the posterior basal ganglia or small areas of cortex. The appearance of swelling on MRI is in itself not very prognostic and it is more important to consider the evidence of injury once swelling has abated.

Cortical highlighting represents laminar necrosis and capillary proliferation and may be accompanied by signal change in the adjacent sub-cortical white matter. It is particularly seen around the central gyrus and along the mid interhemispheric fissure and insula. The appearances may be maximal in the second week post insult and resolve thereafter (Rutherford et al. 1995). The subsequent outcome depends largely on the presence and extent of other lesions.

The basal ganglia and thalami are particularly prone to injury associated with global HI insult and MRI evidence of injury is associated with motor impairment particularly cerebral palsy (Rutherford et al. 1996). The characteristic abnormalities of short T1 and short T2 are found in the posterior and lateral lentiform nuclei and venterolateral nuclei of the thalami. The

severity of the imaging changes are in proportion to the degree of subsequent impairment (Cowan et al. 2003; Rutherford et al. 2004; Rutherford et al. 1998). Mild basal ganglia changes accompanied by a normal appearance of the posterior limb of the internal capsule (PLIC) may also be seen and are associated with late motor problems. Moderate focal changes in the posterior and lateral lentiform nucleus with or without PLIC changes are associated with athetoid CP. Widespread, severe basal ganglia changes affecting all areas including the head of the caudate nucleus and associated with abnormal PLIC with or without brain stem lesions are associated with an abnormal outcome characterised by secondary microcephaly and spastic CP.

Patchy white matter abnormalities alone may be associated with a normal clinical outcome but infants with extensive white matter lesions usually have overt clinical impairment (Rutherford et al. 1996). Characteristic patterns predicting white matter injury include streaky changes on T1 in the centrum semiovale and marked loss of grey white matter differentiation. Affected regions are often situated in the watershed distribution. Early after injury diffusion weighted sequences may be used to identify infarcted areas but these changes will resolve by five to seven days when abnormality becomes evident on conventional MRI imaging. Outcome following white matter injury depends on the on the site and extent of the lesion. Infants with only mild to moderate changes may have a normal outcome. Whilst those with extensive abnormality but solely in the white matter may have cognitive impairment with no motor deficit (Cowan et al. 2004). In a further group of infants with both

white matter and basal ganglia lesions a mixture of cognitive and motors deficits will result.

The posterior limb of internal capsule is usually of high signal intensity on T1 weighted images after about 38 weeks' gestation. The absence of this finding is strongly associated with an abnormal outcome (Rutherford et al. 1998). In a single centre study of 75 infants, abnormally low signal in the posterior limb of the internal capsule in T1 weighted images had a sensitivity of 91%, specificity of 100% and a positive predictive value for severe neurodevelopmental impairment at one year of age of 100% (Rutherford et al. 1998). In infants with stage two or moderate NE, where prognosis is most difficult to ascertain from clinical staging, the sensitivity was 90% and specificity 100%. However, it is important to note that developmental changes make this sign unreliable at less than 38 weeks of gestation.

Other areas of the brain may be affected but difficult to demonstrate using MRI. Changes are difficult to detect in the cerebellum despite damage being present at post mortem and hence are not reliable for prognostic purposes. Similarly damage is not easy to detect acutely in the hippocampus but the presence of atrophy may be seen at a later stage. Severe abnormality of the brain stem is a poor prognostic sign when seen. Haemorrhage is of uncertain prognostic value as small amounts of subarachnoid or subdural bleeding are commonly detected and not, on their own, of significance. Extensive intraparenchymal haemorrhage, perhaps in an infant with severe systemic upset is important. Thrombosis in the main sinuses is of variable prognostic

importance and may be hard to diagnose unless a conventional or magnetic resonance venogram is performed.

Overall MRI has a major role in documenting injury and is useful in counselling parents but due to problems with infant stability and the time taken to acquire the image it can not be utilized to select for trial entry. Prognostic accuracy may be improved when MRI is used in combination with EEG (Biagioni et al. 2001). In this study a normal MRI was always associated with normal EEG background activity and normal outcome; in contrast severe abnormalities on MRI and marked EEG abnormalities were associated with an abnormal outcome. Furthermore, when the MRI showed moderate abnormalities, the EEG in all cases but one identified patients with normal and abnormal outcome.

1.5.3.3.4 Computed tomography

Cerebral computed tomography (CT) is a useful technique for demonstrating haemorrhage (Ment et al. 2002) but can also document neurological injury following perinatal asphyxia. Several studies from the 1980s used CT in the sick newborn infant but accurate data on prediction of outcome may be best obtained from studies restricted to the term infant with overt perinatal asphyxia or clinical encephalopathy.

The characteristic CT finding after asphyxia is a variable degree of decreased attenuation with or without haemorrhage (Fitzhardinge et al. 1981), (Guardia et al. 1986; Khare and Merchant 1990). Quantification of the extent of low

attenuation may attempted using a low density index (Lipper et al. 1986) or classified as normal, patchy or generalised (Lupton et al. 1988). In general infants with normal CT scans rarely develop major neurological deficits and CT scans with extensive areas of hypodensity are associated with adverse outcome (Fitzhardinge et al. 1981; Lipper et al. 1986; Lupton et al. 1988). An important caveat to this is that hypodensities may take time, perhaps up to 10-14 days, to develop. Hence CT is not useful in the selection of infants for intervention in the first six hours after birth although it may have some utility in assessing the prognosis particularly when performed beyond the first week of life.

The published studies on the use of CT to predict outcome are predominantly from the 1980s. It is likely that the quality of images obtained by modern machines is somewhat better than those obtained in these studies. The use of repeated scans to improve the accuracy of outcome prediction (Lipp-Zwahlen et al. 1985) has been reported but there are concerns regarding radiation exposure, particularly in a repeated fashion. Therefore imaging with a non ionising modality would be preferred for repeated scanning. Indeed current practice guidelines suggest the primary imaging modality for a term infant with NE should be MRI and that CT use should be limited to excluding major haemorrhage (Ment et al. 2002). Notwithstanding this fact some centres have difficulty getting access to MRI and use CT preferentially.

1.5.3.3.5 Cerebral metabolism

Magnetic resonance spectroscopy (MRS) can be used to document cerebral energy metabolism after perinatal hypoxia-ischaemia. Using 31p MRS a

decline in the levels of high energy phosphates may be demonstrated in the brain eight to 24 hours after asphyxia (Hope et al. 1984). This 'secondary' or 'delayed energy failure' is characterised by a fall in the concentration ratio of phosphocreatine to inorganic phosphate ([PCr]/[Pi]). In one study [PCr]/[Pi] value greater than two standard deviations below the mean predicted death or multiple neurological impairment with a sensitivity of 72%, specificity of 92% and a positive predictive value of 91% (Roth et al. 1992). A refinement on this technique allows absolute concentrations of phosphorus metabolites to be measured by MRS, and confirmed a significant correlation between [PCr] or [ATP] at birth and neurodevelopmental outcome (Martin et al. 1996).

Proton MRS may be used to predict outcome and offers a potential advantage in demonstrating abnormality soon after birth. A significant association between cerebral lactate measured by this technique at a median of 13 hours after delivery and the severity of subsequent energy failure has been reported (Hanrahan et al. 1996). Also increased relative concentrations of lactate soon after birth correlate with later neuro-developmental impairment (Amess et al. 1999; Hanrahan et al. 1999). Although nuclear magnetic resonance spectroscopy provides useful information on brain function and is predictive of outcome it is not routinely available outside of a research setting. Furthermore infant handling and safety issues are similar to those concerning MRI.

1.5.3.3.6 Positron emission tomography

Positron emission tomography (PET) may be used to measure cerebral blood flow (Volpe et al. 1985) or cerebral metabolism for glucose. Regional cerebral

metabolism for glucose has been studied in asphyxiated infants with encephalopathy (Thorp et al. 1988) (Blennow et al. 1995b) (Thorngren-Jerneck et al. 2001) and the deep subcortical area, thalamus, basal ganglia, and sensorimotor cortex are the most metabolically active regions (Thorngren-Jerneck et al. 2001; Thorp et al. 1988). Initial studies could only report relative values but more recently quantification has become possible using this technique. The total cerebral glucose metabolism is inversely correlated with the severity of NE (p less than 0.01) (Blennow et al. 1995b). Also the quantitative measure was lower in five of six infants who developed cerebral palsy compared with the infants with no neurodevelopmental impairment at two years (Blennow et al. 1995b).

PET gives functional information that may be used to complement morphological data from imaging. However there is no experience in using the technique early to select infants for intervention and the utility in predicting outcome is limited by the fact that only small numbers of infants with only short term follow-up are reported and most have been studied after one week of age. Furthermore the technique is expensive, requires specialist support, is not commonly available and exposes the infant to a dose of ionising radiation similar to that from a CT scan thus is likely to remain a research tool.

1.5.3.4 Use of biochemical markers of cerebral damage to predict encephalopathy or outcome

Changes in the level of a variety of substances measured in either blood or cerebrospinal fluid (CSF) have been related to perinatal asphyxia. Elevated

levels of the excitatory amino acids aspartate and glutamate have been demonstrated to be present in the CSF following perinatal asphyxia (Hagberg et al. 1993b). For both amino acids a higher level was found in the group with severe compared with mild hypoxic-ischaemic encephalopathy (Hagberg et al. 1993b). Although this data may aid the understanding of neurological injury there is no proposal to date to use it in predicting outcome or selecting infants for neuroprotection trials.

1.5.3.4.1 Blood and cerebrospinal fluid lactate

Lactate is formed from pyruvate during hypoxia and poor tissue perfusion and its measurement has an accepted role in management of critically ill infants after cardiac surgery (Charpie et al. 2000). The role of a single or repeated measurement of lactate in predicting long term outcome following perinatal asphyxia is less clear. A single blood measurement expressed as either a lactate/pyruvate ratio or as a concentration has been correlated with moderate or severe encephalopathy (Chou et al. 1998; da Silva et al. 2000). Similar findings were obtained by Shah using retrospective data (Shah et al. 2004). However, lactate production is not exclusive to hypoxia and is also stimulated by other non-hypoxic factors such as glucose infusion and catecholamines. Even when used in the specific situation of hypoxia it may not have a major advantage over base deficit, with which it has a close association, in the prediction of encephalopathy (da Silva et al. 2000). Moreover it is a poor predictor of subsequent neurodevelopmental outcome, with sensitivity of 12% and positive predictive value of 5%, when used in a non-selected population (Ruth and Raivio 1988). This limits the utility for accurately counselling

parents and probably also for selection of infants for intervention trials even if the intervention is assumed to be low risk.

1.5.3.4.2 Urinary Lactate

An alternative to blood lactate that has been explored is the ratio of lactate to creatinine in the urine measured by proton magnetic resonance (Huang et al. 1999). This ratio was altered within six hours in infants who developed an encephalopathy and, using a ratio of 0.64 or above, had a sensitivity of 94% and specificity of 100 % for predicting encephalopathy. It was also reported to be significantly higher in the infants with adverse outcomes compared with those who had favourable outcomes at one year. However, the test is limited by the requirement for magnetic resonance spectroscopy facilities often at short notice and "out of hours".

1.5.3.4.3 Other biochemical markers of neurological damage

Two other studies have examined interesting candidate substances and attempted to correlate the levels with outcome. Cerebrospinal fluid nerve growth factor levels were studied in 10 infants with asphyxia at birth and reported to be lower compared to a reference group (Riikonen et al. 1999). Similarly increased levels of CSF interleukin 6 were reported in neonates with adverse outcomes compared to those with favourable outcomes (Martin-Ancel et al. 1997). These studies are limited by the small numbers of infants recruited and although giving some hope for the future these measurements cannot yet be relied upon in clinical practice for either early or late prognostic information.

Creatinine kinase BB is found in both neurons and astrocytes. Levels in CSF have been studied in relationship to abnormalities in fetal monitoring (Feldman et al. 1985) and short term outcome (De Praeter et al. 1991; Ruth 1989) but there are problems with interpretation (Kumpel et al. 1983) and the efficacy in prediction of outcome is mixed (Nagdyman et al. 2003; Nagdyman et al. 2001); therefore use is not recommended.

More recently there has been interest in several proteins that are predominantly found in brain tissue, thus called Brain Specific Proteins (BSPs). Release of these BSPs into the blood or CSF implies damage to the cell with release of the BSP from where it should normally be confined (Leviton and Dammann 2002). Neuron specific enolase (NSE) is a glycolytic enzyme present predominately in neurons and neuroendocrine cells (Marangos and Schmechel 1987) but also found in oligodendrocytes (Sensenbrenner et al. 1997). Increased levels are reported in asphyxiated infants up to 32 hours following delivery. Although infants with the highest NSE levels died (Thornberg et al. 1995a) and the levels of NSE also corresponded well with aEEG abnormalities there was overlap in severity of encephalopathy. When serial CSF levels of NSE were measured at 12 and 72 hours the former was found to be more accurate in predicting adverse neurological outcome (Garcia-Alix et al. 1994). Notwithstanding this the technique has a limited application in either selection or counselling parents on prognosis.

Glial fibrillary acidic protein (GFAP) is a structural protein of the intermediate filament of astroglia and is present almost exclusively in astrocytes (Leviton and Dammann 2002). It is found in the CSF following a variety of acute brain disorders. In perinatal asphyxia levels have been reported to be five fold higher than a reference group within 12-48 hours of birth (Blennow et al. 1995a). The rise in GFAP also correlated with the severity of the encephalopathy and presence of adverse outcome, at 10 to 40 months, defined by abnormal tone, reflexes or disability was predicted with sensitivity 78% and specificity of 50% (Blennow et al. 1995a). The S-100 protein family consists of 19 members but only S-100B is considered to be a specific marker of neurological damage (Herrmann et al. 2000). It is released from astrocytes and when found in the blood reflects altered membrane integrity and increased permeability of the blood brain barrier. In a study of 29 asphyxiated infants serum protein S-100 in combination with creatinine kinase BB performed well in predicting moderate to severe encephalopathy (Nagdyman et al. 2001). Similarly in a further study of 22 infants with asphyxia the concentrations of protein S-100, neuron-specific enolase plus two other markers glial fibrillary acidic protein and neurofilament protein were found to be elevated in the CSF. Furthermore, the concentration correlated somewhat with outcome. Those who died had significantly increased levels (Blennow et al. 2001). However, given the small study numbers further studies are required before this can be considered a robust test.

Recently Bartha et al. have studied infants with neonatal encephalopathy and reported elevated levels of cytokines IL-1Beta, IL-6 and IL-8 in samples

collected in the neonatal period from infants with adverse outcome (Bartha AI 2004). As yet there have been no prospective studies using these measures to predict outcome and further work is also required before these measures could be used in the clinical rather than research situation.

1.5.3.5 Use of clinical scoring systems to predict encephalopathy or outcome

Attempts have been made to predict multiple organ morbidity using scoring systems early in the neonatal course. Carter (Carter et al. 1998) utilized both immediate intrapartum and postpartum measures and acid base status close to delivery to assist in rapidly identifying the term and near term newborn at risk for multiple organ morbidity after acute perinatal asphyxia. Thompson (Thompson et al. 1997) reported a scoring system to predict neurodevelopmental outcome at one year following hypoxic ischaemic encephalopathy in the neonatal period. In prospective assessment of 45 infants it was highly predictive for outcome. A peak score of 15 or higher had a positive predictive value of 92% and a negative predictive value of 82% for abnormal outcome, with a sensitivity and specificity of 71% and 96%, respectively. A third system the *Encephalopathy Score*, as described by Miller et al. (Miller et al. 2004) has also been shown to be useful in predicting subsequent neurodevelopmental outcome. However, these scoring systems were not applied within the first six hours. Hence at present they would be limited in use to counselling parents and should not be used in selection for intervention.

Ekert (Ekert et al. 1997) reported a retrospective cohort and found the important predictors of severe adverse outcome in the first four hours to be delayed onset of breathing, administration of chest compressions, and seizures. At 60 minutes of age the sensitivity of the predictive model was 85% and specificity 68%. An alternative similar model (Toh 2000) based on clinical features, and tested retrospectively, reported a sensitivity of 66.7% and specificity of 100% to predict either death or major motor disability at 18 months of age. Another early clinical score obtained at 30 minutes of age and based on evaluation of consciousness, respiration pattern, Moro and grasping reflexes has been proposed but only short term outcome data has been reported (Wayenberg et al. 1994).

Finally generic scoring systems, such as SNAP or CRIB, that are not specifically aimed at neonatal encephalopathy may predict mortality and morbidity in sick ventilated term infants (Sutton et al. 2002). Scoring systems and models such as these may be used to counsel parents but there are no data on their performance in comparison with other data such as that obtained using MRI or aEEG. If confirmed to be robust when used prospectively in the first three to six hours then they could have a role particularly for trial entry.

1.5.4 Summary of methods for predicting encephalopathy or outcome

Fully accurate early prediction of ultimate neurodevelopmental outcome remains elusive. This is perhaps due to factors such as plasticity of the brain and the ability to compensate for quite extensive injury. However, there is also the problem that many existing studies do not relate to generalised asphyxia

but rather to single organ reperfusion insults and many measures detect the degree of systemic insult using this as a proxy for neurological insult. Many of these metabolic parameters do not correlate well with the degree of damage to the central nervous system.

1.6 Pathways of neurological injury

In order to logically develop potential interventions that may improve outcome after HI insults it is essential to understand the cellular and basic mechanisms that lead to final neurological damage. The healthy fetus is able to adapt remarkably well to moderate or short periods of asphyxia (Westgate et al. 2007). Moreover, all infants who undergo a normal labour and delivery experience some reduction in both partial pressure of oxygen and pH during the birth process, and thus technically, it may be said that all infants are exposed to "physiological asphyxia". Fortunately, this is well tolerated in the great majority of cases, and only a tiny minority of infants are adversely affected, either because of overwhelming severity of the insult, or pre-existing placental compromise or neural sensitisation to injury. At the most basic level, in order for injury to occur, there must have been a sustained period of time when the brain cells were unable to maintain cellular homeostasis, leading to depolarisation and cell swelling (cytotoxic oedema). This period is referred to as the "primary" phase of injury.

During this phase, when oxygen delivery is inadequate to maintain oxidative metabolism, the brain and other organs can maintain some production of high energy metabolites such as ATP by anaerobic metabolism of glucose to lactate. This is highly inefficient since each glucose produces only 2 instead of

36 or 38 ATP. The amount of lactate produced during hypoxia is often used as an indicator of the severity of the insult. In practice, it is critical to appreciate that there is only a very rough relationship between the severity of lactic acidosis, and the severity of the neuronal insult. This poor correlation partially reflects that circulating lactate is produced largely in the periphery, not just the brain, and during moderate insults, perfusion of the periphery is reduced to help support cerebral perfusion. Further, as insults become more severe, hypoxic compromise of the heart leads to hypotension and *reduced* delivery of glucose to the brain, and hence *reduced* production of lactate.

The combination of profound hypoxia with hypotension and reduced perfusion (i.e. hypoxia-ischemia) leads to impairment of residual cerebral anaerobic metabolism and hence more rapid development of hypoxic depolarisation; not surprisingly most significant neural injury is closely associated with systemic hypotension (Johnston et al. 1995; Vannucci and Vannucci 1997).

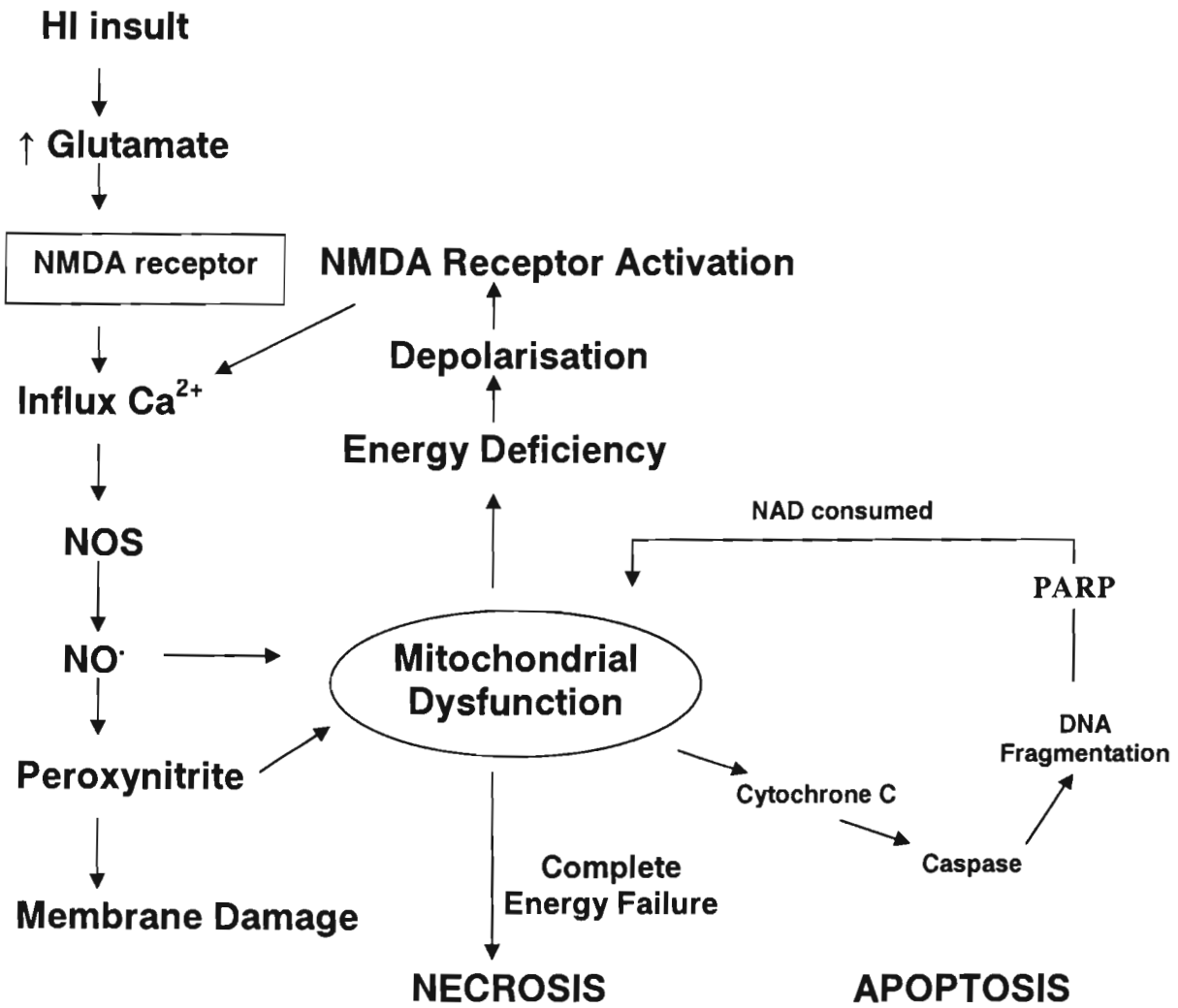
The critical insight that suggested the possibility of therapeutic intervention after this primary insult was the finding that although cell death can occur during a sufficiently severe or prolonged insult, in many cases the insult triggered a complex sequence of biochemical events leading to cell death hours or even many days after the primary injury. This pattern of transient recovery in a so-called 'latent' phase, followed by a delayed, 'secondary' deterioration has been well documented in both newborn infants and newborn and adult animal models (Ginsberg and Busto 1989; Marks et al. 1996b;

Penrice et al. 1997b; Thoresen et al. 1996b; Vannucci and Vannucci 1997; Williams et al. 1991; Williams et al. 1992).

Thus resuscitation from the primary insult with restoration of oxygen and cardiac output can be followed by a latent phase when the primary cytotoxic oedema resolves and oxidative metabolism can be normal. During this period EEG activity is depressed, and cerebral blood flow initially recovers and then shows a delayed but transient relative reduction. As late as 6 to 15 hours later there can be a progressive deterioration as shown by developing seizures, extracellular accumulation of excitotoxins such as glutamate (Tan et al. 1996), secondary cytotoxic oedema and increased cerebral blood flow. Human and animal studies have confirmed that these events are associated with a biphasic pattern of impaired energy metabolism on MRS, with initial recovery of the energy failure associated with the initial insult after resuscitation, followed by a delayed (secondary) fall in energy state (Azzopardi et al. 1989; Blumberg et al. 1997; Lorek et al. 1994; Roth et al. 1997). The secondary phase of injury lasts from about six hours to about 72 hours after the insult and is responsible for a significant proportion of final cell death (Beilharz et al. 1995; Lorek et al. 1994; Williams et al. 1991; Williams et al. 1992). The two pathways of neurological injury are summarised in the figure below (Figure 1).

As it may take hours or even days for the full extent of neuronal loss to become manifest following HI this may provide a window of opportunity during which intervention may decrease secondary injury.

Figure 1. Schematic representation of mechanisms involved in HI neurological injury in the newborn infant. Adapted from Johnston (Johnston et al. 2001).



1.6.1 Primary energy failure

Mitochondrial dysfunction and cerebral energy failure are fundamental to the primary phase of injury. The HI insult causes depletion of high energy phosphate compounds such as ATP and phosphocreatine (Vannucci and Duffy 1977). This in turn leads to an interruption in intracellular homeostasis

due to failure of the membrane bound sodium/potassium ATPase pump, which is energy dependent. The resulting wave of neuronal depolarisation then allows cations, particularly sodium and calcium, to enter into the cells. This flux of cations is associated with water entry into the cell that causes cell swelling (cytotoxic oedema). In severe cases immediate cell lysis occurs but it is also possible for the cell to recover.

1.6.2 Toxic excitatory neurotransmitters

Dysfunction of excitatory synapses is an important feature of the damage process (Johnston et al. 1995). An excitotoxic cascade occurs that is sustained by excessive stimulation from excitatory amino acids (Delivoria-Papadopoulos and Mishra 1998; McDonald and Johnston 1990). Glutamate is an abundant neurotransmitter within the brain. Reuptake of this excitatory amino acid via peri-synaptic glial sodium dependent pumps is responsible for maintaining the usual low levels of neurotransmitters (Silverstein et al. 1986). However, this process is energy dependent and so the primary phase of HI is associated with glutamate and glycine accumulation and resolves rapidly with resuscitation (Hu et al. 1991). Consistent with this, increased levels of excitatory neurotransmitters are found in the CSF of infants with NE (Hagberg et al. 1993b; Riikonen et al. 1992). Given the role of glutamate it is not unexpected that areas of the brain with a high density of glutamate receptors, namely the basal ganglia, thalamus and some parts of the cerebral cortex, are particularly vulnerable to HI injury.

Intriguingly, a less well understood secondary increase in excitatory neurotransmitters also occurs during the secondary phase, likely reflecting the failure of cellular oxidative metabolism, leading to failure of reuptake. The significance of this secondary phase remains unclear. Although the elevated levels result in over stimulation of the N-methyl-D-aspartate (NMDA) receptor and are closely linked to the onset of seizures (Tan et al. 1996), blockade of the NMDA receptor in this secondary period leads to only a modest improvement in neuronal damage.

1.6.3 Oedema

Oedema is a recognised phenomenon following any major cerebral insult. It results from two main mechanisms; cell swelling (i.e. cytotoxic oedema) and fluid leak that accumulates around the cells (i.e. vasogenic oedema). There is evidence of both mechanisms. As noted above the primary and secondary phases of energy failure are closely associated with cytotoxic oedema, while later oedema as the secondary phase resolves is primarily extracellular and simply reflects cell lysis/necrosis with an osmotic increase in water (Hill 1991; Lupton et al. 1988), i.e. that it is an effect of injury, not a cause.

In humans, serial intracranial pressure (ICP) measurements, using a Ladd monitor, demonstrated that seven of 32 asphyxiated term infants had increased levels in the first week (Lupton et al. 1988) with the maximum levels reached between 36 and 72 hours after birth. Similarly, Levene et al used subarachnoid catheters to measure ICP and found 70 % of infants had levels above 10 mmHg for at least one hour (Levene and Evans 1983).

1.6.4 NMDA receptor

The NMDA receptor is important in normal brain development, playing an essential role in activity dependent neuronal plasticity, but is also central in mediating neurological damage in the newborn infant following HI (Hagberg 1999; Vannucci 1990). Channel opening is dependent on glutamate, glycine and membrane depolarization. In the immature infant the receptors are more easily activated by glycine and have a higher calcium flux than in the adult. These characteristics, particularly the enhanced passage of calcium, link the receptor with subsequent brain injury. Indeed the NMDA receptor may be part of a vicious circle of dysfunction (Strijbos et al. 1996) and injury as the low energy state influences both the membrane potential and mitochondrial dysfunction (Novelli et al. 1988). Furthermore, neuronal depolarization via alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors can further deplete energy. These processes lead to maximal opening of NMDA channels and increased calcium entry into the cell (Choi 1988).

1.6.5 Calcium mediated neuronal damage

The healthy neuron maintains intracellular calcium levels at a very low level and an increase in intracellular calcium has deleterious effects. Although there is some buffering capacity for intracellular calcium this is unable to cope with excessive entry. Intracellular accumulation of calcium leads to impaired ionic gradients and inappropriate activation of intracellular enzyme systems that can cause activation of free radical production, mitochondrial injury, membrane damage and energy failure that for some neurons is lethal. For other neurons the accumulation is not so severe but calcium mediated

activation of neuronal nitric oxide synthetase (nNOS), free radical damage and other enzymes cause ongoing damage to the cell membranes and DNA (Rordorf et al. 1991).

1.6.6 Neuronal Nitric Oxide

Nitric oxide (NO) has a wide range of physiological and pathological actions. It is derived from l-arginine and produced by a family of three different nitric oxide synthases (NOS) (Szabo 1996). It has important functions in the immature central nervous system, playing a role particularly in blood vessel dilatation and neuronal plasticity but also in memory formation and a number of neuroendocrine functions (Szabo 1996). Although NO is a short lived molecule, it can diffuse sufficiently quickly to act as a neurotransmitter and acts particularly on adjacent neurons and astrocytes. The predominant second messenger in the brain is cGMP (Ignarro 1991; Ignarro et al. 1991).

The mechanism of neurological damage associated with NO is complex with the three different forms of NOS: endothelial (eNOS); inducible (iNOS) and neuronal (nNOS) playing very different roles. In summary, it is generally believed that activation of nNOS and iNOS can contribute to neurological damage whereas eNOS activation is mainly protective (Calabrese V 2007).

In perinatal hypoxia, NOS (eNOS) is activated during moderate insults to maintain or increase cerebral blood flow by dilating the cerebral vessels (Calabrese V 2007). The effect is calcium-calmodulin dependent, with NO production regulated, at least in part, via calcium concentration. Nitric oxide

also has a role in the late vasodilatation that occurs 24 to 48 h following HI and inhibition of nitric oxide synthase attenuates the rise in cerebral blood volume and may exacerbate injury (Marks et al. 1996a).

After severe HI nNOS may be activated by entry of calcium via the NMDA receptor channels. The nitric oxide that is produced combines with superoxide to produce peroxynitrite, which is toxic to both cell membranes and DNA. DNA repair mechanisms including cleavage of poly(ADP-ribose) polymerase (PARP) become activated. The ongoing production of NO associated with developing injury after resuscitation is linked with both pre synaptic release of glutamate and neurotoxic events including mitochondrial dysfunction (Tseng et al. 1997). Finally after 12-24 hours inducible NOS (iNOS) becomes active. This late sustained increase in iNOS is associated with invading glial cells (Iadecola 1997).

The multifaceted actions of NO in the mature central nervous system have been reviewed (Calabrese V 2007; Matsui 1999; Moncada 2006; Szabo 1996). It is of note that NO has the potential to be neuroprotective or harmful and may cause neurological damage secondary to an acute insult or through chronic neurodegeneration. This paradox may be explained by differences in levels of NO with physiological concentrations being protective but toxic effects predominating at higher concentrations (Calabrese V 2007) or by the differing timing and origin of NO as per above discussion (Iadecola 1997). In a newborn pig model, selective blocking of nNOS and iNOS by 2-iminobiotin immediately post-insult provided improvement in cerebral energy state,

vasogenic oedema and apoptosis related cell death (Peeters-Scholte et al. 2002).

There is some, limited data from clinical studies of NO production in asphyxiated human newborns. In one study of 17 term asphyxiated infants cerebrospinal fluid levels of NO were reported to be elevated at 24-72 hours and higher levels were associated with more severe encephalopathy (Ergenekon E 1999). Interestingly, there was no correlation between CSF and serum NO levels but the study may not have had the power to find a correlation, or perhaps there is a disparity in the trigger for NO release with the stimulus being more potent within the CNS (Ergenekon E 1999). A larger subsequent study (Shi Y 2000b) reported 33 infants with some evidence of HI (either pH < 7.2 or 1-minute Apgar < 4 or 5-minute Apgar < 7), of whom 28 developed encephalopathy, and 30 controls. Plasma NO and Carbon monoxide (CO) levels were reported to be related to both the degree of encephalopathy and subsequent neurological outcome. Finally two studies, from the same group, have examined brain (Groenendaal et al. 2006) or spinal cord (Groenendaal et al. 2008) tissue of full-term neonates who died after severe perinatal asphyxia. They demonstrated that nitrotyrosine, a reaction product of peroxynitrite and proteins was present in these tissues, which suggested that excessive nitric oxide had been present and could have played a role in the neurological injury (Groenendaal et al. 2006; Groenendaal et al. 2008). In contrast, the CSF of infants with mild encephalopathy did not show elevation of either NO or nitrotyrosine (Gücüyener et al. 2002). Finally, despite the potential for NO to react with superoxide and form peroxynitrite,

the use of NO as a pulmonary vasodilator is generally considered to be safe with few adverse systemic effects because of its rapid interaction with haemoglobin (Finer 1997).

1.6.7 Carbon monoxide

An understanding of CO biology may inform understanding of the mechanisms of neuronal injury. However, as exogenous CO poisoning is not a common clinical problem for newborn infants it is intended that this review is brief and limited to evidence of how CO is involved in neurological injury, particularly interrelationship with other mechanisms at a cellular level (see (Gorman D et al. 2003) for a review of CO as a toxin).

Carbon monoxide is similar to NO in that it has a significant signalling role in the body and the CNS (Snyder SH 1998). These two gaseous molecules, together with hydrogen sulphide, function as neural messengers in the brain and are sometimes referred to as *gasotransmitters* (Szabó C 2007). This neurotransmitter role for CO has been linked with both memory and cognitive function (Shinomura T 1994).

CO is present in the body as both COHb and a dissolved fraction in plasma. Although either fraction is a marker of CO production, plasma CO levels reflect pathological effects so may be more important (Shi Y 2000b). CO is produced via haemoxgenase which occurs in two forms: inducible HO-1 and constitutive HO-2. It is recognised that both CO and NO production is influenced by the inflammatory cytokines IL1, I6 and TNF (Shi Y 2000b) and

they are involved in pathological conditions such as sepsis and endotoxic shock (Shi Y 1997; Shi Y 2000a). There is increased expression of HO-1 during hypoxia (Shi Y 2000b) and there is reasonable evidence that the NO/NO synthase system in the brain works in partnership with CO (Takahashi K 1996). HO-1 is induced by NO donors and HO inhibition suppresses NO production (Okada D 1996). Also endogenously generated CO is a physiological modulator of the ventilation response to hypoxia via its actions on carotid bodies and perhaps at brainstem neurons (Prabhakar NR 1998).

Three mechanisms have been proposed whereby CO may have a role in neonatal encephalopathy (Shi Y 2000b). These are a) via regulation of vascular tone with increased CO production altering vascular tone via cGMP and contributing to cerebral oedema; b) altered CO levels via HO-1 expressed by macrophages, which subsequently may affect antioxidant function; and c) a putative action via the neural messenger role. More basic research is required to evaluate the significance of these possibilities.

1.6.8 Primary cell death

At the time of, and shortly after, the initial insult there is primary cell death. This phase is foremost in the first eight or so hours after the insult and the degree of cell loss is proportional to the precipitating insult (Williams et al. 1992). The predominant mechanism of this is necrosis which affects all cell types and involves adjacent cells causing death of both neurons and glia in a non-selective manner (Walker et al. 1988).

1.6.9 Secondary cell death

A phase of secondary or delayed cell death starts around 8 hours after the injury and lasts for 72 hours or possibly longer (Gluckman and Williams 1992). The pathways for cell death include both necrosis and apoptosis. Necrosis involves a loss of cell membrane integrity and a random pattern of DNA degradation. Swelling of the cytoplasm and organelles occurs but the nucleous is initially spared. Some cells recover initially but then go on to die by apoptosis. This is an active process involving cell degradation with karyohexis and nuclease mediated DNA degradation. It results in death and removal of the cell without associated inflammation and requires energy. Apoptosis may be initiated by a variety of pathways leading to activation of a family of proteases, the caspases (Lipton 1999), and results in the final pathway of endonuclease mediated DNA fragmentation (Beilharz et al. 1995).

The division between necrosis and apoptosis may not be as clear as initially thought. Both processes are reported in infants dying after perinatal asphyxia (Blumberg et al. 1997). Moreover, slowly evolving cell death can occur by necrosis (Colbourne et al. 1999) and mitochondrial calcium overload may be involved in both apoptotic and necrotic cell death. Therefore, it may be more appropriate to consider the process as more of a spectrum including both apoptosis and necrosis, with one or the other being more prominent.

1.6.10 Other proposed factors

In addition to the mechanisms of cell death and damage outlined above there may be other factors, which have a definite, but hard to quantify contribution.

The loss of trophic growth factors and activation of microglia may contribute to cell loss following insult (Lees 1993). In addition, macrophages from the circulation migrate to the site of injury and express cytotoxic cytokines including TNF alpha as well as nitric oxide and hydrogen peroxide. This neuroimmune response may have primarily an anti-infective role but also has the potential to be neurotoxic (Lees 1993). The brain has several endogenous protective mechanisms that broadly fall into one of four categories: cardiovascular responses, that maintain oxygen delivery to the brain and heart during an insult (Reid et al. 1991); release of neuromodulators, including inhibitory neurotransmitters, cellular factors and neurotrophins; cerebral cooling and preconditioning.

1.6.10.1 Protective cardiovascular and cerebrovascular responses

A fundamental principle of cardiovascular regulation is the matching of blood flow to meet metabolic demands. The fetal brain has a characteristically a high rate of oxygen consumption compared with the adult due to the need for growth and synthesis. However, it has a relatively low arterial partial pressure of oxygen and metabolic activity is closely linked to oxygen delivery (Koehler RC 1984).

The knowledge of compensatory responses to hypoxia in adults and mature animal models comes largely from studies of either carbon monoxide (CO), dilution of oxygen with inert gases or altitude. These conditions are different to the fetal milieu, where hypoxic stress is usually associated with or caused by some limitation in blood flow. There are also differences in response to hypoxia due to maturity. However, the general principles of cardiovascular

and cerebrovascular responses to hypoxia in the mature animal model will be briefly summarised before reviewing, more specifically, the fetal / neonatal responses. In the mature animal model the first response to hypoxia, from either hypoxic hypoxia or CO exposure is a compensatory increase in cerebral blood flow that maintains oxygen delivery and permits oxygen uptake to be maintained (Ludbrook GL 1992; Zhu N 1994). Associated with the increase in cerebral blood flow is a concomitant increase in coronary artery blood flow (Ludbrook GL 1992; Zhu N 1994) and increased sympathetic activity that causes peripheral vasoconstriction, increased heart rate and increased rate of breathing. There is also redistribution of cerebral blood flow away from the white matter towards the cortex, which is mediated by HO and NOS (Gorman et al. 2005). In addition, the oxygen carrying ability can be improved via an increase in circulating red blood cell mass secondary to release of stored red cells from the spleen (Gorman et al. 2005).

In the fetus, acute hypoxia produces a mixture of vascular, behavioural and metabolic responses (Jensen A et al. 1987; Jensen A 1999; Pearce W 2006; Peeters et al. 1979). The close coupling of "supply and demand" is maintained by an increase in cerebral blood flow and an inhibition of oxidative metabolism (Lutz PL 1992). Hypoxia causes an increase in sympathetic and parasympathetic activity (Parer 1983; Parer JT 1984) and an associated increase in release of catecholamines and other mediators (COMLINE RS et al. 1965; Jones CT and Ritchie JW 1978a; Jones CT and Ritchie JW 1978b; Lagercrantz H and Slotkin TA 1986; Rurak DW 1978), which induce the cardiovascular and metabolic responses. There is also significant adenosine

release, which in addition to causing vasodilatation through A_2 receptors depresses fetal cerebral oxygen consumption through neuronal A_1 receptors (Pearce W 2006).

The vascular responses that occur in the fetus secondary to hyopoxia are well studied (Jensen A et al. 1987; Jensen A 1999; Peeters et al. 1979; Sheldon et al. 1979). Brief hypoxia causes transient bradycardia then tachycardia with an increase in cardiac output. As with the adult, the early responses include cerebro-vasodilatation, mobilisation of stored red blood cells and redistribution of cardiac output with preferential flow to the vital organs including the brain, heart and adrenal glands, at the expense of flow to the skin, gut and kidneys but flow to the placenta is preserved (Cohn et al, 1974, Rudolph, 1984 Kjellmer, 1988). Vasodilatation in response to actute hypoxia may be demonstrated in fetal lambs and in premature human infants (Daven JR et al. 1983; Lan J et al. 2000). It develops early and is present at less than 0.7 gestation (Kurth CD and Wagerle LC 1992). Although the response is age dependent peaking in the early postnatal period (Bilger A and Nehlig A 1993) and early in gestation the process is unlikely to fully compensate for decreased arterial oxygen content (Gleason CA et al. 1990). A further feature of hypoxic vasodilatation is the heterogeneous distribution within the fetal brain (Koehler RC et al. 1985) with the effect being most evident in the brain stem (Tolcos M et al. 2003).

Adenosine accounts for about 50% of the vasodilatation that occurs in response to hypoxia (Pearce W 2006). The remaining effect is largely due to hypoxia-induced nitric oxide release and direct effects of hypoxia on cerebral

arteries. Another route of hypoxia-induced vasoreactivity is that mediated via endothelial function. This has a relatively minor effect in the fetus (Zurcher SD et al. 1998) but becomes more significant after birth (White CR et al. 2005) and throughout early postnatal life with the endothelial contribution to hypoxic vasodilatation becoming prominent in adult cerebral arteries (Zurcher SD et al. 1998). The main mode of action is endothelial release of nitric oxide (Coumans AB et al. 2003; Hunter CJ et al. 2003). Finally, hypoxia may also have a direct effect on vascular smooth muscle (Pearce WJ 1995). Potential mechanisms include altered membrane potential and calcium influx (Cornfield DN et al. 1994) or another nitric oxide-independent mechanism (Armstead WM 1997). It is recognised that immature cerebral arteries are less resistant to the direct effects of acute hypoxia than adult arteries (Pearce W 2006) and that smaller peripheral cerebral arteries relax quickly and completely in response to hypoxia compared to the larger proximal arteries, such as the carotid, which maintain tone much better (Pearce W 2006).

If compensatory mechanisms such as increases in cerebral blood flow (CBF) and oxygen extraction are not able to cope then decompensation occurs and bradycardia ensues, as it is not possible to decrease myocardial oxygen requirements during hypoxia. The bradycardia may be sustained and accompanied by a decrease in blood flow that may persist for some time. Once cerebral blood flow becomes compromised then the only enduring protective response is the shunting of the remaining flow to the brainstem, which maintains autonomic function (Ashwal et al. 1984). Further decrease in oxygen content causes cerebral vascular resistance to rise, resulting in an

additional decrease in cerebral blood flow until oxygen delivery falls below a critical threshold, at which point cerebral oxygen consumption then falls (Jones et al. 1977). In contrast to the adult, the fetal heart may be the last organ to fail during hypoxia. Neonatal cardiac dysfunction is a recognised complication of severe hypoxia and may manifest as cardiogenic shock, left ventricular failure as a result of global myocardial ischaemia.

1.6.10.2 Protective Neurotransmitters

In contrast to the excitotoxic properties of glutamate, other neurotransmitters and modulators have inhibitory properties, Gamma amino butyric acid (GABA) and adenosine particularly reduce neural activity and antagonise the excitotoxic responses thus decreasing metabolic demand. The extracellular level of GABA is increased following HI (Hagberg et al. 1987) and studies in the fetal lamb demonstrate a major increase in levels during the primary injury phase with a subsequent rapid fall and no significant increase during the secondary phase of injury. This pattern of decrease in levels may be one factor determining the onset of the secondary phase of injury (Tan et al. 1996). Similar increases are seen in adenosine (Gidday et al. 1995) and other neurotransmitters, including noradrenaline and dopamine (Blennow et al. 1995c), that are released following HI insult. They may have protective properties and blocking of these are reported to be associated with a more severe injury (Koos and Doany 1991; Park et al. 1988; Penning et al. 1991).

Adenosine particularly may have a key role in suppression of neural activity (sometimes referred to as neuronal hibernation) and subsequent

neuroprotection. Moreover, as discussed, in addition to its important affects on metabolic activity it also influences blood flow so potentially may act on both "supply and demand" (Blood AB 2002; Blood AB 2003; Karimi A 1996; Winn HR 1981). In the mature animal model, Adenosine has been recognised for some time to have potential for a neuroprotective effect (Evans MC 1987; Von Lubitz 1999). Stimulation of the adenosine A1 receptors causes suppression of neural activity (Dunwiddie and Masino 2001) and A1 antagonists increasing neuronal damage (Von Lubitz 1999). The data from immature animal models are somewhat less consistent with some studies failing to demonstrate a protective effect (Adén U et al. 2001) (Bona et al. 1997). However, in both the fetal sheep model and dog model EEG intensity rapidly decreases at the onset of asphyxia (Gunn AJ et al. 1992; Vannucci and Duffy 1977) which suggests active suppression of neuronal activity. Although this could be possibly secondary to profound tissue hypoxia it is considered more likely that the suppression is due to an actively mediated protective response (Von Lubitz 1999). Support for the active inhibition of activity comes from data obtained in an asphyxiated dog model, which reports the EEG to be isoelectric within two minutes but cerebral high-energy phosphates and ATP levels to be maintained for a further six minutes following induction of asphyxia (Vannucci and Duffy 1977). Further support for adenosine being the important mediator of this comes from the fact that adenosine levels increase during hypoxia (Koos BJ 1997) and that there are increases in local cortical blood flow (Blood AB 2002; Blood AB 2003) and an associated decrease in whole-body oxygen consumption reported in the fetal sheep (Karimi A 1996). Finally, blocking the

A1 receptor results in both a delayed decrease in EEG activity and more histological neuronal injury following HI insult (Hunter CJ et al. 2003).

The K-opioid system in the brain has a role in neuromodulation as well as diuretic and vasoconstrictor actions. Although activity increases after HI insults and putatively has protective effects, the action is not clear and opiate antagonists are also protective (Lou et al. 1989).

1.6.10.3 Cellular Factors

There is a transient increase in expression of the calcium-binding protein calbindin and a biphasic production of neurotrophins after HI. Calbindin may be protective by buffering the increased free calcium in neurons. This effect is supported by the finding of high levels of calbindin in cells resistant to ischaemic injury. Also a small rise in neurotrophins from the nerve growth factor family occurs in the initial phase, soon after termination of the HI insult and is followed by a significant upsurge in broader-spectrum growth factors including insulin-like growth factor-I (IGF-I), which reaches maximal levels at three to five days after injury.

1.6.10.4 Head Temperature After Birth

After birth it is normal for a mild degree of cooling to occur in the infant, with a drop in core temperature from 37.5°C to 36.5-37°C. This may be a natural protective process. There is animal evidence of attempts to reduce brain temperature following hypoxic ischaemic insult. In several animal models exposure to hypoxia is associated with an effort to select a lower ambient temperature and to increase heat losses (Gordon and Fogelson 1991; Wood

1991; Wood and Gonzales 1996). Also historical studies in term newborn infants found that moderately asphyxiated infants had a core temperature about 1.5 degrees lower than the non asphyxiated ones despite being nursed in the same way (Burnard and Cross 1958). These findings have led to the suggestion that hypothermia may be an adaptive response to insult (Thoresen and Wyatt 1997).

1.6.10.5 Preconditioning

A complex phenomenon of preconditioning is recognised whereby a single, subthreshold insult not severe enough to produce damage protects against subsequent more severe insults. The maximum protective effect occurs at about 24 hours and then rapidly diminishes. Although the mechanism of protection is uncertain, it is known that several anti-apoptotic agents are induced including bcl-2, growth factors, free radical scavengers, inhibitory neurotransmittors and heat shock proteins (Kato et al. 1994; Kirino 2002; Kitagawa et al. 1991).

1.7 Neonatal management

The management of a newly born infant delivered after a period of perinatal asphyxia should aim firstly to limit the impact and duration of primary insult and secondly to minimise any secondary injury. Adequate resuscitation is the initial priority but after this there is a need to provide supportive care, perform accurate serial evaluations and communicate effectively with the family.

1.7.1 Resuscitation

Rapid and effective resuscitation is paramount in the management of an asphyxiated newborn infant. However, some aspects of resuscitation may predispose to further injury via free radical mediated injury, co-morbidities such as lung disease or other mechanisms such as drug effects.

1.7.2 Resuscitation with 100% oxygen versus room air

There has been considerable research interest in the use of room air for resuscitation. Preliminary data obtained from a mixed group of term and preterm infants reported higher five minute Apgar scores for the group resuscitated with room air with no difference in median time to first breath, first cry and need for ventilation (Ramji et al. 1993). A subsequent international randomised controlled trial performed in over 600 infants showed similar primary outcomes of death within seven days or moderate / severe encephalopathy (Saugstad et al. 1998). Moreover, in a third study of room air versus oxygen, the room air resuscitated group recovered more quickly as measured by Apgar score, time to the first cry, and sustained pattern of respiration (Vento et al. 2003). In addition to these clinical outcomes, the reduced-to-oxidized-glutathione ratio was significantly reduced and erythrocyte superoxide dismutase and catalase activity were respectively 69% and 78% higher in the oxygen resuscitated infants than the controls at 28 days (Vento et al. 2003). Clinical experience of room air to resuscitate the asphyxiated newly born term infant has recently been published (Vento et al. 2001) and although the current ILCOR guidelines (Anonymous 2000; Richmond S 2007) suggest there is currently insufficient evidence to specify

the concentration of oxygen to be used at initiation of resuscitation further data the research outlined above may lead to reconsideration of this in the future.

1.7.3 Other relevant areas of research in newborn resuscitation

1.7.3.1 Meconium

Meconium aspiration syndrome is a common co-morbidity with neonatal encephalopathy. The management of an infant born through meconium stained liquor has been well researched and evidence based guidelines have been published. Some infants will have meconium in the respiratory tract despite suctioning indicating in utero aspiration (Falciglia 1988; Rossi et al. 1989). Notwithstanding this if the infant has evidence of compromise at birth suctioning under direct vision is indicated to decrease risk of secondary pulmonary sequelae (Greenough 1995). However if the infant is vigorous then suctioning manoeuvres do not decrease the rate of subsequent respiratory complications (Wiswell et al. 2000).

1.7.3.2 Adrenaline (Epinephrine)

Adrenaline is the primary drug in neonatal resuscitation. Exogenous catecholamines increase in all aspects of Apgar score except skin colour (Irestedt et al. 1984). In clinical practice the indication for adrenaline in the newly born infant is bradycardia unresponsive to cardiac massage and respiratory support. The dose is in large part derived from animal models and the adult human data, with a lack of randomised data obtained in human infants (Ziino et al. 2003). Current recommendations for use are 0.1-0.3 mL/Kg of a 1:10,000 solution via either endotracheal tube or intravenously

and there is inadequate data to support the use of higher doses (Anonymous 2000). Indeed one animal study suggests an excess mortality for higher doses of adrenaline (Neumar et al. 1995).

1.7.3.3 Cooling

Therapeutic cooling after resuscitation is the subject of this thesis and will be considered in subsequent sections. However, it should be noted that a newborn infant has a relatively large surface area to weight ratio, particularly of the head, and is wet with liquor hence under normal circumstances undergoes a cooling process after birth. If an asphyxiated infant is nursed under an overhead heater then the normal gradient between core and brain temperature is abolished (Gunn and Gunn 1996). Thus the cerebral cortex immediately below the scalp is potentially at a higher risk of adverse effect due to the relative lack of cooling (Gunn and Bennet 2001). Although the latest recommendations on neonatal resuscitation (Anonymous 2000) state that there is not, as yet, enough evidence to actually cool infants during the resuscitation there is a clear recommendation to avoid hyperthermia. Moreover, this recommendation may change as further trial data is published. In clinical practice it is reasonable to protect the head and scalp from excessive radiant heat and important not to cause excessive heating.

1.7.4 Management of the infant in Neonatal Unit

1.7.4.1 General measures

The infant assessment should include a history of risk factors including maternal medical conditions; pregnancy complications; fetal anomalies detected in utero; fetal status in labour, including cardiotochographic

abnormalities and scalp pH; maternal indicators of infection, presentation and method of delivery. Examination of the placenta may also prove helpful (Marks et al. 1989) (Altshuler 1993; Altshuler 1996) (Scher et al. 2002; Torfs et al. 1990; Williams and Lucci 1990). Physical examination should assess cardiorespiratory status, growth and the presence of any congenital anomalies. A detailed and careful neurological examination is central to discussion of the future prognosis (Shankaran 2002). Urgent investigation should be performed to exclude treatable non-asphyxia causes of encephalopathy including infection or metabolic disease or identify complications of asphyxia including bleeding.

In most cases conventional management for an infant with multi-system sequelae of perinatal asphyxia is largely supportive (Levene 1993; Vannucci and Perlman 1997; Whitelaw and Thoresen 2002). The infant's cardiorespiratory status should be monitored and signs of multiorgan system dysfunction sought and treated where appropriate. Respiratory insufficiency may occur due to central depression resulting from the encephalopathy but associated pulmonary complications are also common. These include pneumonia, meconium aspiration syndrome and pulmonary hypertension. Infants with encephalopathy respond variably; some will hyperventilate, some breath normally and some hypoventilate. Thus monitoring for hypoxia, acidosis and hypercarbia and appropriate intervention with respiratory support for hypoventilation due to depressed consciousness or apnoea is central to optimal outcome. Ventilation also has a role in airway protection if bulbar

function is compromised, if seizures are prolonged or if drugs are used that depress the respiratory drive.

Abnormal blood gas parameters are associated with an exacerbation of neurological injury. An increased plasma carbon dioxide has two potentially adverse effects on the brain. Firstly, increased concentrations may worsen intracellular acidosis. Secondly, hypercarbia is associated with vasodilatation and increased flow that may contribute to a risk of haemorrhage (Milley et al. 1984) (Gleason et al. 1989). Conversely hypocarbia results in vasoconstriction (Rosenberg 1992; Rosenberg et al. 1982) thus further compromising the brain that requires adequate oxygen and substrate supply to recover from injury. Clinically both severe hypocapnia and severe hyperoxia during the first two hours of life were associated with a significantly increased odds of an adverse outcome following perinatal asphyxia (Klinger et al. 2005). The aim is thus to achieve normocarbia and avoid hypoxia.

1.7.4.2 Specific Measures

Persistent pulmonary hypertension is associated with the failure of the circulation to adapt to ex-utero circulation. It is frequently associated with fetal, intrapartum or post-partum asphyxia but may also be associated with sepsis, parenchymal lung disease and congenital anomalies. It manifests clinically with hypoxia and cyanosis. Affected infants have raised pulmonary vascular resistance with right to left shunting on echocardiography. The severity of the outcome to some extent depends on the cause. One note of caution is that in infants treated for pulmonary hypertension the prolonged duration of

hyperventilation was linked with adverse neurdevelopmental outcome (Bifano and Pfannenstiel 1988).

Cardiac compromise should be looked for and cardiac echocardiography may identify valvular incompetence, hypovolaemia, poor myocardial contractility and low flow states. Inotropes may be used for hypotension or poor cardiac output although it should be recognised that blood pressure is a poor predictor of low cardiac output. Dopamine is the most commonly used agent if early hypotension is present or a low flow state is documented. It is effective in increasing cardiac performance and raising systolic BP (Cabal et al. 1980; DiSessa et al. 1981). However, it also increases systemic vascular resistance. Dobutamine is an alternative inotrope and a combination of these two agents is frequently used. Adrenaline may also be used in refractory cases but causes vasoconstriction, may worsen tissue perfusion and thus be associated with metabolic acidosis. It is important to avoid both hypertension and hypotension as asphyxia may be associated with pressure passive circulation and further perturbations may predispose to bleeding or exacerbation of the primary injury. Epidemiologically cardiac compromise is a marker for adverse outcome and the need for dopamine is associated with a doubling of rate of mortality compared with asphyxiated infants not requiring pressor support (Daga et al. 1998). Therapeutically, the use of dopamine to prevent morbidity and mortality in term infants with suspected perinatal asphyxia has been the subject of a Cochrane review (Hunt and Osborn 2002) but in the one trial identified (DiSessa et al. 1981) there was insufficient data to suggest any improvement in long-term outcome.

A persistent severe metabolic acidosis may be corrected with sodium bicarbonate, which is an effective buffer but requires adequate ventilation to remove carbon dioxide via the lungs. If bicarbonate is administered to an infant not adequately ventilated (either spontaneously or mechanically) it may cause hypercarbia and a paradoxical respiratory acidosis (Ostrea and Odell 1972) that may result in adverse effects on cerebral blood flow (Lou et al. 1978).

Renal impairment is common in asphyxiated full-term infants and manifests as oliguria and or electrolyte disturbance. Many infants also have inappropriate anti diuretic hormone (ADH) secretion and avoidance of fluid overload is important. Management is with strict fluid balance and regular measurement of electrolytes and creatinine, particularly looking for evidence of fluid overload or hyperkalaemia. Infants with anuria or oliguria should receive 40-60 mls / Kg /day until adequate urine output is documented. Theophylline, a nonspecific adenosine receptor antagonist, may have a role in protecting the kidney by inhibiting the vasocontrictive effect of adenosine following hypoxaemia and/or ischaemia. In a recent randomised trial in 51 asphyxiated infants intravenous theophylline (8 mg/Kg) given in the first 60 minutes of life decreased the rate of severe renal dysfunction. (Jenik et al. 2000)

If there is evidence of excessive bleeding or petechiae then a platelet count and coagulation profile should be performed and abnormalities corrected with platelet transfusions or replacing clotting factors with fresh frozen plasma.

The decision to feed will depend on a clinical assessment of the severity of asphyxia and associated system dysfunction. Feed intolerance is common and necrotising enterocolitis may complicate perinatal asphyxia; thus breast milk is preferred.

Hypoglycaemia should be recognised and corrected. The current treatment aim is to restore normal levels of glucose during both the resuscitation and recovery stage. In adults hyperglycaemia exacerbates brain injury following asphyxia. The effect is not so clear in immature animal models where hyperglycaemia superimposed on hypoxia-ischaemia has been reported to be both protective (Hattori and Wasterlain 1990) and harmful (Sheldon et al. 1992). Other studies suggest hyperglycaemia may be neuroprotective before an insult but detrimental if induced after an asphyxial episode (Vannucci and Yager 1992). Clearly this should be qualified by the differences between animals and humans plus the differences between the mature and immature brain. Hypoglycaemia effects may depend on the mechanism (Shalak and Perlman 2004). Insulin induced hypoglycaemia may have detrimental effects but if hypoglycaemia is induced by fasting it may be protective (Yager 2002). This is presumed to be secondary to increased ketone bodies. In a recent report initial hypoglycaemia was noted to be an important association with adverse outcome following fetal acidaemia, with an odds ratio of over 18 for adverse neurological outcome if hypoglycaemia occurred (Salhab et al. 2004).

1.7.4.2.1 Post Asphyxial Seizures

Post-asphyxial seizures usually start before 24 hours after birth (Williams et al. 1990) (Ekert et al. 1997; Gluckman et al. 2005). Persistent seizures are associated with an increased risk of poor outcome (McBride et al. 2000; Miller et al. 2004). In animal models repeated seizures may exacerbate cerebral injury from HI (Wirrell et al. 2001; Yager et al. 2002) and disturb growth and development (Holmes et al. 1998). It is generally accepted practice to treat overt clinical seizures but the evidence is lacking for treatment of electrical events without clinical correlate (Booth and Evans 2004; Levene 2002; Mizrahi 1989). Initially phenobarbitone is used but if this fails a variety of second and third line agents including, phenytoin, lignocaine and benzodiazepines may be added (Evans and Levene 1998; Rennie 1997; Rennie and Boylan 2003). Intravenous paraldehyde is described to be helpful for pervasive seizures (Armstrong and Battin 2001). It is important to note that although overt clinical fitting may cease the electrical seizures may persist (Hellstrom-Westas et al. 1985) suggesting a potential role for ongoing EEG monitoring. However, treatment of electrical seizures without clinical correlate has not been shown to change long term clinical outcome in this context.

1.7.4.2.2 Interventions aimed at cerebral oedema

The role of intervention with corticosteroids to reduce cerebral oedema remains unproven. Experimentally, pre-treatment with dexamethasone in a five day old rat model prior to asphyxia results in less severe cerebral effects (Adlard and De Souza 1974). However, there are no randomised trial data on corticosteroids given after insult in newborn infants. Case series demonstrate

a temporary fall in intracranial pressure but no improvement in outcome of neonates with encephalopathy. Svenningsen used a regimen described as "brain orientated intensive care in severe perinatal asphyxia" which included two mg of dexamethasone at six hourly intervals (Svenningsen et al. 1982). Although an improvement in outcome was described over time, the regimen included several other interventions including ventilation and it was not possible to conclude that there was a discrete protective effect of dexamethasone.

In addition to the lack of evidence supporting any benefit of corticosteroid use there are well recognised negative effects such as hypertension, hyperglycaemia and adverse effects on the immature nervous system. Thus the use of steroids in infants with neonatal encephalopathy of presumed HI origin is not justified.

1.7.4.2.3 Mannitol

Mannitol is an osmotic diuretic. These increase the osmotic pressure in the circulation so move fluid across the blood-brain barrier away from the brain. In an animal model mannitol has been demonstrated to decrease the water content of the brain if given immediately after the asphyxial event but not to have an effect on subsequent degree of brain injury (Mujsce et al. 1990). In humans an uncontrolled study of 1 g/Kg infusion performed in 225 asphyxiated infants demonstrated those who received the intervention before two hours of age had a better survival and better neurological outcome than those treated after two hours [Marchal C et al. 1974. Original in French cited

in (Whitelaw 2000)]. In a further non randomised study of 1g/kg infusion of mannitol a reduction in intracranial pressure was demonstrated but there was no benefit in long term outcome (Levene et al. 1987). One randomised study reported no benefit from treatment with mannitol in 25 severely asphyxiated infants (Adhikari et al. 1990).

Although cerebral oedema is a potential complication of asphyxia and mannitol may have a short term lowering effect on pressure there is a problem with no available non invasive method of measuring the intracranial pressure. Moreover, monitoring and aggressive treatment of increased intracranial pressure has been reported to have no significant benefit (Levene et al. 1987).

1.7.4.2.4 Anticonvulsants for Prevention of Mortality and Morbidity

Barbiturates were among the first drugs to be used in an effort to improve the outcome following perinatal hypoxic ischaemic injury. There are three randomised controlled trials of prophylactic barbiturates. Goldberg (Goldberg et al. 1986) studied 32 consecutive infants with low Apgar scores and early encephalopathy requiring ventilation. Half of the group were treated with 35 mg/Kg thiopentone divided into five doses starting between one and three hours of age. Ruth (Ruth et al. 1991) studied infants with Apgar scores below four at five minutes or requiring ongoing ventilation at 30 minutes and gave 30 mg/Kg phenobarbitoine load then 15mg/kg four hours later and this was followed by 5 mg/kg for five days. Hall (Hall et al. 1998) gave 40mg/kg based on criteria that included (1) an initial arterial pH less than or equal to 7.0 with a

base deficit 15 mEq/L or more, (2) Apgar score less than or equal to three at five minutes of age, or (3) failure to initiate spontaneous respiration by 10 minutes of age.

There are some methodological problems with these studies. None used a placebo and many infants in the non intervention arm also received anticonvulsant therapy, usually phenobarbitone, for seizures. In the Hall study the control group received a mean of 27 mg/kg although it was started later than in the intervention group. Finally even though follow-up occurred at the appropriate time and using the appropriate neurodevelopmental tests, it was not always satisfactorily documented. Loss to follow-up was 3% in the Goldberg study, 23% in the larger Hall study and no data on completeness of follow-up are given in the Ruth study.

Overall, the Hall study was the only one to demonstrate any reduction in the risk of severe developmental delay or death in the barbiturate treated arm but issues with the large loss to follow-up and lack of concealment undermine this. Furthermore meta-analysis of all 77 infants from the three studies does not show a significant effect on death, death or severe neurodevelopmental outcome or severe neurodevelopmental disability separately (Evans and Levene 2000). Trials published subsequent to this have been small and do not alter the metanalysis findings(Singh D 2004; Vargas-Origel A et al. 2004). On the negative side, infants treated with thiopentone have increased requirements for pressor support (Goldberg et al. 1986). Hence at present

there are no data to support the use of prophylactic barbiturates for neonatal encephalopathy.

1.7.4.2.5 Allopurinol

Allopurinol is an inhibitor of xanthine oxidase and a free radical scavenger. It has been shown to ameliorate experimental brain damage via the blocking of free radicals (Palmer et al. 1993; Palmer et al. 1990; Thordstein et al. 1993) but in the preterm infant a randomised controlled trial failed to show a reduction in the incidence of white matter injury (Russell and Cooke 1995). It is unclear whether there is a protective role in brain injury occurring at term. Van Bell (Van Bel et al. 1998) studied the effect of high dose allopurinol in a randomised trial of 22 infants. No toxic effects were observed with a dose of 40 mg/Kg and aEEG activity in the first eight hours decreased in the control group but remained stable in the treated group. In this small study and one other study (Benders 2006) no statistically significant protective effect was demonstrated even on combining death and developmental delay as a poor outcome (Veen et al. 1999). Further study is required before this therapy can be recommended in infants with NE.

1.7.4.2.6 Excitatory amino acid inhibitors

Competitive antagonists block the glutamate site directly but are polar substances so do not readily cross the blood / brain barrier. The use of non-competitive antagonists such as Ketamine, MK801 and dextromethorphan has been explored experimentally.

MK 801 has been demonstrated to have significant neuroprotective effects in newborn animals but it has a toxic profile so has not been studied in humans (Levene 1992). Dextromethorphan has been shown in the rabbit model to have a protective effect (Steinberg et al. 1993) and has been used in humans undergoing neurosurgery (Steinberg et al. 1996) but use in term infants with perinatal asphyxia has not been formally assessed in a trial.

The use of calcium channel blockers has also been reported. In the fetal sheep model flunarizine given in low dose 30 minutes before the insult has some efficacy in terms of reduction in overall cerebral damage and seizure frequency. However high drug levels were associated with hypotension and increased mortality in fetus (Gunn et al. 1994). In humans similar cardiovascular problems also occurred after Nicardopine was administered to four severely asphyxiated infants. Three developed severe hypotension and two died after cardiovascular collapse (Levene et al. 1990). Hence, extreme caution is required when using these drugs in infants with NE and no calcium blocker is currently being actively assessed in NE.

Magnesium sulphate is a voltage dependent non-competitive NMDA antagonist that blocks glutamate induced toxicity. In the animal model administration of magnesium sulphate was well tolerated but there was a lack of benefit either in terms of severity of delayed cerebral energy failure (Penrice et al. 1997a) or degree of tissue damage in the near-term fetal lamb (de Haan et al. 1997) and piglet asphyxia models (Greenwood et al. 2000).

In human infants magnesium sulphate (400 mg/kg) was associated with respiratory depression for three to six hours and had a high risk of causing hypotension. However, if 250 mg/kg MgSO4 was given then mean arterial blood pressure tone and heart rate were unaffected (Levene et al. 1995).

A pilot trial of magnesium sulphate 250mg/kg was suspended after problems with the preparation of magnesium sulphate resulted in the infants receiving an excessive dose. At the time two infants had been reported to have unexpected adverse effects out of a total of 56 infants recruited from nine countries. Another small trial of 250 mg/kg magnesium sulphate daily for three days has been performed in 33 infants selected by Apgar score of less than seven at five minutes and either failure of spontaneous respiration at 10 minutes or seizures within 24 hours of birth. Survival with normal results of cranial computed tomography, electroencephalography and establishment of oral feeding by 14 days of age, was significantly more frequent in the treated group than in the control group (12/17 vs 5/16, P = 0.04). No significant differences in blood pressure, heart rate or respiratory rate were observed between groups (Ichiba et al. 2002).

Electrophysiologically magnesium sulphate was found to have had no immediate effect on amplitude integrated EEG-patterns but on later recordings at 12 hours of age, in six of the eight treated infants the aEEG was more depressed compared with the three hour recording (Groenendaal et al. 2002). Overall magnesium sulphate is of no proven benefit and in higher doses has an unacceptable risk of hypotension. Further trials comparing magnesium

sulphate to placebo are required before it can be recommended for clinical use.

1.7.4.2.7 Naloxone

Although animal data (Ting and Pan 1994) suggests that naloxone, a specific opiate antagonist, may have a potential role in improving outcomes following perinatal asphyxia there is insufficient human data to support clinical use. A randomised controlled trial (Chernick et al. 1988) that compared the use of naloxone with placebo in newborn infants with an Apgar score of six or less at one minute after birth was identified in a recent Cochrane review (McGuire et al. 2004). However, there was insufficient data available to evaluate the safety and effectiveness of the routine use of naloxone for newborn infants of greater than 34 weeks gestation with suspected perinatal asphyxia.

1.7.4.2.8 Novel treatments needing further evaluation

Several other potential therapies exist that have not yet been studied in a clinical trial in human infants. Lazaroids are 21-amino steroids that have neither glucocorticoid nor mineralocorticoid actions. They may assist with reducing free radical damage, particularly lipid peroxidation, but may also have a role in maintaining the blood brain barrier following insult and are considered non-toxic (Kavanagh and Kam 2001). Other potential compounds include long-acting analogues of adenosine which, if given prior to the insult, have been reported to reduce neuronal loss, in some (Dragunow and Faull 1988) but not all studies (Araki et al. 1989). Finally, there has been considerable interest in exogenous IGF-I which has a potent anti-apoptotic effect (Gluckman et al. 1993; Gluckman et al. 1998; Guan et al. 2004). In

animal models (adult rat, and the fetal lamb) a single intraventricular dose was associated with reduced secondary neuronal death (Guan et al. 2003; Guan et al. 2000; Guan et al. 2001). IGF-I is cleaved at its N- terminal to produce a tripeptide glycine-prolint-glutamate (GPE) and des(I-3)-IGF-I. The latter is protective at high dose which confirms that protection is mediated via neuronal IGF-I receptor activation. GPE is also protective although the exact action is unclear.

It is also worth considering mechanisms that enable animals to survive hypoxia. A logical extension to the concept of suppressed neurological activity secondary to the release of inhibitory neurotransmitters is a more generalised regulated metabolic depression. Hibernation is an adaptive response, seen in some animals, to an adverse environment including low oxygen availability. Indeed this may allow some species to survive major oxygen deprivation for extended periods of time (Boutilier 2001; Carey et al. 2003; Ramirez et al. 2007). During deep topor most animals do not maintain body temperature, so cool to around 2-10 degrees C and their basal metabolic rate drops to 2-4% of normal (Carey et al. 2003). This is accompanied by major physiological changes that may include a decrease in heart rate from 200-300 beats per minute to 3-5 per minute and decrease in respiratory rate from 100 to 3-5 breaths per minute (Carey et al. 2003). In fact hibernation is a complex hypometabolic state that in addition to reducing oxygen requirement prevents cellular injury and maintains vital functions (Ramirez et al. 2007). The body temperature is not constant throughout the whole hibernation period and there are periods of increased temperature and arousal. These arousals are quite complex and during these episodes most functions briefly return to normal

levels. For some animals this may involve activities such as eating food (Carey et al. 2003). The process of controlling hibernation is quite complex and must occur in a co-ordinated manner as some cells remain metabolically active whilst others are inactive or rely on anaerobic metabolism (Ramirez et al. 2007).

In addition to hibernation, there are adaptive features found in animals such as increased stores of glycogen or high levels of myglobin and short term manipulation of temperature (Ramirez et al. 2007). Animals such as seals and ducks can reduce their brain temperature by approximately 3 degrees during diving (Ramirez et al. 2007). This reduces the metabolic rate and oxygen consumption by about 20%. It should be noted that both hibernation and these short term adaptive changes rely on a response that is quite diverse in its cellular and sub-cellular components but that occurs in a coordinated manner. Thus the ongoing challenge is in trying to harness such knowledge from animal models to protect brain function in the term newborn infant.

1.7.4.2.9 Candidate therapies

This section has hitherto reviewed mechanisms and agents with the potential to be neuroprotective following perinatal asphyxia. It is notable that there were few therapies that had been studied using randomised controlled trial (RCT) methodology. Table 7 summarises the available data from RCTs that have been discussed in this section (Chernick et al. 1988; Goldberg et al. 1986; Hall et al. 1998; Ichiba et al. 2002; Ruth et al. 1991; Van Bel et al. 1998). It is evident that, at the time of embarking on the hypothermia studies, there were no other candidate therapies that were ready for clinical trials.

Table 7. Summary of randomised controlled studies examining the effect of an intervention on outcome following perinatal asphyxia.

Study (references given in the text)	Total number infants in study	Drug	Adverse Outcome Treatment Group	Adverse Outcome Control Group	Effect on long term neurodevelopmental outcome (RR and 95 % CL)
Goldberg	32	35 mg/Kg thiopentone	10/17	10/15	RR = 0.98 (.55, 1.74)
Ruth	38	Phenobarbitone 30 mg/kg	8/21	5/17	RR = 1.3(0.52, 3.24)
Hall	40	Phenobarbitone 40 mg/kg	3/20	10/20	RR = 0.3 (0.1, 0.93)
Van Bel	22	Allopurinol 40 mg/kg	11	11	No benefit but "under powered"
Ichiba	33	Magnesium sulphate 250 mg/kg	11/16	5/17	14 day outcome only reported
Chernick	85	Naloxone ~0.4 mg/kg	44 received naloxone	41 received saline	No benefit in resuscitation plus no data on follow-up
Chernick	108	Naloxone ~0.4 mg/kg	54 received naloxone	54 received saline	No benefit in resuscitation plus no data on follow-up

1.7.5 Hypothermia

Prior to undergoing clinical trials of efficacy in human infants any potential neuroprotective intervention, such as hypothermia, must satisfy some basic criteria in the animal model. Firstly, the potential for a protective effect needs to be clearly demonstrated. Secondly, the intervention must be well tolerated and have a reasonable safety profile. Thirdly, there needs to be an established and workable treatment regimen. For hypothermia this would need to include a clear idea of the optimal time of onset, depth and duration of cooling. The following section will review the available literature with regard to these three criteria.

1.7.5.1 Cerebral protective effect of hypothermia

Before reviewing the experimental literature regarding hypothermia it is helpful to clarify the nomenclature regarding degree of hypothermia. In clinical practice, the temperature range 0-25 °C is recognised as deep or profound hypothermia. Similarly the range below 0 to minus eight °C is recognised as super cooling or freezing. However, for the purposes of neuroprotection in the newborn a lesser degree of cooling is more appropriate. Unfortunately there is no consensus on the boundary between mild and moderate hypothermia. Although it is common to consider anything less than three degrees of cooling to be mild hypothermia there is no universally accepted definition and the literature may vary in terminology used. Moreover, when reviewing animal experimental data it is important to be familiar with the normal core temperature of the model. Therefore, in order to be as clear as possible the basal and induced temperatures will be specified in the following review of experimental and clinical studies.

1.7.5.2 Hypothermia prior to insult or at same time

The protective effect of hypothermia instituted either before or at the time of HI insult, particularly focal HI insult analogous to stroke, is well recognised. Detailed review of the experimental data obtained from adult animal paradigms is beyond the scope of this review but has been well appraised by Miyazawa (Miyazawa et al. 2003). In the clinical context, deep hypothermia (< 28 °C) has been used for half a century during open-heart and some neurosurgical procedures, where it is recognised to have a protective effect and is accepted as standard clinical care (Jonas 1998; Jonas 2002). However, the use of hypothermia as a neuroprotective agent after a HI insult,

particularly in the newborn is both a more contemporary and to some extent more contentious issue.

1.7.6 Effect of hyperthermia on HI injury

It is evident from animal data that temperature appreciably modulates the damage process following a HI insult (Yager et al. 2004). Preventing hyperthermia from occurring in rat pups during induced seizures is associated with a significant reduction in brain damage compared to controls (Yager et al. 2004). An increase in temperature of just two °C significantly increases brain damage in rodent models of HI injury (Minamisawa et al. 1990). Overall, hyperthermia produces a similar effect to an increased duration of insult but may also induce infarction in the neocortex and basal ganglia. The possible mechanisms that have been advanced to explain this increase in damage include; increased release of excitotoxic neurotransmitters, specifically glutamate and glycine which are reported to be increased and remain elevated for longer (Baena et al. 1997; Takagi et al. 1994) and an increase in permeability of the blood-brain barrier (Dietrich et al. 1990).

In parallel with this experimental evidence there is a small amount of important human epidemiological data. In adults with stroke the severity of the lesion and the clinical outcome has been correlated with temperature (Reith et al. 1996). Similarly in children with a birth weight above 2500 grams the rate of CP is increased nine fold if maternal temperature was above 38 °C during labour (Grether and Nelson 1997).

1.7.7 Effect of hypothermia on HI injury

The basis of a protective role for hypothermia started with a series of animal experiments in the 1940s. It was observed in several immature animal species that hypotherrnia increased the anoxic tolerance as measured by survival time when breathing 95% nitrogen and 5% carbon dioxide. In the kitten, rabbit and dog pup the hypothermic animals recovered from periods more than twice as long as that which killed the normothermic controls (Miller et al. 1964). In the guinea pig, which is relatively more mature at birth, the increase was less impressive, from five minutes to only nine minutes (Miller and Miller 1954; Miller et al. 1964). This data was used as the foundation to the early clinical studies that are described later.

A more contemporary interest in hypothermia as a neuroprotective strategy restarted in the 1980s. An important paper by Busto et al (Busto et al. 1987) reported that small differences in brain temperature during HI insult determined the extent of ischaemic neuronal injury. Although this study was performed in an adult animal model it demonstrated the potential for hypothermia to provide neuroprotection. This provoked considerable interest in neonatology where protection of the immature brain following term perinatal asphyxia had been hitherto elusive.

There is now a body of data supporting a protective effect for hypothermia in newborn animal models of global asphyxia, although the initial results were not consistent and depended on the experimental paradigm used. This fact illustrates the complex interrelationship that exists between depth of cooling,

duration of cooling, time of cooling commencement and subsequent outcome following a standardised insult. Studies performed in a seven day old rat model showed that cooling from 38 to 32 °C and maintaining this for three hours provided a degree of protection to the brain when assessed by histology at one week (Thoresen et al. 1996a). Another study, using the same model but cooled by only four degrees failed to demonstrate protection when assessed at four weeks (Trescher et al. 1997). These results suggested that mild to moderate damage might be delayed but not prevented by hypothermia. However, in a third study it was shown that if the rats were cooled by five degrees for a period of six hours protection was then evident (Bona et al. 1998). This study also provided some long term outcome data. Although sensorimotor function was not significantly improved by hypothermia if all animals were included, in female pups the total functional score was higher in the hypothermia group and corresponded to a marked reduction in the neuropathology score in this subgroup. Thus, these data suggested for the first time that hypothermia had potential to improve behaviour outcome.

The rat clearly has major differences to human infants, particularly with respect to maturity at birth and brain size. Therefore it is important to examine results from larger animal models. In the piglet cooling to a rectal temperature of 35.4 +/- 0.5 compared with the normal of 38.3 +/- 0.4 °C during HI insult provided protection both in terms of less severe stages of impairment and less damage on histology (Laptook et al. 1997). Encouragingly, protection was also provided when modest hypothermia for one hour was started immediately after HI insult ceased. In this experiment piglets were studied with their rectal

temperature maintained at either 38.3 +/- 0.3 °C or 35.8 +/- 0.5 °C during the first hour after 15 minutes of brain ischaemia. Again, hypothermia was associated with less severe stages of encephalopathy and less histological evidence of neuronal injury in temporal and occipital cortex and in the basal ganglia compared with the normothermia group. However, if a relatively short period of one hour of modest hypothermia was delayed until 30 minutes post-ischaemia it did not provide neuroprotection. (Laptook et al. 1999). Significant neuroprotective effects on moderate, but not severe, hypoxic-ischaemic insults levels were also reported by other authors using three hours at 35 °C (Haaland et al. 1997). A more prolonged cooling period, 12 hours at a rectal temperature of 35 °C, in the piglet model demonstrated cerebral lactate as measured by proton (1H) MRS was reduced.(Amess et al. 1997). A significant protective effect on energy failure as measured by [PCr]/[Pi] or [NTP]/[EPP] on MRS over the first three days was also observed (Thoresen et al. 1995).

Ideally to provide adequate neuroprotection with minimal risk of systemic adverse effects only the brain would be cooled. Although this has been demonstrated in experimental preparation using cardiac bypass procedures (Wass et al. 1998a; Wass et al. 1998b). This is clearly impractical for routine practice and a more practical approach is required. A pragmatic option would be selective head cooling utilising a cooling cap on the scalp whilst the body is warmed, to prevent excessive systemic hypothermia. Potential ways of warming the body include placental heat exchange in the fetal sheep (Gunn et al. 1997) or an overhead heater in infants (Gunn et al. 1998b; Simbruner et al. 1999).

In Auckland an elegant series of studies has been performed using selective head cooling (Gunn et al. 1999a; Gunn et al. 1997; Gunn et al. 1998b; Westgate et al. 1999). These studies established the feasibility and efficacy of the technique. In addition, the extradural temperature produced by the technique was measured invasively and the studies critically examined time of cooling onset so demonstrating a relationship between extent of neurological damage and time of initiation of cooling (Gunn et al. 1997).

In the first study (Gunn et al. 1997), anaesthetised near term fetal sheep were subject to 30 minutes of cerebral ischaemia induced by bilateral carotid artery occlusion. At one and a half hours they were randomised to either cooling or sham cooling for 72 hours. Intrauterine cooling was induced by circulating cold water through a coil around the fetal head. A fall in extradural temperature of 5 to 10 °C, and a fall in oesophageal temperature of 1.5 to 3 °C resulted. In the hypothermia group there was reduced secondary cortical cytotoxic oedema and at five days there was greater residual EEG activity. On histology there was a dramatic reduction in the extent of cortical infarction and neuronal loss in all regions. In the second experiment (Gunn et al. 1998b) the onset of head cooling was delayed until shortly before the onset of postasphyxial seizures. Unanaesthetised near-term fetal sheep in utero were subjected to 30 minutes of cerebral ischaemia. At five and a half hours they were randomised to either cooling, as described previously, or sham cooling for 72 hours. The hypothermia group had better recovery of EEG activity and reduced neuronal loss on histology compared to the controls but the degree of protection was

less than if cooling was commenced at 90 minutes. In the third experiment (Gunn et al. 1999a), unanaesthetised near-term fetal sheep received 30 minutes of cerebral ischaemia. Then eight and a half hours later, they received either cooling or sham cooling until 72 hours after the insult. Although cerebral cooling attenuated the rise in impedance in the short term there was no significant difference in EEG activity after five days recovery or in parasagittal cortical neuronal loss. The conclusion was that delayed prolonged head cooling begun after the onset of postischaemic seizures was not neuroprotective.

In summary hypothermia, usually only a reduction in temperature of 2-3 °C, has in several studies using perinatal animal models, been demonstrated to improve a variety of outcomes following HI insult. The improved outcomes include short term or proxy endpoints such as energy status but also performance testing and histological evidence of neuronal loss. In a series of experiments in the fetal lamb, a significant reduction in histological neuronal loss was associated with extradural temperatures below 35 °C (Gunn et al. 1997) and it was suggested that a cooling regimen, continued for 72 hours, needed to be commenced before six hours in order to be effective. These fetal lamb studies were used as a basis for the subsequent human infant studies at National Women's Hospital in Auckland.

Since commencing the human studies further important animal data has been published. Clearly the fetal sheep model differs from the clinical use in human infants in that it is an in utero model. Hence two studies in the piglet model are

of consequence. The first used the piglet HI model to confirm the feasibility of utilising the head-cooling cap to cool the brain more than the body for a 24-hour period while keeping the core temperature mildly hypothermic (Tooley et al. 2002). The second demonstrated the feasibility of achieving a protective effect using head cooling in combination with a mild degree of systemic cooling for a period of 24 hours immediately after the insult in the piglet model of hypoxic-ischaemic encephalopathy (Tooley et al. 2003). Finally the presence of stress and the use of sedation may also be an important consideration in the effectiveness of selective cooling. When the cooling process was conducted in unsedated piglets for 24 hours the distribution and degree of injury within the brain was similar in the cooled and non cooled groups (Thoresen et al. 2001). It was noted that the cooled piglets shivered and were more active than the controls. They also had plasma cortisol levels that were significantly higher during the cooling period compared with basal levels. It was speculated that the stress of shivering and feeling cold interfered with the previously shown neuroprotective effect of cooling.

1.7.8 Major side effects of hypothermia

It is important to note that hypothermia does have a significant profile of potential adverse effects. These include metabolic, cardiovascular, pulmonary, coagulation and immunologic complications (Gunn and Gunn 1998; Schubert 1995). These adverse effects are mostly proportional to the degree of cooling and appear to become clinically significant at temperatures below 34 ºC but are even more likely to occur below 32 to 30ºC. To some extent this is supported by the experimental data in the adult dog, where

cooling to 34 °C after cardiac arrest was more protective than 28-32 °C hypothermia, while deep hypothermia (15-25 °C) was deleterious, particularly with regard to cardiovascular complications (Weinrauch et al. 1992). This may suggest the importance clinically of minimising the degree of systemic hypothermia.

Pulmonary effects include shift of the oxygen dissociation curve to the left. Furthermore there may be an increased tendency to pulmonary vasoconstriction (Benumof and Wahrenbrock 1977; Stern and Braun 1970). Clearly in the clinical situation it may be difficult to be certain of the exact contribution of hypothermia as these issues may also be associated with perinatal asphyxia. However caution would be necessary if hypothermia exacerbated such pulmonary compromise that occurred secondary to perinatal asphyxia, including pulmonary hypertension. Cardiovascularly there is a decreased cardiac output. The relationship between cardiac output and organ perfusion is particularly complex given the decreases in metabolic activity that occur with decreasing temperature.

Other important factors are the potential for coagulation problems due to platelet and clotting factor abnormalities (Chadd and Gray 1972), although recent evidence suggests that effects of selective cerebral cooling on coagulation are mild(Ferguson et al. 2005). It is recognised that clotting activity may be independent of factor levels and may reflect the temperature effect on enzyme activity (Reed et al. 1990; Rohrer and Natale 1992). The production of thromboxane B2 by platelets is temperature dependent and

cooling produces a reversible platelet dysfunction. Thus a negative relationship is seen between the bleeding time and temperature (Valeri et al. 1987). However, three important points should be noted. First, as temperature decreases there is an increase in viscosity that could cause adverse effects on the microcirculation if not accompanied by changes in coagulation. Second, an abnormal coagulation may occur as a complication of perinatal asphyxia (Chadd et al. 1971). Third, the contribution of hypothermia to the haemorrhagic diathesis may be easily overlooked since coagulation studies are performed at 37 ^{o}C not the infants' actual temperature.

Despite what is known about the potential adverse side effects, mild to moderate hypothermia appears to be well tolerated in a variety of animal experimental models. (Gunn et al. 1997; Haaland et al. 1997; Thoresen et al. 1995) suggesting that it may also be tolerated in human infants.

1.7.9 Mechanism of neuroprotection

The protective mechanisms of hypothermia are complex. From basic science, van't Hoff's rule states that the velocity of chemical reactions is related to a rise in temperature. It is also well recognised that there is a linear decrease in metabolic activity with decrease in temperature (Bigelow WG 1950; Westin et al. 1962). This equates to approximately six % per degree (Erecinska M 2003). Although this probably has some part to play it is not the only factor and other actions are also important. In this way cooling has some advantage over drug therapies that target just one step in the cascade of neurological injury. Specific actions that are likely protective include decrease in the release of and increase in the reuptake of glutamate, an excitatory amino acid

(Busto et al. 1989; Nakashima 1996; Thoresen et al. 1997); lowering production of toxic nitric oxide and free radicals (Thoresen et al. 1997); decrease in seizure frequency and duration (Thoresen M 2003; Tooley et al. 2003); less disruption of the blood brain barrier (Clifton GL 2004); less severe cerebral oedema (Dempsey RJ 1987) and decreased rate of cell commitment to apoptosis (Edwards et al. 1995).

1.7.10 Clinical use of cooling

The use of cooling, mostly in the form of topical cooling as a local treatment for minor lesions such as sprains or as a local anaesthetic has been reported for many centuries (Swan 1973). However, this is quite different to use as a neuroprotective therapy after an HI insult.

1.7.10.1 Use in the newborn

From the late 1950s to early 1970s there were a number of reports of the use of hypothermia to treat newborn infants presenting with asphyxia. Mostly these infants were described as "unresponsive" and were immersed in cold water maintained between 8 and 14 °C. A special bath was used so that the whole body was immersed with the exception of the nose and mouth. Cooling was continued until spontaneous breathing commenced. Thereafter the infant was removed from the bath and allowed to spontaneously rewarm. It was possible to combine such treatment with other interventions such as transfusion of oxygenated blood and, as the technique evolved, with positive pressure ventilation. However this was not performed in all cases.

In total, over 150 infants from a number of centres were reported to be cooled in this manner (Miller 1969). In the 89 infants who weighed over 2500 grams at birth and had a five minute Apgar score of 0 to 3, the mortality was 7.9 % (7 infants) compared to the approximately 45% in a similar group of infants treated conventionally (Drage and Berendes 1966b). The typical time in the bath was around eight minutes (range 3-28 minutes) and average time to warm up was eight hours (3-12 hrs) (Laptook and Corbett 2002). The lowest infant temperature reported as being produced by this regimen was between 20 and 32 ^{o}C. The longest period before regular breathing was established was over three hours. In another case apnoea lasted for 79 minutes; although the infant did breath he was reported to die at 30 hours from respiratory distress. Although an encouraging outcome was reported, this data was limited by some methodological issues particularly the fact that no control group was used and follow-up data was only reported for a small sub-group (Westin et al. 1962).

Four important papers published in 1950s and 1960s suggested a worse outcome for preterm infants nursed in a cooler compared with a warmer environment (Buetow and Klein 1964; Day et al. 1964; Jolly et al. 1962; Silverman et al. 1958). Observational data also exist to suggest that term infants presenting with hypothermia had a worse outcome than those presenting with a temperature within the normal range (Gunn and Outerbridge 1978). However, it should be stressed that many infants would have co-morbidity such as growth restriction or other conditions leading to both excessive heat loss and adverse outcome. Moreover no similar prospective

trial data were obtained in term infants to demonstrate a worse outcome from hypothermia as an induced therapy. Occasional cooling studies in term infants continued to be published until the early 1970s then stopped. Several authors have suggested that practice of hypothermia stopped at that time due to the influence of the published data on preterm infants. This may be true in terms of influencing the majority of neonatal practice but it is worth noting that JA Miller, an active researcher and proponent of research, retired in 1970. He did continue to publish and as late as 1973 was extolling the safety of hypothermia in term infants (Cordey et al. 1973). Further retrospective studies (Clardy et al. 1985) demonstrated an increased infection rate following childhood hypothermia in the 1980s and research efforts moved away from hypothermia to methods of resuscitation and methods of predicting adverse outcome.

1.7.10.2 Cooling in Adults

Cardiac arrest in adults is a situation similar in some ways to perinatal asphyxia but differentiated by the relative intolerance to asphyxia of the adult brain. In this situation there is a clear mandate to use cooling (Bernard 2004) supported by two major randomised controlled trials (Bernard et al. 2002; Hypothermia after Cardiac Arrest Study 2002), a Cochrane review (Holzer et al. 2005), and a recommendation from the International Liaison committee on Resuscitation (Nolan et al. 2003). However, despite this wealth of evidence hypothermia has not yet become standard clinical practice in this situation (Abella et al. 2005). In other forms of adult brain injury there is not a firm evidence base to support the use of hypothermia. Three Cochrane systematic reviews examine the use of systemic cooling on outcome in human adults with

head injury (Gadkary et al. 2002); acute stroke (Correia et al. 2000); and coronary artery bypass surgery (Rees et al. 2001). There is currently no evidence from randomised controlled trials and these systematic reviews to support hypothermia for treatment of either head injury or acute stroke, or for prevention of neurological injury after coronary artery bypass surgery. However, it must be recognised that these conditions may differ quite considerably from perinatal HI injury. It should also be noted that this literature suggests that hypothermia may be harmful by increasing the risk of sepsis (Gadkary et al. 2002) and mortality (Rees et al. 2001). The safety and efficacy of hypothermia in the term newborn infant cannot be inferred from adult studies thus a full and complete assessment is required.

In the term infant with perinatal asphyxia the first safety study of selective head cooling was reported in 1998 (Gunn et al. 1998a). This study demonstrated for the first time in a randomised, controlled study that selective cerebral cooling with mild systemic hypothermia, in contrast to historical concern, appeared to be a safe and convenient method of reducing cerebral temperature. Although this work was the first to demonstrate the apparent safety of the technique it was part of a cautious stepwise approach evaluating the effects of lower temperature at one temperature range before moving to a lower range. From the experimental literature (Gunn 2000), a lower rectal temperature of approximately 34 to 35ºC would be needed to protect the deeper brain structures. I became involved with the hypothermia project at this time and this thesis covers a body of further clinical research, as outlined below, that followed this initial safety study.

1.8 Summary and structure of thesis

This literature review aims to highlight several important facts, identify some deficits that exist in the current knowledge and pose potential research questions. The main findings of the review are:

1) NE remains a significant problem in New Zealand both in number of cases per year and resultant morbidity and mortality. Although not all cases of CP result from NE, any available intervention that altered disease pattern would help an important minority of infants, possibly around 15% of NE cases.

2) The majority of therapies with potential neuroprotective properties are either not yet ready for clinical trials or have been investigated and found to be wanting, either because they are ineffective or they have adverse side effects.

3) Hypothermia has a potential protective effect that is well demonstrated in a variety of animal models. Thus is appropriate for clinical trials and, prior to commencing the studies reported in this thesis, some safety data was available. However, prior to the data included in this thesis, there was still a need for short term safety at temperatures likely to provide neuroprotection and longer term outcome data from cooled infants.

4) Although there was correlation between imaging appearances on MRI or CT scan and neurodevelopmental outcome following perinatal asphyxia there was no data available on the interpretation of imaging following cooling and how this may aid prediction of final neurodevelopmental outcome.

5) There is no perfect method of assessment and selection of infants for therapeutic trials of neuroprotection but electrophysiological techniques, particularly the aEEG appears to be the best way of augmenting clinical assessment. Importantly aEEG has been assessed in the first six hours after birth and has been correlated with subsequent outcome but, as yet, there is no data available on what happens to infants not selected for trial entry.

As stated above the basis of this thesis will be a number of studies undertaken at National Women's Hospital in term infants with perinatal hypoxia ischaemia. The overall aim of this work was to assess selective head cooling as a potential method of improving outcome for infants with perinatal asphyxia. Although a single centre could not recruit enough babies to have sufficient power to judge effect on outcome, this work would be required as a pilot to a multicentre trial that could do this. The subsequent chapters will describe this process in more detail including review of short term safety and neonatal outcome data; the 18 month neurodevelopmental outcome; imaging data and correlation with clinical measures of outcome after cooling; outcome of infants not selected for cooling; some interesting clinical vignettes around cooling experience and a review of infants who had NE but did not qualify for the randomised controlled CoolCap trial (Gluckman et al. 2005).

2 Methods

2.1 Recruitment

During the period, January 1996 until September 2002, term infants admitted to the neonatal unit at National Women's Hospital and considered to have experienced perinatal asphyxia were prospectively evaluated for signs of neonatal encephalopathy (NE). The families of eligible infants were approached about participation in a series of selective cooling studies. The initial studies were safety studies (Battin et al. 2003; Gunn et al. 1998a) but later infants were entered into a multicentre randomised trial of selective cerebral hypothermia (Gluckman et al. 2005). The initial study group was recruited by Professor Tania Gunn but I performed the follow-up studies on these and subsequent infants (Battin et al. 2001), recruited infants to the later safety study (Battin et al. 2003), and was the site investigator for National Women's Hospital in the randomised controlled CoolCap trial (Gluckman et al. 2005).

The North Health Regional Ethics Committee issued ethical approval to perform selective head cooling in term infants with hypoxic ischaemic encephalopathy. Written parental consent to study the individual infants was obtained in each case.

Study infants were either inborn at National Women's Hospital, referred from local Level One hospitals or referred from the community following home birth. All infants were evaluated before five and a half hours after birth as monitoring and/or cooling was required to have started before six hours; therefore infants who presented to National Women's Hospital neonatal unit after this time were excluded. Infants with obvious major congenital abnormalities or metabolic diseases were also excluded. Details of the individual studies, including the specific inclusion criteria and dates of study, are given in the subsequent chapters and this section will cover only generic methods common to several chapters including: technique of cooling, monitoring, follow-up, imaging and statistical analysis.

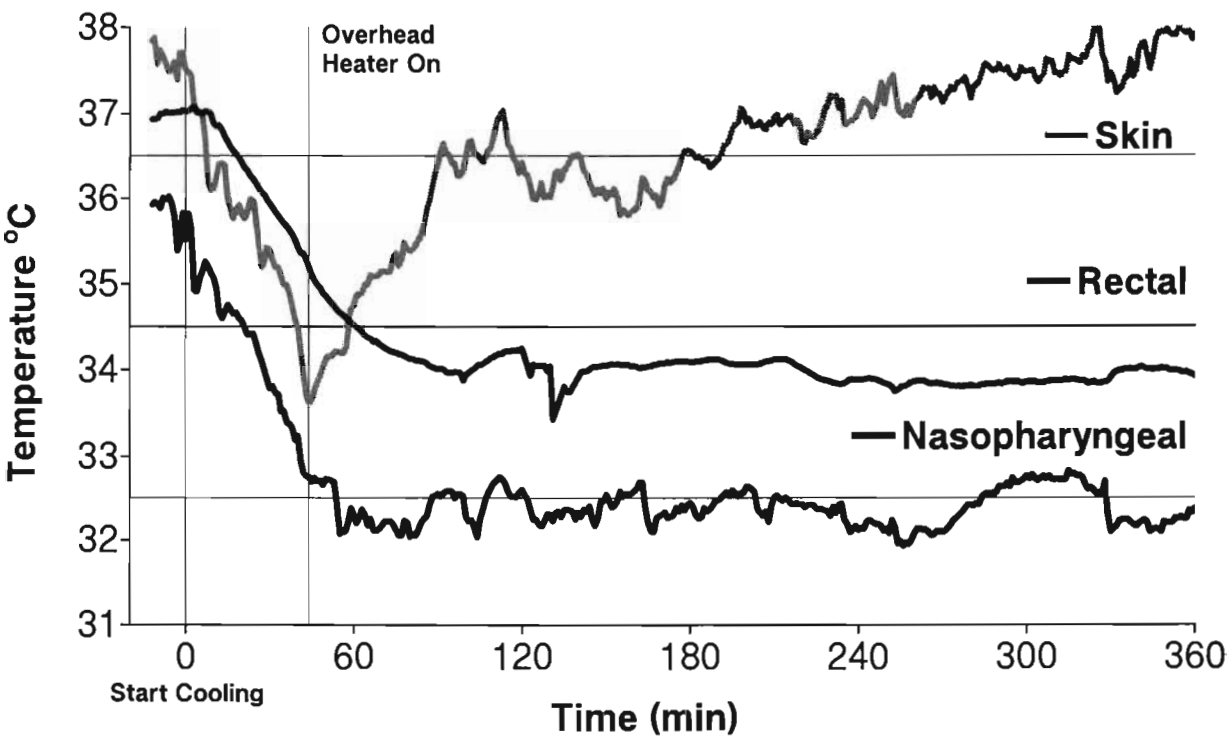
2.2 Cooling technique

In the hypothermia group of infants, selective head cooling was accomplished by circulating cooled water through a cap placed on the infant's head. Overhead heaters, servo-controlled to the abdominal skin temperature, were adjusted to maintain the allocated rectal temperature. The initial 17 babies were cooled using a cap made of Silclear tubing (Degania Silicone Ltd, Degania Bet, Israel) coiled to fit around the scalp of the infant and held in place by a baby bonnet. Subsequently, a commercial device (Olympic Medical, Seattle, WA, USA) was used. The cooling devices both worked on the same principle of a small thermostatically controlled cooling unit and a pump that circulated the water through the coil. The Olympic Medical device differed in the extra provision of monitoring and alarms as an integral part of the equipment. The temperature of the water circulated could also be adjusted

within a specified range. Blinding or masking was not considered possible due to concern that a sham cap could produce a warming effect with a potentially deleterious rise in cerebral temperature.

2.2.1 Initiation of Cooling

Figure 2. Initiation and first 6 hours of cooling in a spontaneously breathing infant



The initiation of cooling was performed in a standardised prescribed manner. The infant was first placed on an open cot with a radiant heater and any other sources of heat, such as under blanket warmer, were removed. The infant was exposed except for a nappy and placed flat on the mattress, which was in the horizontal position (see also Appendix 1, Nursing care of cooled infants). The overhead heater was then switched off then the cooling device switched on and infant temperature monitored. When the rectal temperature dropped

below 35 °C the overhead heater was switched on to warm the exposed skin. Whilst the infant nasopharyngeal and rectal temperatures plateaued the skin temperature rose steadily until between 37 and 38 °C. The cooling process typically took about 40 minutes before the core temperature was below 35 °C. Thereafter there was a 1.5 to 2 °C gradient between the rectal and nasopharyngeal temperatures unless the infants were receiving warmed humidified gases via an end tracheal tube or CPAP circuit as part of their respiratory support.

The initial water temperature was set at 10 °C. The choice of this temperature was based on the experience gained in the safety study (Gunn et al. 1998a). Appropriate adjustments from 10 °C (between 8 and 12 °C) were made as required to maintain the allocated rectal temperature.

In the cooled infants the rectal temperature was maintained within the prescribed range for 72 hours. Thereafter the cooled infants were slowly rearmed by stopping the circulation of cold water then removing the cap. The temperature was allowed to rise at 0.5 °C per hour until within the normal range. Rewarming was achieved without significant overshoot in temperature. The cooling was discontinued earlier at 48 hours only if the infant was judged by the attending clinician to have significantly recovered on a neurological examination at this time.

2.2.2 Monitoring

The rectal, fontanel, and nasopharyngeal temperatures were continuously monitored with thermostats (IncuTemp1, Mallinckrodt Medical, St Louis, MO or YSI Precision 440, Yellow Springs Instrument Co, Yellow Springs OH). In addition, standard clinical monitoring appropriate to neonatal intensive care was performed. All infants had continuous electrocardiograph monitoring and pulse oximetry but umbilical arterial catheters for blood gas and blood pressure were only inserted if clinically indicated. If indwelling catheters were not used to measure blood pressure invasively then standard Doppler oscillometric methods (Dinamap, Critikon, Tampa, Florida, USA) were utilised.

Temperature monitoring was performed using a multi-channel recorder and computer separate to the cooling unit in the original 17 babies. In the subsequent infants temperature was monitored using the Olympic Medical device. Temperature data was displayed continuously with one display set to always show the core rectal temperature whilst the other could show any one of the following temperatures: water in, water out, cooling block, scalp, skin or nasopharyngeal temperature. In addition to the continuous display each of these temperatures was recorded and stored in the machine memory as a simple text file. This data was then imported in to a standard spreadsheet for analysis, using the comma delimited format.

2.3 Clinical care

Early cranial ultrasound was performed in all infants to exclude major intracranial bleeding or malformation. Follow-up cerebral computer

tomographic scan (CT) and electroencephalographic (EEG) studies were obtained five to seven days after delivery when clinically possible. Blood and if indicated surface cultures were performed and the infants were treated with antibiotics until the culture results were known. Seizures were diagnosed clinically and managed using a loading dose of 20 mg/kg of phenobarbitone and if needed further phenobarbitone, 20 mg/kg of phenytoin and/or paraldehyde. If the heart rate fell to less 80 bpm during the cooling run an ECG was performed to allow study of QT interval. The study infants were all kept nil by mouth during cooling but the rate of feeding of cooled infants after rewarming and the feeding of control infants was at the discretion of the attending clinician who directed all other aspects of clinical care.

2.4 Follow-up

For the infants recruited to the pilot cooling studies, clinical follow-up after discharge was performed in the outpatient clinic until the child was 18 months old. Developmental progress was serially assessed by history and structured neurological examination was performed to elicit abnormalities of tone, tendon jerks, gait and fine motor skills. Head circumference and weight gain were also assessed serially. Formal audiology assessments were routinely performed (using the standard clinical service) in those infants with an encephalopathy that was judged moderate or severe.

Formal developmental assessment using the Bayley Scales of Infant Development Second Edition (Bayley 1993) at 18 months of age was

performed by a psychologist (Dr Anne Dezoete), who was blinded to treatment group allocation of the infants.

2.5 Data and statistical methods

Data are presented as mean ± standard deviation if normally distributed, or as median (range) as appropriate. Incidences were compared by Fisher's exact test or by Chi squared. Study groups were compared by two-way Mann Whitney U test or Student's t-test as appropriate. Analyses were performed using Statview version 5.0.1 (SAS Institute Inc, Cary, NC).

3 Safety Data In Pilot Studies Of Selective Cerebral Hypothermia

3.1 Background

Selective cerebral cooling utilises a cooling cap applied to the scalp whilst continuing to warm the body by some method; an overhead heater was used in clinical studies. The intention of this approach was to provide optimal cerebral cooling but with a lesser degree of systemic cooling. The technique used in human infants was similar to that which in the fetal sheep model resulted in dramatic cerebral protection if cooling was initiated 1.5 hours after insult (Gunn et al. 1997). The initial human studies of selective cooling aimed to demonstrate the safety of the technique (Gunn et al. 1998a); hence these early studies were necessarily conservative in the temperature range studied (36.5-36 ^{O}C and 35.9-35.5 ^{O}C). The current study aimed to build on this by testing safety at temperature ranges (35 ± 0.5 ^{O}C and 34.5 ± 0.5 ^{O}C) that were, from animal data, considered likely to have neuroprotective effects.

3.2 Methods

The study period ran from November 1997 until December 1998. Term infants with a history of perinatal asphyxia were prospectively evaluated for NE

following admission to the neonatal unit. Infants were assessed against the following inclusion criteria: 1) gestational age greater than 37 weeks; 2) five minute Apgar score below six or cord/1st arterial pH < 7.1; 3) encephalopathy consisting of lethargy/stupor, hypotonia, abnormal reflexes including an absent or weak suck. Of note, at this stage, the selection criteria did not include any assessment of aEEG so the infants could be more mildly affected than those in the CoolCap Trial (Gluckman et al. 2005).

After obtaining parental consent, the infants were randomised by sealed envelopes to either a control group or cooling group. In order that experience be gained at one temperature range before exposure of babies to the lower temperature range the two cooling groups were sequential. Six infants were randomised to be cooled to 35 ± 0.5 °C and three infants were randomised to be controls. The use of 2:1 randomisation was to maximise experience gained in cooling infants during the time allocated for pilot studies. Two of the infants were recruited to the study by Professor Tania Gunn as I was unavailable at the time of presentation. In view of the lack of adverse reactions to hypothermia and encouraging short term outcome of cooling in the first group ethical permission was then given to allow the final group of infants to be allocated to 34.5 ± 0.5 °C (n=7). As this study followed a previous study using the same enrollment criteria for the period 1996-97 data were utilized from an additional 10 infants who were randomised to be used as control infants in this previous study period (Gunn et al. 1998a). Thus in total data were available from 13 infants treated with hypothermia and 13 selected matched control infants.

Selective cerebral hypothermia was achieved using the previously described technique. The rectal temperature was maintained within the prescribed range (34.5 ± 0.5 °C, 35 ± 0.5 °C or normothermic) for 72 hours. Thereafter the cooled infants were slowly rewarmed at 0.5°C per hour until their temperature was within the normal temperature range.

Due to the small sample size and minor difference in temperature between the two cooling groups (35 °C n=6 and 34.5 °C n=7) the groups were combined for comparison with the control group. Thus results are given for the cooling group as a whole (i.e. all 13 cooled infants) unless otherwise stated. Blood gases were analysed at 37°C then adjusted as appropriate (Jonas RA 1993) (Gunn et al. 1998a). When results of blood gases are given for the whole group values closest to 1, 2, 3, 6, 12, 24, 48, 72 and 76 hours were analysed in order to reduce the effect of disproportionate sampling in the sickest infants.

3.3 Results

Twenty-six infants (15 female and 11 male) were enrolled in the study following initial clinical evaluation. Thirteen of the 26 infants received selective head cooling, 12 infants for 72 hours and one infant for 48 hours. There were no significant differences in gestational age, birth weight, initial pH or Apgar scores at five minutes for the individual groups (Table 7). Cooling commenced at a mean of 4.9 (1.3) hours after birth. The mean age of initiation

of cooling was 4.8 (1) hours for inborn (n=4) and 5.2 (0.9) hours for outborn (n=9) infants.

Table 8. Clinical characteristics for each study group.

Group	Gestation (Weeks)	Birth Weight (Grams)	Cord pH	Cord base excess	5 min Apgar (range)
Controls	39.8(1.4)	3371 (383)	6.88 (0.20)	-19 (8.0)	5 (2-7)
35.0 ± 0.5 0C	40 (2.0)	3333 (496)	6.88 (0.19)	-19 (7.4)	5 (2-7)
34.5 ± 0.5 0C	40.2 (1.2)	3892 (628)	7.02 (0.15)	-14 (10.0)	4 (0-6)

No infant died whilst undergoing cooling. However, one cooled infant and three control infants died. The cooled infant had severe persistent pulmonary hypertension (PPHN) and died at five days of age following a rapid deterioration. Cooling had been discontinued 48 hours prior to death. Although the infant was sick at this stage, with an oxygenation index of between 30 and 35, there had been no significant clinical change or deterioration in oxygenation during the rewarming phase. There were three deaths in the control infants. Two of these deaths occurred in the first 24 hours. One infant was a home birth with severe respiratory failure and another had multisystem failure including hypotension and PPHN that failed to respond to intensive care. The third death occurred in a profoundly handicapped infant with microcephaly and spastic quadraparesis who was discharged home for terminal care. Thus, 12 of the cooled and 10 of the control infants were discharged home on full sucking feeds. There was no significant difference between the groups in age at discharge of survivors with the cooled infants discharged at a mean (SD) of 12 (5) days and the controls at 13 (12) days.

No infants required warming up for clinical instability during the cooling run. Respiratory support was required in 10 of the 13 cooled infants (Table 8). The inspired oxygen requirements did not substantially alter during the first six hours of the cooling period, with the exception of one infant where the fraction of inspired oxygen increased from 0.27 to 0.50.

The only cooled infant who died from respiratory causes was the aforementioned growth restricted post term infant with meconium aspiration syndrome and PPHN. He received high frequency ventilation, nitric oxide, sodium bicarbonate infusion and inotrope support, but given the overall condition was not treated with ECMO and died two days after rewarming. The other infants with PPHN were uneventfully rewarmed after 72 hours. Positive pressure ventilation for greater than 72 hours after birth was required in four cooled infants and one control infant. Three of these four cooled infants and the control infant demonstrated clinical and echocardiographic evidence of PPHN, in the first 24 hours following admission, and were treated with inhaled nitric oxide. The fifth infant ventilated beyond 72 hours of age was cooled to 34.5 ^{o}C and required prolonged support primarily for neurological reasons. Pulmonary haemorrhage occurred in two of the cooled infants with PPHN and none of the controls. In one case it occurred during the cooling run, at nine hours of age, but in the other infant it occurred at three hours before the initiation of cooling.

Table 9. Respiratory support required by the study infants.

	Target Rectal Temp (°C)	Respiratory support	FiO2 (max)	PPHN	NO	Pulmonary haemorrhage (1st episode)
1	35.25	IPPV	1.00	PPHN	yes	9 hours
2	35	IPPV	1.00	PPHN, MAS	yes	3.6 hours
3	35	IPPV	1.00	PPHN, sepsis	yes	No
4	35	None	.21	no	no	No
5	35	CPAP	.35	no	no	No
6	35	None	.21	no	no	No
7	34.5	None	.21	no	no	No
8	34.5	CPAP	1.00	no	no	No
9	34.5	CPAP	.80	no	no	No
10	34.5	Oxygen	.25	no	no	No
11	34.5	Oxygen	.33	no	no	No
12	34.5	IPPV	.21	no	no	No
13	34.5	CPAP	.21	no	no	No
14	37	CPAP	1.00	no	no	No
15	37	None	.21	no	no	No
16	37	IPPV	1.00	MAS	no	No
17	37	IPPV	1.00	no	no	No
18	37	IPPV	1.00	MAS, PPHN	no	No
19	37	IPPV	.87	MAS	no	No
20	37	IPPV	1.00	no	no	No
21	37	IPPV	1.00	no		No
22	37	None	.21	no	no	No
23	37	Oxygen	.48	no	no	No
24	37	None	.21	no	no	No
25	37	IPPV	1.00	PPHN	yes	No
26	37	CPAP	.40	no	no	No

IPPV = intermittent positive pressure ventilation; PPHN = persistent pulmonary hypertension; MAS = meconium aspiration syndrome; NO= nitric oxide

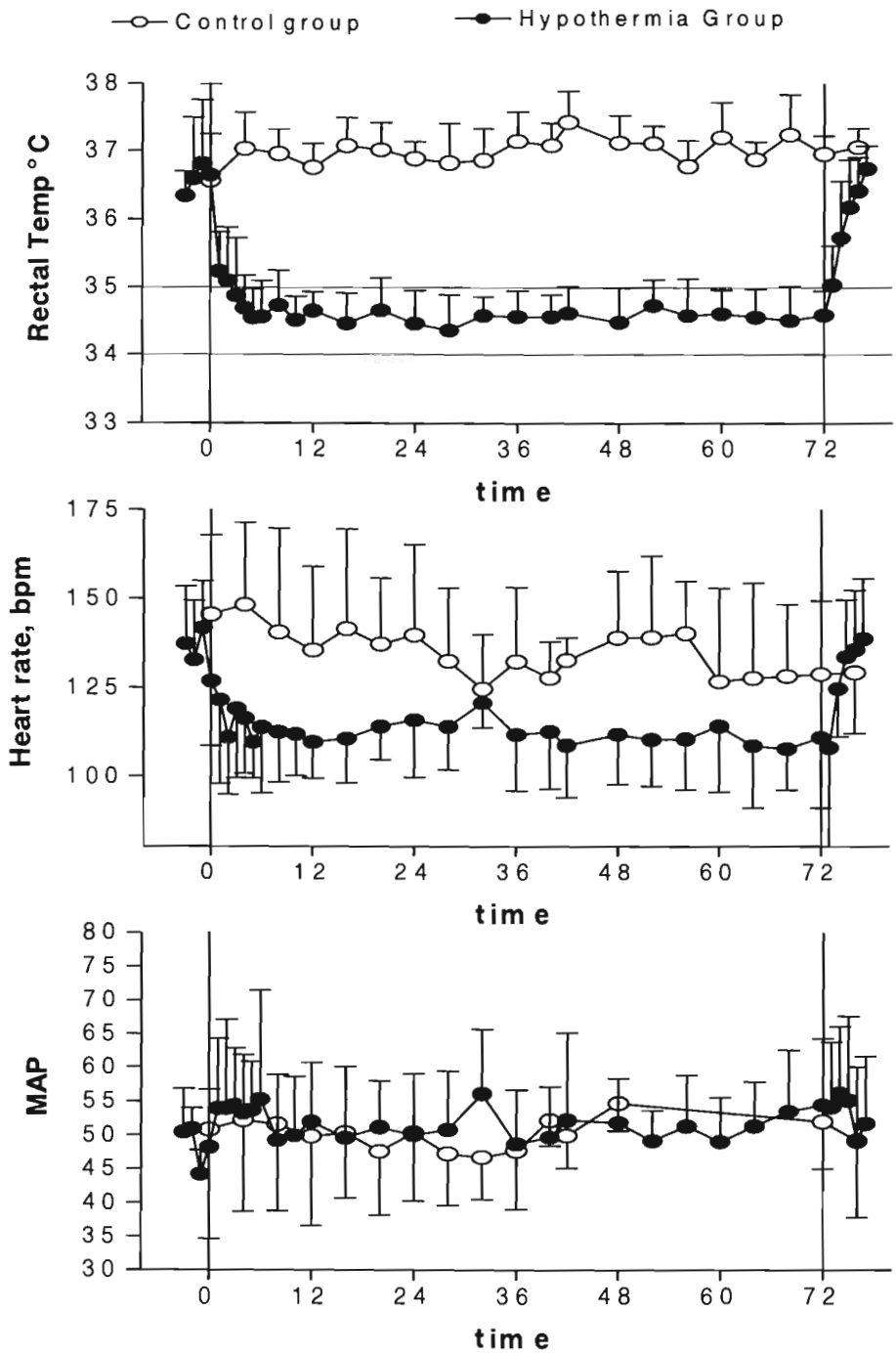


Figure 3. Changes in rectal temperature, heart rate and mean arterial blood pressure over time. There is a fall in heart rate during cooling of about 30 bpm but no significant change in MAP. Data are displayed as mean and standard deviation.

Although heart rate decreased during cooling there were no significant differences in the incidence of hypotension or use of volume and inotrope support between the cooled and control infants (Figure 2). Six of the cooled infants and four of the control infants demonstrated hypotension defined as a mean arterial blood pressure (MAP) less than 40 mmHg during the first 72 hours (p=0.69). Severe hypotension below 35 mmHg despite volume and inotropic support occurred in two cooled and two control infants. Volume expansion was used in the initial resuscitation of two cooled infants and four control infants (p=0.64). During the next 72 hours further volume support was required by both of these cooled infants and one other cooled infant but no controls. Inotropes were used to maintain mean arterial blood pressure in three cooled and two control infants during the first 72 hours (p=0.6). In two of the cooled infants this was started prior to cooling and dose was not adjusted at the time of cooling. Although volume and inotrope support were required in several infants, both cooled and controls, early in the clinical course there was no requirement for volume expansion or increased inotrope during the slow elective rewarming of the cooled infants (Table 9).

Table 10. Cardiovascular support required by study infants.

	Target Rectal Temp (°C)	MAP <40mmHg	MAP <35mmHg	Inotropes	Volume pre-cooling	Volume during cooling	Bradycardia <80 bpm
1	35.25	yes	yes	Adren, Dop, Dob	45 ml/kg	RBC + PLTs	yes pre-cooling
2	35	yes	yes	yes pre-cooling	20 ml/kg	10 ml/kg	no
3	35	no	no	2 hrs after starting	no	22 ml/kg	no
4	35	no	no	no	no	no	no
5	35	yes	no	no	no	no	no
6	35	no	no	no	no	no	no
7	34.5	no	no	no	no	no	no
8	34.5	yes	no	no	no	no	yes
9	34.5	no	no	no	no	no	no
10	34.5	no	no	no	no	no	yes
11	34.5	yes	no	no	no	no	no
12	34.5	no	no	no	no	no	no
13	34.5	yes	no	no	no	No	no
14	37	no	no	no	no	no	no
15	37	no	no	no	no	no	no
16	37	yes	no	Dop	no	no	no
17	37	no	no	no	no	no	no
18	37	yes	yes	no	10ml/kg	no	no
19	37	no	no	no	no	no	no
20	37	no	no	no	30 ml/kg	no	no
21	37	no	no	no	yes > 30ml/kg	no	no
22	37	no	no	no	no	no	no
23	37	no	no	no	no	no	no
24	37	yes	no	no	no	no	no
25	37	yes	yes	Dop + Dob	20ml/kg	no	yes
26	37	no	no	no	no	no	no

Dop = Dopamine; Dob = Dobutamine; Adren = Adrenaline; RBC = Red Blood cells; PLTs = Platelets

All the cooled infants demonstrated a fall in heart rate during cooling (Figure 2). In two cases the rate dropped below 80 bpm. However, no infant had a

rate less than 70 bpm, and none demonstrated an abnormal rhythm or appeared to be clinically compromised by the change in heart rate. One infant had a bradycardia of less than 80 bpm before cooling. One infant cooled to a rectal temperature of 34.5°C had a prolonged QT interval of 570 msec associated with a heart rate of 85 bpm on ECG aged 34 hours. The QT interval returned to normal the day after rewarming (Gunn et al. 1999b). Five other cooled and two non-cooled infants had an ECG performed during the cooling run or the first three days of life. The V5 lead QT intervals in these infants were not prolonged compared with published normal values (Davignon A 1979) (Table 10).

Table 11. QT and Corrected QT (QTc) intervals for cooled infants.

Infant	Age(h)	Temp(°C)	HR(bpm)	QT(msec)	QTc
7	34	34.3	85	570	679
7	84	37.4	125	300	434
8	30	34.4	140	320	488
9	12	33.9	115	330	460
9	42	34.1	107	380	510
9	63	34.2	73	420	460
9	85	37.4	136	260	390
11	24	34.5	94	440	
11	60	34.5	116	360	
12	36	34.8	110	360	

HR= heart rate

Thrombocytopenia defined as platelets less than 150 x 10^9 /L occurred in four cooled and four control infants. The minimum values during the first three days of life were 16, 45, 61, 111 x 10^9/L in the cooled and 73, 106, 134, 136 x 10^9/L in the control infants. Thrombocytopenia was treated with platelet transfusion if the count was below 30 x 10^9/L or symptoms of excessive

bleeding were present. In the infants without thrombocytopenia, only one (cooled) infant required platelet transfusion and no excessive bleeding was observed from venepuncture sites or mucous membranes in either study or control group. Formal clotting studies were performed in two cooled and four control infants when clinically indicated for overt bleeding. The results were abnormal in two cases with an INR of 2.4 (normal = 0.8 - 1.2) in a cooled infant who died and 4.5 in a control infant who died. All other results were within normal limits. Hypoglycaemia, defined as blood glucose below 2.6 mmol/L, was a common problem present in three cooled and four of the control infants during the first 72 hours. However, levels normalised in all infants over the first 24 hours despite the use of restricted fluids. All infants had clinical or biochemical evidence of renal impairment which resolved in all surviving infants. There was no significant difference between the mean maximum creatinine in the cooled group and the control group (0.145 (0.068) and 0.146 (0.060) mmol/L respectively). The median (interquartile range) for serum potassium in non haemolysed samples taken in the first 72 hours was 4.6 (4.2-5.1) in cooled infants and 3.5 (3.2-4.5) mmol/L in the controls. The mean sampled pH for the cooled group during cooling was 7.33 (0.1); however three of the cooled infants had received sodium bicarbonate infusions as management of PPHN. When results from infants receiving sodium bicarbonate infusions were excluded the mean pH was 7.31 (0.04) during cooling and 7.36 (0.20) in the controls during the first 72 hours. No infant had clinical evidence of hepatic or gastrointestinal complications although a transient rise in liver enzymes was seen in both groups.

Blood culture positive infection with *B. fragilis* occurred in one of the cooled infants and one of the controls had a blood culture positive for group B streptococcus. These blood cultures were taken prior to cooling. In addition the cooled infant was neutropenic at birth and it is likely that the infection was at least in part responsible for the clinical presentation. All infants received antibiotics for 48 hours until the results of admission cultures were known. Five cooled and three control infants had the course of antibiotics continued for a median of 5 (range 4-7) days at the discretion of the attending neonatologist.

For the cooled infants the mean cap temperature used during the cooling process was 10.0 (1.0) °C. The minimum cap temperatures used were 8.0 °C in a 5-Kg infant with seizures and 8.3 °C in another 3.5-Kg infant who presented following maternal pyrexia and had pervasive seizures. In both infants the cap temperature had to be increased once seizures were controlled and the production of heat was decreased. (Figure 3). The maximum cap temperature used in any infant was 12 °C.

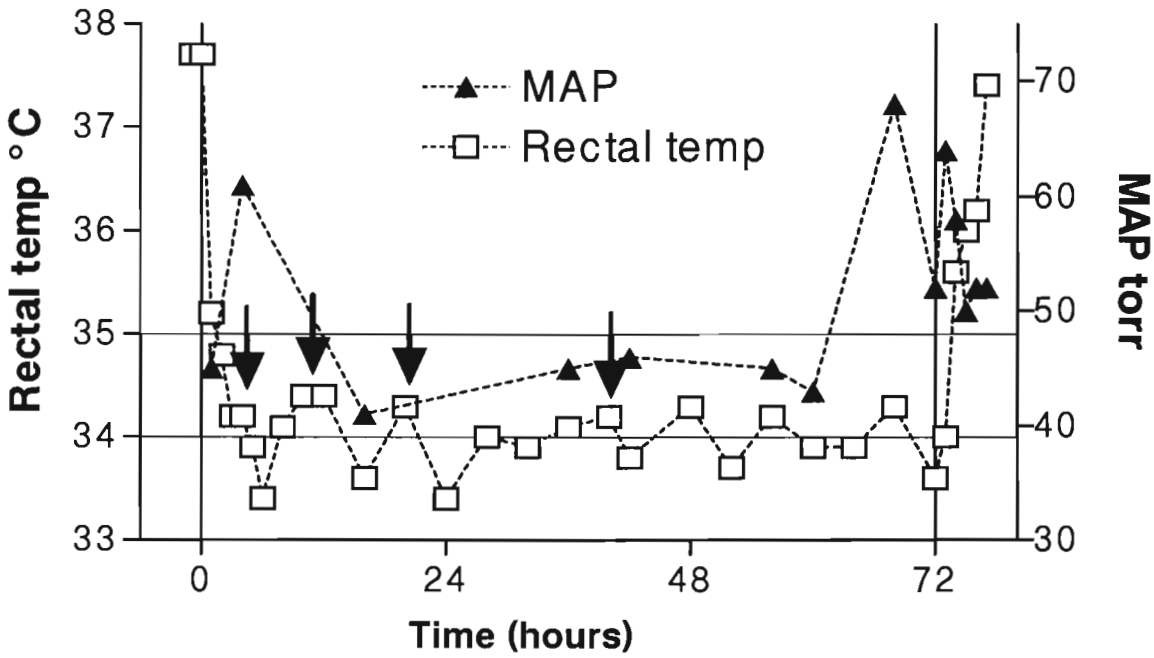


Figure 4. Effect of anticonvulsants on temperature and mean arterial blood pressure. After each dose of anticonvulsant movements cease and rectal temperature falls. Initial cap temperature 8.3 ^{O}C and increased in two increments to 10 ^{O}C at 24 hours.

↓ Indicates time of anticonvulsant administration

Once cooling had been achieved the mean difference between rectal and nasopharyngeal temperature was 1.4 °C in the infants not receiving respiratory support. However, this gradient could not be measured in those receiving warmed gases via the nasal passages either as nasopharyngeal CPAP or nasal ventilation. Rewarming proceeded over four hours and occurred smoothly (Figure 4). On removal of the cap all the infants demonstrated scalp oedema that resolved during the following 24-36 hours. Following rewarming four of the cooled infants had mild overshoot in temperature above 37.3 °C to a maximum of 37.7 °C rectal.

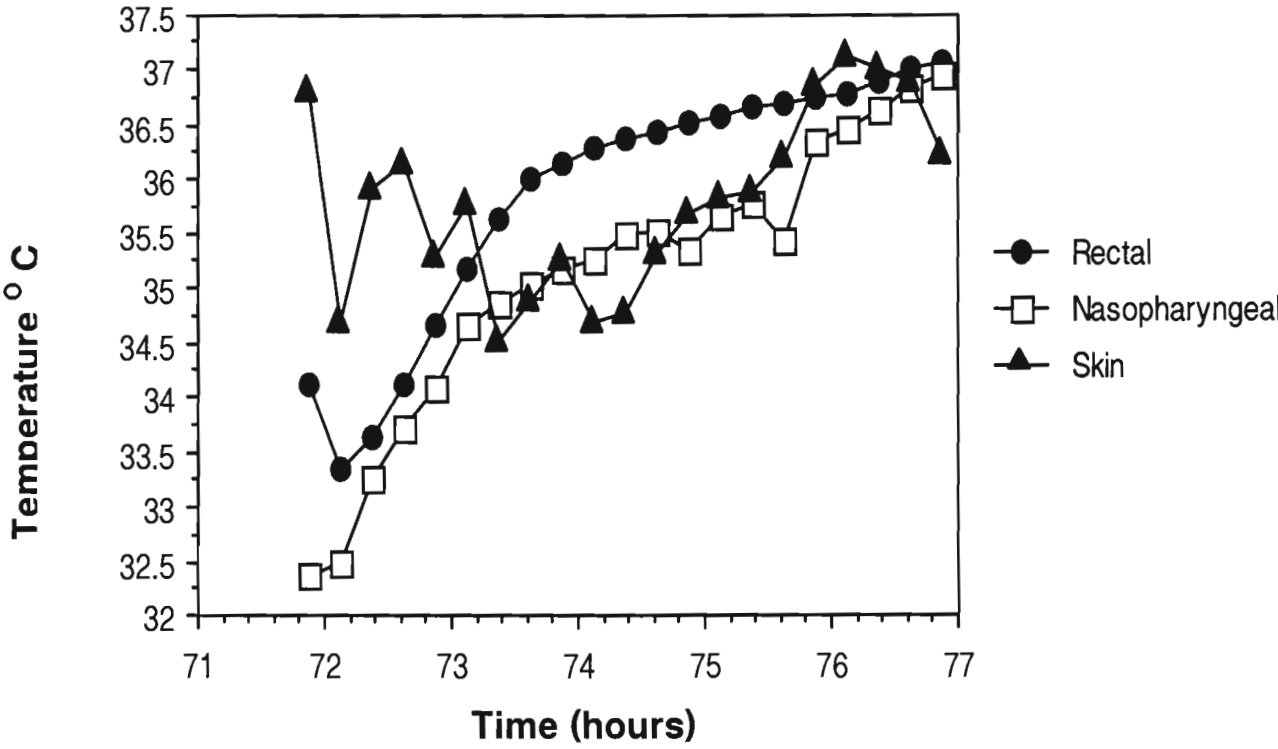


Figure 5. Changes in rectal, nasopharygeal and skin temperature during rewarming in a typical infant.

Seizures treated with anticonvulsants were common in both groups of infants occurring in 9/13 (69 %) cooled infants and 5/13 (38 %) controls. Although there was a trend toward more frequent seizures in the cooled group this was not significant (p=0.1) and the mean age at onset was similar with cooled infants starting at 9.3 (7) and controls at 12 (11) hours respectively. The median (range) number of anticonvulsants drugs used was also similar 1(1-3) in the cooled and 1(1-3) in the cooled group. Data are not available on total duration of seizures as the episodes were diagnosed clinically and aEEG monitoring was not used under the cap. Follow-up EEG at one week was performed in 19 of the 26 infants. Four infants had died and three infants (one cooled and two control infants) did not have an EEG performed. In cooled

infants the result was within normal limits in six cases and in the remaining five infants demonstrated either persisting abnormalities of background or excessive sharp waves. In the control infants the follow-up EEG was within normal limits in seven cases and abnormal with an excess of sharp wave and spike activity in one.

3.4 Discussion

Perinatal asphyxia remains a significant cause of adverse outcome in terms of both neonatal death and long term disability. There are few therapeutic interventions available and the care of infants with NE is largely limited to supportive measures and anticonvulsants (Levene 1993; Vannucci and Perlman 1997). The work described in this chapter demonstrates clearly that the innovative technique of selective head cooling with systemic hypothermia of 34.5 or 35 °C is practical and may be safely performed, for up to 72 hours, in a population of term infants with NE and a variety of other multisystem effects of perinatal asphyxia. Although the safe use of this technique has been previously reported (Gunn et al. 1998a) the degree of cooling had been cautious and the current study reports safety within a temperature range applicable to large clinical trials of neuroprotection.

Potential for adverse effects of hypothermia have been recognised for many years. Although much of this concern came from studies in preterm infants (Buetow and Klein 1964; Day et al. 1964; Silverman et al. 1958) it has been extrapolated to the term infant. Furthermore, there are some data that demonstrate an association between hypothermia and adverse outcome in the term infant (Gunn and Outerbridge 1978). Although this relationship is

clearly one of association not causation there has been a long- standing clinical practice to avoid hypothermia in infants. Concern regarding respiratory status may be of particular importance given the frequency of respiratory problems in infants with systemic sequelae of asphyxia in addition to NE (Martin-Ancel et al. 1995; Shankaran et al. 1991). In the current study there was a requirement for respiratory support, within the first 72 hours of life, in 20 of the 26 infants. Although a small effect can not be excluded, the cooled infants did not have an excessive requirement for respiratory support compared with controls and the support was often weaned during cooling, suggesting that the process was well tolerated. Furthermore, the majority of cooled infants did not demonstrate an increase in oxygen requirement during the initial cooling phase. PPHN is a frequent association with perinatal asphyxia; however it is also recognised that moderate to deep hypothermia may be associated with an increase in pulmonary vascular resistance (Benumof and Wahrenbrock 1977). One other case series reported a moderate but consistent increase in oxygen requirement in association with cooling (Thoresen 2000). In contrast the current study did not demonstrate this and it is possible that each centre had differing practices with regard use of the overhead heater (Gunn and Battin 2000) or other minor variations in personal practice to account for this difference. There were also some differences in selection of infants for the pilot series with the Thoresen study having more severely unwell infants. Three cooled infants had echocardiographic evidence of PPHN which was treated with nitric oxide. Although their status was already poor prior to initiation of cooling formal assessment of pulmonary artery pressure was not performed prior to onset of

cooling due to urgency in initiating cooling. The lack of an apparent change in either ventilation or oxygen requirements during cooling suggests that selective cooling, as used in this study, did not significantly affect the development of PPHN.

In the current study, there was no clinically significant change in mean arterial blood pressure either during the cooling or rewarming. Other studies have reported concern regarding peripheral vasodilatation and resultant hypotension during rapid rewarming (Thoresen 2000). However, their experience was different in two ways. Firstly, in contrast to the practice in Auckland, they did not maintain a stable balance with the overhead heater usually on maximum during the cooling. Secondly, no Auckland infant was rewarmed for clinical instability hence local infants underwent rewarming slowly at a rate of 0.5 ^{o}C per hour. Following this regimen there was no requirement for volume expansion or increased inotropic support during rewarming. Although a fluid bolus during rewarming is likely not to be harmful and may be helpful in infants with a rapid redistribution of blood flow it was not needed as a routine.

As would be expected from both previous experience (Gunn et al. 1998a) and what is known about electrophysiological effects of hypothermia, all infants demonstrated a decrease in heart rate during the cooling run. In the cooled group the mean decrease was approximately 30 bpm. In addition, one infant demonstrated prolongation of the QT interval on ECG when cooled to 34.5 ^{o}C. The experience with this infant has been previously published (Gunn et al.

1999b). It is known that lowering of temperature decreases the heart rate (Schubert 1995). Moreover, both hypothermia and lowered heart rate are linked with an increase in QT interval. No other infant had a bradycardia below 70 bpm and in no infant was a clinically important arrhythmia detected. Nevertheless, it would be considered prudent to perform continuous close cardiac monitoring during hypothermia and formal ECG has a role in detecting QT interval prolongation if there is any doubt, particularly if the heart rate is below 70 bpm.

Hypokalemia may occur in animal models during cooling (Sprung J 1991) and was reported in the series of whole body cooled infants (Azzopardi et al. 2000). In the current study the median serum potassium was 4.6 mmol/L in the cooled infants but there was a wide range of values including some below 3.0 mmol/L. Furthermore low values were recorded in both cooled and control infants. This may reflect a caution in the use of maintenance potassium in addition to intracellular shift during cooling; review of our data reveals that only one infant received added potassium in their intravenous fluid during the first 24 hours after presentation and in most cases there was a delay in starting maintenance until the creatinine had been measured and was reassuring. This caution may be justified given the potential for rebound severe hyperkalemia (Koht et al. 1983), or sensitivity to hyperkalaemia (Sprung et al. 1992). In adults rebound hyperkalaemia may also occur (Sprung et al. 1990) and has been described to result in cardiac arrhythmia on rewarming (Zydlewski and Hasbargen 1998).

Although excessive haemorrhage was not a feature of either the initial safety study of selective cooling (Gunn et al. 1998a) or other early safety studies of whole body cooling (Simbruner et al. 1999), the clotting cascade is known to be affected by alterations in temperature. In the current study there was a limited investigation of coagulation with formal coagulation testing, performed at 37 °C, only when clinically evident problems occurred. In one pilot study of whole body cooling (Azzopardi et al. 2000) a quarter of the infants had abnormal coagulation treated with fresh frozen plasma and in one who subsequently developed haemorrhagic infarction the coagulation status became abnormal again after rewarming. The infants studied by Azzopardi were kept on average a degree cooler than in the current study at a rectal temperature of 33.2 ±0.6 degrees for 48 hours. This area will need to be further monitored.

One point for discussion was that the blood gas analysis presented was not corrected for temperature. In practice correction would make only a modest change in the pH. One method of correcting the results is to use the Rosenthal Correction Factor (Rosenthal 1948). This will increase the pH by 0.015 units per degree C change in temperature. For example, if an infant is at a temperature of 34°C and has PH of 7.360 with the blood gas electrode temperature of 37°C then the corrected pH is 7.405.

A further discussion point is that this study used a selected control group as several potential adverse effects of cooling may also occur in NE. The selected controls included some infants who were from a time period that did

not completely match the cooled infants; specifically 10 of the controls were recruited in the proceeding year. Nevertheless the group was considered to be an appropriate control as inclusion / exclusion criteria and management protocol had not changed during this period. The small number of infants in each group means there is potential for uncommon sequelae of cooling to be missed in this study.

The apparent non-significant excess in the frequency of seizures seen in the cooled infants compared with the controls is also likely a function of the small numbers. With small numbers there is certainly the potential for differences in disease severity between groups and it is not possible to stratify by disease severity. Although the seizure frequency was intriguing, the implications were debatable and a couple of points need to be highlighted. First, it should be noted that these are clinically diagnosed seizures not electrographic. Second, seizures are clearly a proxy outcome and, although a concern, may not accurately reflect long-term outcome.

The importance of the first point is that there are recognised problems in the accurate detection and diagnosis of seizures in newborn infants. At the time of the reported pilot studies we did not have any form of EEG monitoring in routine clinical use and it is recognised that there is a poor correlation between EEG and clinical manifestations of seizure (Boylan et al. 1999). Continuous EEG monitoring at the cotside has been available for 25 years, although useful in producing an EEG signal that was largely free of artefact these systems did not provide real time analysis and display (Eyre et al.

1983). Amplitude integrated EEG has also been available for some time. Although this is largely used as a tool to predict outcome in term asphyxiated infants, it may also be used at the bedside to monitor for seizures (Rennie et al. 2004). The experience is largely with term infants but even in this group seizure detection is not ideal with approximately half of seizures not identified by staff particularly if focal, low amplitude, or of short duration (Rennie et al. 2004; Toet et al. 2002). One approach to improving the real time recognition of seizures has been to provide long term monitoring and include automated seizure detection software (Boylan and Rennie 2006). Indeed, a recent review called for greater attention to the problem of neonatal seizures with emphasis on application of recent advances in monitoring methods in addition to the use of newer anticonvulsants agents in this age group (Rennie and Boylan 2007).

A major problem with monitoring either EEG or aEEG in infants undergoing selective head cooling is that the electrodes would need to be placed under the cap if the normal montage is used. A modified approach using frontal electrodes may not pick up some seizures apparent using the standard approach. A further impediment to routine cotside use is the fact that devices frequently use many channels that require a large number of electrodes to be placed. Other techniques use a combination with video recordings. Studies using this technique have demonstrated that a high number of seizures occur without clinical episodes recognised by even experienced staff as seizures (Murray et al. 2007). However, the system may be unwieldy in the neonatal care environment. An alternative approach has been to combine EEG and

ECG, which produces a significant improvement in yield in automated neonatal seizure detection (Greene et al. 2007). Although less cumbersome such systems are still quite specialised. In recent years the approach has been to use the BrainZ EEG system, which is a simple two-channel device that is used widely in New Zealand and Australia, mainly in term infants with encephalopathy. It has automated seizure detection software that compares favourably with other systems such as Gotman's algorithm (Navakatikyan et al. 2006). Although at present we do not have experience in using this in conjunction with selective cooling.

Apropos, the issue of seizures being a proxy outcome there is a lack of data on the relationship between this and long term neurodevelopmental outcome in cooled infants. Data from local follow-up is presented in the next chapter and does not suggest a risk of adverse outcome. Other small studies have reported a variable effect of cooling on seizures. Eicher (Eicher et al. 2005) reported an increased rate of seizures in a controlled study of 62 infants in whom half received whole body cooling. Of note the seizures were of later onset in the cooled group. Also the seizure episodes were clinically diagnosed in that study and there was also an increased rate of tremors in the cooled infants. Tooley (Tooley et al. 2003) in a study of cooled piglets with EEG monitoring reported a similar rate of seizures in those receiving selective head cooling to the controls but the number of prolonged seizures was less in the cooled group. A similar finding of shorter total seizure duration was reported by the group in cooled human infants (Thoresen M 2003).

Overall, the small numbers and chance seem to be the most likely the explanation for the trend towards a discrepancy in seizure frequency between control and cooled infants seen in the pilot data but not evident in the larger trials (Gluckman et al. 2005)(Shankaran S 2005).

There is still much to be learnt regarding the clinical use of hypothermia. In the Auckland protocol, a servo-controlled overhead heater that provided near maximal skin warming was used, thus minimising potential for rapid changes in temperature. In common with other studies (Thoresen 2000) the need for close observation is recognised during key phases of cooling and rewarming to avoid over-cooling or conversely overswing on rewarming. Such observation and minor adjustment may also be needed during ventilation changes that institute or discontinue use of warmed humidified gas affecting temperature balance. The use of medications such as sedatives, anticonvulsants and paracetamol all have the potential to interact with temperature control and cause excessive cooling, whilst the onset of seizures may cause heat generation leading to spontaneous rewarming. Another minor point is that the cooled infants' scalp was oedematous following removal of the cap. Although this resolved spontaneously over 24 hours, parents needed to be informed about this. Furthermore, no object should be placed below the cap in contact with the scalp and the scalp should be checked each day to ensure no areas of pressure damage occur.

In conclusion, this study provides valuable safety data regarding the use of selective hypothermia but these data should not be over interpreted. It was

performed as a safety study and is without adequate power to look at efficacy. However, it does represent an important step providing the safety data that is necessary before committing large numbers of infants to an adequately powered randomised trial.

4 Neurodevelopmental Outcome Following Selective Cerebral Hypothermia

4.1 Background

In the previous chapter only short term outcomes were examined. Convalescent CT scan and neurological course prior to discharge may be used as proxy outcomes but do not replace the need for longer term outcome data. The current chapter aims to examine outcome at 18 months in both cooled and control infants.

4.2 Methods

During the period, January 1996 until December 1998, term infants admitted to the neonatal unit at National Women's Hospital and considered at high risk of perinatal asphyxia were prospectively evaluated for signs of neonatal encephalopathy. Infants were enrolled into the study based on a clinical history consistent with perinatal asphyxia and the presence of all of the following entry criteria: 1) gestational age greater than 37 weeks; 2) 5 minute Apgar score below 6 or cord/1st arterial pH < 7.1; 3) encephalopathy consisting of lethargy/stupor, hypotonia, abnormal reflexes including an absent or weak suck. Infants with obvious major congenital abnormalities or metabolic diseases were excluded. All infants were evaluated between two

and five hours after birth and monitoring and/or cooling was started before six hours after birth.

After parental consent had been obtained, the infants were randomised by computer generated numbers in sealed envelopes and allocated to either a control group (n=13) or cooling group (n=18). In order that experience be gained at one temperature range before exposure of babies to the lower temperature range the cooling groups were sequential. The initial cooling group were cooled to a rectal temperatures of 36.5-36 °C (minimal cooling group, n=6)(Gunn et al. 1998a). As no adverse effects were found at this temperature, infants were then studied with mild hypothermia, at rectal temperatures of 35.9 - 35.5°C (n = 6) and 35 ± 0.5 °C (n=6). In view of the lack of adverse reactions to hypothermia and encouraging short term outcome of cooling in the first three groups ethical permission was given to allow the final group of infants to be sequentially allocated to 34.5 ± 0.5 °C (n=7) (Battin et al. 2003). Outcome data were also collected from two further, normothermic, infants who fulfilled the study entry criteria but were not randomised due to non-availability of the researcher.

Demographic and clinical data were collected including documentation of pregnancy and labour complications, mode of delivery, Apgar scores, umbilical cord arterial blood gases, details of resuscitation and any other relevant clinical details. Clinical follow-up was as detailed in the methods chapter. In order to study the effect of cooling on outcome it was considered, from available animal data, that the minimal cooling group was unlikely to

show significant neuroprotection. Hence, for comparison with the normothermia group, the groups with mild systemic hypothermia (35.5-35.9, 35 ± 0.5 and 34.5 ± 0.5 ºC) were grouped together.

4.3 Results

All infants had evidence of perinatal asphyxia with depressed 5 minute Apgar scores and low cord or first arterial pH (Table 11). However the degree of encephalopathy that subsequently developed varied from mild to severe. Thirty-four of the 40 study infants survived until discharge and there was one subsequent death in late infancy in a control infant with severe spastic quadriparesis. Of those who died in the neonatal period, three were in the normothermic group of infants and three were cooled; two in the 36-36.5 ºC group and one in the 35 ± 0.5 ºC group. The deaths were from primary respiratory failure with associated persistent pulmonary hypertension and/or meconium aspiration in four cases, and profound multisystem, including renal failure, in two cases.

Table 12. Clinical characteristics of study groups

Group	Gestation (wks)	Birth Weight (g)	Cord pH	5 min Apgar (range)
Control	39.4 (1.7)	3458 (438)	6.85 (0.25)	5 (2-7)
36.5-36 ºC	39.3 (1.4)	3356 (568)	6.98 (0.21)	5 (0-8)
35.9-35.5 ºC	39.1 (1.1)	3192 (481)	6.93 (0.11)	6 (4-8)
35±0.5 ºC	40.0 (0.9)	3333 (410)	6.92 (0.19)	4 (2-7)
34.5±0.5 ºC	39.8 (0.8)	3892 (628)	7.02 (0.13)	3 (0-6)

Data are mean (S.D) or median (range)

Physical examination and Bayley assessment at 18 months was undertaken in 29 of the 34 surviving infants. Two infants with severe cerebral palsy and

microcephaly were reviewed at this age but in view of their severe neurological impairment did not undergo a formal Bayley assessment. Three infants were only reviewed prior to 18 months. One cooled infant (36.5-36 ºC) underwent formal neurological examination at 12 months with no neurological abnormality or developmental delay detected, but the family then moved away and declined to return for Bayley assessment. The other two infants who were not reviewed at 18 months were normothermic controls who were initially seen in the first year of life but thereafter did not attend the clinic. One family were contacted by phone and stated that there were no parental concerns with development at 18 months, but declined to attend for formal hospital review, the other infant was lost to follow-up.

Adverse outcome, defined as one or more of the following: death; cerebral palsy; Bayley scores greater than two standard deviations from the norm; special sense abnormality including blindness, or hearing impairment requiring amplification, occurred in 14 out of 40 study infants (Table 12). Of the 15 normothermic infants, four had an adverse outcome, including three deaths in the neonatal period and 1 death in late infancy associated with severe cerebral palsy, microcephaly and seizures. In addition, one infant had a mental development score of 71. The minimally cooled group (36-36.5 °C) had an adverse outcome in four of the six infants with two neonatal deaths and two cases of severe cerebral palsy. In the remaining 19 infants treated with mild systemic hypothermia, there was one death, three cases of cerebral palsy, and one infant with isolated hypotonia and developmental delay at 18 months. No infant had hearing impairment requiring amplification but three

infants with developmental delay and cerebral palsy (infant no.17, 18, 31) were reported to have mildly increased auditory thresholds. Two other infants (no.5, 39) had evidence of conductive loss due to middle ear effusion. Seizures beyond the neonatal period occurred in only three cases (no.13, 17, 18) all of whom had severe spastic quadriparesis and microcephaly.

Six normothermic, one minimally cooled, and four mildly cooled infants had mild early encephalopathy (Stage 1). Only one of these infants had an adverse outcome. She had developmental delay and on clinical follow-up, at age four years, she was given a diagnosis of autistic spectrum disorder. Amongst infants with early Sarnat stage 2 or 3 encephalopathy, an adverse outcome was found in four of nine normothermic infants (44%) and four of five minimally cooled infants (80%), whereas in the combined mildly cooled groups an adverse outcome was found in four of 15 infants (26%). This equated with an odds ratio of 0.46 compared with normothermia but due to the relatively small numbers the 95 % confidence limits (0.08, 2.56) were wide and crossed unity.

Within the groups of infants cooled to 35 ± 0.5 ºC or 34.5 ± 0.5 ºC there were two infants (no. 28, 38) with a very poor initial neurological condition and evidence of having undergone very severe perinatal insults, who had remarkably good outcomes when compared with the anticipated course. The first infant (no.28) required resuscitation in the delivery suite and after admission to the neonatal unit still had an arterial pH of 6.7. Clinical condition was very poor and the infant required cardiac massage and repeated doses of

adrenaline and sodium bicarbonate for refractory bradycardia. Despite very severe encephalopathy, and a four week stay on the neonatal unit, follow-up revealed no abnormal neurological findings and a Bayley assessment within the normal range. In the other case (no.38) the infant was an apparent stillbirth with resuscitation abandoned at 20 minutes due to lack of response. Shortly thereafter precordial activity was detected and resuscitation was recommenced. The infant developed a severe encephalopathy with absent brainstem reflexes on early neurological examination, and yet the clinical outcome at 18 months showed a normal neurological examination and Bayley assessment within the normal range.

Table 13. Neurological and neurodevelopmental outcome on follow-up.

No.	Study group	Sarnat stage	Neurodevelopmental outcome	Bayley MDI	Bayley PDI
1	Normothermic	2	Within normal limits	87	91
2	Normothermic	1	Within normal limits	71	94
3	Normothermic	2	Severe spastic quadriparesis, microcephaly, seizures	N/A	N/A
4	Normothermic	3	Died	died	died
5	Normothermic	1	Within normal limits	105	99
6	Normothermic	1	Within normal limits	100	110
7	Normothermic	1	Within normal limits	80	85
8	Normothermic	2	Within normal limits	106	102
9	Normothermic	3	Died	died	died
10	Normothermic	1	Within normal limits (< 12 months)	DNA	DNA
11	Normothermic	2	Within normal limits	101	99
12	Normothermic	2 to 3	Died	died	died
13	Normothermic	2	Within normal limits	95	103
14	Normothermic	2	Within normal limits	93	95
15	Normothermic	1	Within normal limits (< 12 months)	DNA	DNA
16	36-36.5 °C	2	Died	died	died
17	36-36.5 °C	2	Severe spastic quadripariesis,	<50	<50

			microcephaly, seizures, squint		
18	36-36.5 ºC	2	Severe spastic quadriparіesis, microcephaly, seizures	N/A	N/A
19	36-36.5 ºC	3	Died	died	died
20	36-36.5 ºC	2	Within normal limits	94	90
21	36-36.5 ºC	1	Within normal limits	91	99
22	35.5-35.9 ºC	1	Within normal limits	93	87
23	35.5-35.9 ºC	2	Within normal limits	85	92
24	35.5-35.9 ºC	1	Within normal limits	101	99
25	35.5-35.9 ºC	2	Within normal limits	91	103
26	35.5-35.9 ºC	2	Within normal limits (at 12 months)	DNA	DNA
27	35.5-35.9 ºC	1	Within normal limits	89	94
28	35 ºC	3	Within normal limits	102	100
29	35 ºC	2 to 3	Died	died	died
30	35 ºC	2	Within normal limits	95	94
31	35 ºC	1	Moderate hypotonia, developmental delay	65	50
32	35 ºC	2	Severe spastic quadriparіesis	<50	<50
33	35 ºC	2	Within normal limits	80	103
34	34.5 ºC	2	Within normal limits	97	115
35	34.5 ºC	2	Within normal limits	91	91
36	34.5 ºC	2	Within normal limits	107	103
37	34.5 ºC	2	Spastic cerebral palsy, developmental delay	50	<50
38	34.5 ºC	3	Within normal limits	104	102
39	34.5 ºC	3	Severe spastic quadriparіesis, Microcephaly	<50	<50
40	34.5 ºC	2	Within normal limits but mild ligament laxity	85	91

MDI = Mental Developmental Index; PDI = Psychomotor Developmental Index; N/A = not available; DNA = did not attend; Sarnat stage allocated retrospectively after 3 days

4.4 Discussion

This study documents the neurodevelopmental outcome of a group of term infants with evidence of post-asphyxial encephalopathy who received either head cooling with varying levels of mild systemic hypothermia or standard, normothermic care. Although a number of small studies were performed in the 1950s and 1960s where infants not breathing spontaneously at five minutes were immersed in cold water until respiration resumed. These studies did not include a control arm and only limited follow-up data were reported (Westin et al. 1959) (Cordey et al. 1973; Miller et al. 1964). In fact, the outcome for infants depressed at birth was relatively poor at that time with a mortality of around 20% if the 5-minute Apgar was less than 3(Drage and Berendes 1966a). Some short term outcome data have been published following hypothermia for perinatal asphyxia (Battin et al. 2003; Gunn et al. 1998a). However, the current study was the first to publish longer term outcome data (Battin et al. 2001).

The study was not designed to test the efficacy of cerebral cooling; it did not have sufficient power. The primary aim was to detect major adverse effects of hypothermia in infants exposed to perinatal asphyxia rather than detection of a beneficial trend that supported intervention with cooling. For this reason, a stepped design was employed, with increasing depths of hypothermia in successive groups of treated infants. Importantly, there was no evidence of any unexpected late adverse effects of hypothermia. The major abnormal outcomes reported were cerebral palsy, developmental delay, microcephaly, hearing impairment and seizure disorders, consistent with the well known

effects of perinatal hypoxia-ischaemia (Robertson and Finer 1985; Robertson and Finer 1993). The one infant with autistic spectrum disorder was diagnosed later aged four years. In retrospect this may have accounted for her poor developmental progress at 18 months but at that stage it was not possible to confirm the diagnosis.

Formal assessment of outcome was performed at 18 months in the majority of infants. Although it is considered preferable to ascribe outcome status at two years corrected in preterm infants, 18 months was chosen as a compromise to allow formal examination for signs of cerebral palsy but not to cause an excessive delay in obtaining outcome data. Three infants were younger than 18 months when last reviewed but it is unlikely that this influenced the results appreciably. One infant was reviewed at 12 months and signs of cerebral palsy are usually manifest by this age in term infants. The other two infants presented with a mild encephalopathy and initial follow-up findings were reassuring. Within the study group, it was characteristic that clear signs of abnormal development such as abnormal tone and microcephaly were detected well before 12 months in the infants with an adverse outcome. The presence of early onset microcephaly and poor neurological outcome in infants with perinatal asphyxia has been previously reported (Cordes et al. 1994).

The outcomes of perinatal encephalopathy are closely related to the severity of the encephalopathy, with normal outcomes expected amongst infants with mild encephalopathy (Levene et al. 1986; Robertson and Finer 1993).

Although the classic Sarnat staging is made in retrospect, and no systematic data are available for early prospective clinical assessment, the present series clearly suggests that the great majority of the infants with milder encephalopathy did have a normal outcome. Amongst infants with moderate to severe encephalopathy, there was no suggestion of a worsening of outcome in the combined mildly cooled groups, but rather a trend to better outcome (26% adverse outcome versus 44% in controls by 18 months). Further, although it is vital not to over-interpret individual cases, it is of some interest that within the groups cooled to 35 or 34.5 ± 0.5 ºC several infants with a very adverse initial condition and clinical courses were observed to have essentially normal outcomes at follow-up.

Formal assessment of neurodevelopment with adequate power to detect differences is required before any new or innovative procedure is adopted as accepted practice. The sample size required for such a study of the treatment of neonatal encephalopathy is quite large. If it is assumed that hypothermia started before six hours of age is associated with a 30% reduction in death or disability, and the incidence of adverse outcome is 50% in the control arm, then 175 infants would need to complete follow-up, in order to have an 80% power of correctly detecting such an effect. Similar figures to these were used in the power calculation for the multicentre randomised trial (Gluckman et al. 2005).

Several aspects of the present study may be relevant to future studies regardless of any limitation due to sample size. Firstly, despite entry criteria

using strict clinical data a substantial number of cases with an abnormal pH and Apgar scores developed only a mild course. This is consistent with the published literature (Shankaran et al. 1991). As a consequence of this and the small sample size, the severity of encephalopathy and associated systemic disease was variable between study groups. As recently reviewed (Groenendaal and de Vries 2000), combinations of clinical factors at birth can be used to improve the specificity of infant selection for treatment. However, this can exclude significant numbers of infants with an adverse prognosis and many proposed factors are to some extent subjective or dependent on local practice. Using aEEG it may be possible to objectively identify high risk infants. Those infants with abnormal aEEG recordings when tested before six hours of age may have up to an 80 to 90% rate of adverse outcome (al Naqeeb et al. 1999; Toet et al. 1999). Such a high predictive value not only reduces the numbers of infants with a good prognosis who will be unnecessarily cooled, but it markedly reduces the number of infants needed to be recruited. This enables smaller trials that may be completed in a shorter time so potentially could make it more feasible to efficiently trial different cooling strategies.

The infant's temperature before trial entry may also affect the time available to start treatment. There is increasing clinical and experimental evidence that hyperthermia during and after cerebral hypoxia-ischaemia is associated with earlier and more severe neurological deterioration (Reith et al. 1996) and accelerates the development of apoptosis (Guan et al. 2000). It may be relevant that two infants (no.37, 39) who developed cerebral palsy and had a

poor outcome following treatment with hypothermia were born following maternal pyrexia during labor of 38.4 and 38.7 °C respectively. In the first case the mother had taken a hot bath whilst in labour, and there was no sign of prolonged rupture of membranes or maternal infection. In the second case there was a true knot in the cord and prolonged rupture of membranes but negative blood cultures. These two infants had rectal temperatures of 37.7 and 38.6 °C on admission to the neonatal unit and cooling was commenced at 5.6 and 3.2 hours respectively.

In conclusion this neurodevelopmental outcome data obtained from infants treated with selective cerebral hypothermia confirmed the potential for a substantial degree of neuroprotection following perinatal asphyxia. The mixed outcome from the study infants also reinforced the point that even though the short term safety of cooling has been demonstrated, a large formal randomised controlled trial was required in order to demonstrate the long term efficacy of hypothermia in a clinical context. The current data were used to as pilot data for such a trial.

5 Neuroimaging In Infants With Perinatal Asphyxia

5.1 Background

The literature review section of this thesis stressed the importance of long term neurodevelopmental data in judging outcome following perinatal asphyxia. This may be considered to be particularly apposite for a group of infants who have undergone an intervention such as selective hypothermia. However, whilst long term neurodevelopment is considered to be the "gold standard" there is sometimes a need to judge outcome at a time point earlier than 18 to 24 months. In these circumstances, imaging data may act as a proxy for the ultimate neurodevelopmental outcome. Similarly, it is extremely useful for clinical purposes such as counselling parents and directing care of infants with NE, particularly in the case of an infant with a moderate encephalopathy where the prognosis is variable and can not be determined from clinical staging alone. Finally cerebral imaging may be a method of estimating both the pattern and extent of neurological injury in infants who survive following NE.

The aims of the work described in this chapter were:

1) To determine whether cerebral CT scan appearances obtained at one week of age were prognostic for neurodevelopmental outcome in term infants with NE. Accordingly the utility was examined for both the selectively cooled group and the combined group of cooled and control infants.

2) To determine whether the typical patterns of injury observed following perinatal asphyxia were modified by exposure to selective cooling.

Additional unpublished work was performed by a collaborator to examine the intra and inter-observer variability in grading of the CT scans in infants with NE. Although I was involved this was not the focus of my own work; therefore some of the data are discussed but not reported in detail in this chapter.

5.2 Method

A cohort of term infants with NE born during the period January 1996 until December 1998, were reviewed. During this period all term infants admitted to the neonatal unit at National Women's Hospital and considered at high risk of perinatal asphyxia were prospectively evaluated for signs of NE. Infants who satisfied the entry criteria of: 1) gestational age greater than 37 weeks; 2) 5 minute Apgar score $\leq$ 6 or cord/1st arterial pH $\leq$ 7.1; 3) neonatal encephalopathy and had a CT scan performed to assist with prognostication were included in the current review regardless of whether they were cooled or not.

The routine cerebral imaging performed on term infants with NE usually included an early cranial head ultrasound. However, the purpose of this was to exclude major intracranial bleeding or malformation and the findings were not used to predict prognosis. Cerebral CT obtained between five and 10 days after delivery was used to assist outcome prediction. During the study period, access to MRI was not optimal primarily due to difficulties in transport to the unit and coordination with EEG timing. Hence, CT was used in preference to MRI and is the imaging modality reported here.

5.2.1 Scanning Techniques

All CT studies were performed on the same CT scanner (HiSpeed Advantage; GE Medical Systems, Milwaukee, Wis.) without contrast or additional sedation as per a standard clinical scan. Sequential axial images (3mm collimation through the posterior fossa and 5mm or 7mm collimation to the vertex) were obtained in all cases. Standard windowing was used: posterior fossa WW 80 WL 30, anterior fossa WW 60 WL 30. The CT scan images were then reprinted with all identifying features such as name and NHI number removed.

5.2.2 Analysis

The study images were reviewed and graded by a neuroradiologist blind to the neurological outcome. The initial plan was to use the following system of grading for the study: 0) normal; 1) white matter oedema; 2a) mild watershed infarction (1-2 small areas of cortical low density in a parasagittal distribution); 2b) moderate watershed infarction (3-4 areas of > 2 cm cortical watershed low density); 3) severe generalised infarction; or 4) involvement of basal ganglia.

Examples of cases representing these grades of hypoxic ischaemic injury were provided for practice prior to scoring study cases. Although this system was used as the basis for description of injury patterns, the previously mentioned companion study examining the intra and inter observer variability in grading of the CT scans considered that this classification was quite complex with some disagreement between individual reporting neuroradiolgists. Hence for the purposes of analysing the prediction of neurodevelopmental outcome by CT in this chapter, the images were divided into those that showed normal appearances or mild abnormality and those with marked abnormality; thus the scan grades were combined 0-2a versus 2b-4 and these results then correlated with the 18 month outcome data. This binary division of the modified grading system was subsequently supported by good intra and inter-observer agreement (Battin MR 2001). The sensitivity, specificity, positive and negative predictive values for CP or death were calculated from the CT data and clinical outcome for whole group, cooled infants only and moderate encephalopathy infants.

5.3 Results

Thirty-six infants with NE who had undergone CT scan were reviewed. The median (range) birth weight was 3536 (2280-5000) grams and mean gestational age 40 (37-42) weeks. In addition to the clinical signs of an encephalopathy they had clinical features supporting hypoxic ischaemia as the aetiology including a mean (SD) umbilical/1st arterial pH of 6.94 (0.20) and median (range) 5 minute Apgar score of 4 (0-7). The encephalopathy was graded as severe in two infants, moderate in 24 and mild in 10 cases. Twenty-

five of the 36 infants underwent selective cooling. For the purposes of this chapter these infants are not subdivided into individual cooling ranges but grouped together for analysis.

The CT scan was performed at a median age of seven (4-15) days. Although still requiring neonatal care, not all of the infants required ventilation or inotropic support at the time of CT scan. At the time of the study infants were required to be transported to another hospital approximately three Km away in order to undergo CT scan. All infants tolerated both the transport and the scanning process well.

Several infants demonstrated abnormality that was in addition to the typical HI injury described and graded as above. This included one case each of: old cerebral infarct in the middle cerebral artery distribution; dysplasia of the sylvian cortex; subgaleal haemorrhage; isolated thin subdural haemorrhage; and skull fracture with associated subdural haemorrhage. All of these except the middle cerebral infarct occurred in infants who had been cooled.

Overall, the CT scan was graded as normal or mild (0-2a) in 29 (80.5 %) and abnormal (2b-4) in seven (19.5 %) (Table 13). Apropos clinical outcome, one infant with severe encephalopathy was given terminal care and died in the neonatal period. Neurodevelopmental outcome data are available in the remaining 35 infants who survived. At 18 month follow-up 25 infants (69%) were functionally normal and seven infants (19%) had cerebral palsy. Additionally, two infants (6%) had tone abnormalities, at less than 12 months,

but very mild abnormality or normal examination at 18 months. One infant (3%) had developmental delay but normal physical examination at 18 months.

Table 14. CT scan results for the whole group, cooled and non-cooled (control) groups.

Grade	Whole group (n=36)	%	Cooled infants (n= 25)	%	Controls (n=11)	%
0	9	25	7	28	2	18
1	2	5.5	1	4	1	9
2a	18	50	12	48	6	55
2b	2	5.5	1	4	1	9
3	0	0	0	O	0	0
4	5	14	4	16	1	9

The utility of CT scan in prediction of outcome following NE may be looked at in a number of ways. In this study it was reviewed for the whole group, a sub group of cooled infants and a sub group of infants with moderate NE, where it is hardest to predict outcome (Table 14).

Table 14. Analysis of performance of CT in predicting outcome in term infants following perinatal asphyxia.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Whole group (n=36)	78	96	88	93
Cooled (n=25)	83	95	83	95
Moderate NE (n=24)	86	94	86	94

5.4 Discussion

In this study the results of CT scans performed in a population of term infants with NE were examined and correlated with neurodevelopmental outcome. The data suggest that CT scan performed at one week was a helpful adjunct to clinical staging in predicting neurodevelopmental outcome. In particular,

normal or only mildly abnormal CT scan at one week appeared to be very predictive of a good neurodevelopmental outcome. In abnormal CT scans evidence of minor white matter infarction alone was not predictive of neurodevelopmental abnormality but extensive injury or basal ganglia damage was predictive of a poor outcome. These findings are somewhat similar to those described for MRI following NE (Cowan 2000; Rutherford et al. 1996).

The fact that the data suggest CT scan may be useful in predicting outcome, following NE, is in contrast to some of the published literature. One study of cranial CT obtained in infants following perinatal asphyxia correlated CT hypodensity and post-mortem findings (Flodmark et al. 1980). This study reported a poor correlation between imaging findings and ischaemic brain damage other than haemorrhage. However, the study group included a mixture of preterm and full term infants, with only 11 infants born at term. Other studies (Adsett et al. 1985; Fitzhardinge et al. 1981; Lipper et al. 1986) have reported a correlation between poor neurodevelopmental outcome and CT abnormality including extensive hypodensity. Although selected by clinical criteria including fetal distress, cord pH, Apgar scores and gestation it is difficult to judge the actual grade of encephalopathy, due to lack of detail, in some studies (Fitzhardinge et al. 1981; Lipper et al. 1986). Moreover, these studies are nearly 20 years old and there have been considerable improvements in the quality of images obtained by CT scan in that time. More recently a study of CT, ultrasound and MR examination (Blankenberg et al. 2000) has reported a negative predictive value of 58.8% for adverse outcome at two years irrespective of technique used. Although this would suggest little

clinical benefit in performing such imaging the results of this study are difficult to generalise to clinical practice as a variety of presenting neurologic problems were studied in a mixture of premature and term infants. Furthermore only 55% of infants were followed up to two years.

MRI takes time to perform and may not be ubiquitously available, particularly in units where neonatal care is provided in separate maternity hospitals. Furthermore, early imaging with either MRI or CT may involve extra handling and transport of a sick infant. Ultrasound although easily available in the neonatal unit and helpful in the preterm infant has major limitations in prediction of outcome in the term infant with perinatal asphyxia (Cowan 2000). For these reasons some centres have used cranial CT rather than MRI, often with images obtained at about one week of age after the acute condition of the infant has stabilized. At the time of performing this study, CT was the preferred option in Auckland due to a combination of ease in obtaining imaging at a convenient time and co-ordination with EEG investigation. However, since that time several things have happened. Firstly, there has been a body of published literature describing the utility and prognostic yield of MRI (Hunt et al. 2004; Rutherford et al. 2004; Rutherford et al. 1998). Secondly a number of papers have questioned the safety of radiation exposure in children (Slovis 2002a; Slovis 2002b; Slovis 2003) particularly with regard to adult risk of cancer (Berrington de Gonzalez and Darby 2004; McLean 2004) or cognitive function (Meara et al. 2004). Thirdly published neuroimaging practice guidelines have suggested that if MRI is available then this should be the preferred modality and CT use should be restricted to cases

with suspected haemorrhage (Ment et al. 2002). Fourthly, MRI has become more readily available and less expensive.

Notwithstanding the recent change to MRI as the first choice imaging modality for clinical practice, the current study has several methodological strengths. Firstly only term infants who fitted tight clinical criteria for NE were studied. Secondly an experienced neuro-radiologist blinded to the clinical outcome graded the CT images. Thirdly good prospective follow-up data was obtained up to 18 months of age. This time period allows the clinician to clearly identify infants with cerebral palsy. Finally, two thirds of the infants studied had a moderate encephalopathy; the inability to predict outcome from clinical criteria in this group ensures a valid clinical as well as research question. Indeed, the test characteristics for this group, particularly the high negative predictive, indicate a high utility for prediction of outcome in clinical practice.

The study was not designed to, and could not, assess if cooling had a protective effect on either the degree or frequency of central nervous system damage; very large numbers would be required to generate enough power to answer that question. However, useful data on extent of injury could be obtained. Using MRI, the presence of basal ganglia injury, abnormal signal in the posterior limb of the internal capsule or very extensive injury has been reported to be strongly associated with an adverse neurodevelopment outcome following NE (Hunt et al. 2004; Rutherford et al. 1998). Using CT basal ganglia damage or widespread severe injury could be detected although

it was not possible to assess the posterior limb of the internal capsule using this modality.

Although it is conceivable that cooling may have a potential confounding effect on the ability of CT scan to predict outcome, this was considered not to be a major issue. Any neuroprotective effect of hypothermia is unlikely to have a major effect on the ability of imaging findings to predict outcome. All images were obtained between four and 15 days; with the cooled infants normothermic for at least three days prior to imaging. Although hypothermia may be protective, at least in part, against secondary insult imaging 5 -10 days would be after damage due to primary or secondary insult would be manifest. The patterns of injury were consistent with that observed in non cooled NE infants. The most common pattern was 2a (i.e. mild white matter injury) but a significant proportion were also seen with grade 4 (i.e. predominantly basal ganglia damage). These patterns of injury are also consistent with the typical lesions reported in the literature following HI injury including watershed infarction of the parasagittal white matter, cerebral cortical necrosis and basal ganglia injury (Barkovich et al. 1998; Barkovich et al. 1995; Campistol et al. 1999; Christophe et al. 1994; Roland et al. 1990; Rutherford et al. 1996; Rutherford et al. 1994). A recent report by Inder et al (Inder et al. 2004), using MRI to document injury, describes sparing of the cortex but not central grey matter in association with whole body cooling. It is conceded that CT may not be as sensitive as MRI and some minor lesions may be missed by CT. A lesser ability to detect mild cortex or white matter damage would lead to under-estimate of damage in that area rather than

white matter injury being the most common lesion. The different injury distribution could reflect different patterns of neuroprotection using whole body versus selective cooling using a cap. In an animal model variations in patterns of protection have been ascribed to different cooling temperatures (Iwata O 2005). Alternatively the effect may reflect the small numbers in the Inder study (Edwards D 2004). Indeed other imaging studies (Rutherford MA 2005) report a decrease in the incidence of severe cortical lesions in infants treated with selective head cooling.

In conclusion, cerebral imaging has been used in several research studies and has taught us much about hypoxic ischaemic brain injury in term infants. From a research view MRI has advantages in resolution as well as information obtained from sophisticated techniques such as diffusion weighted imaging, spectroscopy and MRI angiography. However, in clinical practice safety is paramount and the lack of ionising radiation with MRI is a major advantage outweighing the potential advantages of speed and cost with CT. Notwithstanding this, the data presented in this chapter suggest that if CT scan is performed then it may document the extent of injury and provide a useful guide to subsequent outcome. In clinical practice the high NPV of a normal or only mildly abnormal scan is also useful in reassuring parents.

6 Clinical Vignettes Associated With Selective Cerebral Hypothermia

6.1 Background

The purpose of this chapter is to review three interesting vignettes that cover clinical events not included in the other chapters but illustrate significant learning points that would otherwise be lost from the reported experience. They are each presented as a case report then discussion.

6.2 Rebound Seizures On Rewarming After Selective Hypothermia

A 41 week gestation male infant was born to a 30-year-old primigravida mother who was previously in good health. The pregnancy had been unremarkable except for the fact that a glucose tolerance test had been performed, the result of which was normal. Labour was induced for post maturity at 41 weeks gestation and an epidural block was inserted for pain relief. In the second stage of labour maternal temperature was noted to be 37.8 ^{o}C and liquor was meconium stained but the CTG was reassuring. Due to failure to progress with persistent occipital-posterior position a trial of Kielland's forceps was attempted. However, this was unsuccessful and an emergency lower segment caesarean section (LSCS) was performed.

The infant was noted to be depressed on delivery and was given Apgar scores of 1, 4, 6, and 9 at 1, 5, 10, 20 minutes respectively. He required positive pressure ventilation but neither drugs nor cardiac massage were given. Cord arterial blood gas demonstrated an acidosis with a pH of 7.08 and

base deficit of 11 mmol/L. Birth weight was 3270 g and postnatal examination on arrival at the neonatal unit revealed the infant to be clinically post term with a lack of subcutaneous fat and post mature looking skin. Initially there was marked hypotonia with a paucity of spontaneous movements but by three hours he was irritable with hypertonic limbs, fisting and some repetitive sucking movements. In view of the encephalopathy he was considered to qualify for the selective cerebral cooling. Parental consent was obtained and he was uneventfully cooled and maintained between 34 °C and 35 °C.

Five hours after commencing cooling he manifested a short period of repetitive jerky movements, lasting about two to three minutes, and he was empirically given phenobarbitone 20 mg/kg dose (at this time it was not possible to record bedside EEG in the unit). However, it was suspected clinically that the movements were more likely to represent irritability than overt seizures. Over the next 60 hours he had no further episodes of abnormal movements but still demonstrated moderate increase in tone, brisk tendon jerks and poor sucking action. At 72 hours of age cooling was discontinued and he was slowly rewarmed at a rate of half a degree per hour. The rewarming process was unremarkable until a temperature of 36.2 °C was reached. At this point he developed a clinical seizure characterised by a 20 second rhythmic jerking of all four limbs associated with jerky eye movements. The blood sugar was normal. He was loaded with phenobarbitone 20 mg/kg but had a further eight short episodes of clinical seizures over the next 24 hours and required additional doses of phenobarbitone up to a total of 40 mg/kg. Thereafter he had no more seizures but was continued on maintenance phenobarbitone therapy for two months. The clinical course was also complicated by a moderate subgaleal haematoma that was detected initially on clinical examination then confirmed on cerebral imaging. In addition to the subgaleal haematoma the initial cerebral ultrasound scan, on day one, also demonstrated an abnormal resistive index (RI) of 0.54 on Doppler. Subsequent CT scan at five days again demonstrated the subgaleal haematoma but did not show any evidence of cortical infarction or intracranial haemorrhage.

On follow-up the subgaleal haematoma resolved spontaneously without sequalae. Formal EEG at one week of age demonstrated a mildly abnormal record with an excess of sharp transients symmetrically over both hemispheres. However, there was no focal abnormality or overt seizures. Assessment performed at 18 months of age included a normal physical examination and Bayley developmental scores of 95 and 89 for cognitive and motor testing.

6.2.1 Discussion

It is recognised that re-warming is a complex process that may, at least potentially, be associated with complications (Gerrits LC 2005). The focus of chapter Two was to illustrate the safety of the 72 hour period of actual cooling. During that study as with the current reported case, care was taken to perform very slow re-warming. Specifically, the desired rate did not exceed 0.5 ^{o}C per hour. Similar recommendations have been used in other pilot studies of cooling (Azzopardi et al. 2000; Shankaran et al. 2002). It was considered that by avoiding rapid changes in temperature potential problems are kept to a minimum. However, in this case rebound seizures were observed despite keeping within the prescribed rate.

In broad terms, there are two main problems associated with re-warming from a hypothermic state. First, rapid warming may precipitate cardiorespiratory instability including a fall in oxygen levels, loss of peripheral vascular tone and increased cardiac work secondary to vasodilation (Thoresen and Whitelaw 2000). Second, rewarming may potentially have neurological effects via reactivation of parts of the cascade of neuro-injury that have been suppressed

by cooling, particularly the release of excitatory amino acid neurotransmitters (Nakashima 1996; Stecker MM 2001).

Following the above clinical experience where an infant had seizures on rewarming, the available sheep data was re-examined to explore the hypothesis that rewarming from moderate hypothermia may be associated with rebound seizure activity. The work was performed by a student and is published elsewhere (Gerrits LC 2005). However a brief summary is included to supplement the current discussion. The aim was to quantify the number, duration and intensity of epileptiform events after rapid rewarming from a period of in utero moderate hypothermia in a previously described (Gunn et al. 1997) chronically instrumented near-term fetal sheep model. In the first six hours after rewarming, electrical seizures were detected in five of nine cooled and only one of 13 sham cooled animals ($p<0.05$, Fisher exact test). Although a significant increase in electrical seizures was evident in the cooled group between two and five hours after rewarming, this difference peaked at two hours and there was no difference between the groups after six hours. Furthermore, for both groups the duration of seizures was short, i.e. less than 30 seconds, and there was no significant difference in either the duration or amplitude of seizures between the groups. Also there was no suggestion that the rebound seizures were associated with a decrease in the protective effect afforded by cooling; on histology the parasagittal neuronal cell loss was significantly more in the controls than in the cooled fetuses.

Clearly there are differences between the very slow rewarming performed during clinical studies in human infants and the rapid rewarming in the sheep model. Indeed in the cooled sheep fetal extradural temperature rose from a mean of 32.5 °C to 39.4 °C by a mean of 47 minutes after the start of rewarming. Nevertheless the animal data indicate that rapid rewarming following cooling was associated with a significant but transient increase in EEG defined seizures. Seizures commonly occur in both animal models of NE (Gunn et al. 1997; Gunn et al. 1998b) and affected infants undergoing cooling (Battin et al. 2003; Shankaran et al. 2002) but are characteristically most severe in the first couple of days then resolve. Cooling is also noted to decrease the frequency of seizures (Thoresen M 2003; Tooley et al. 2003). Accordingly it seems likely that in the clinical situation, using a slow rate of rewarming, that these episodes were true rebound seizures. Animal studies confirm that rapid rewarming may have adverse affects on the nervous system. In adult rats rewarmed quickly over 30 minutes an increase in extracellular glutamate and lactate is reported (Nakamura T 2003) and rewarming over 15 minutes compared with 90 minutes after one hour of hypothermia may aggravate axonal injury (Suehiro E 2001).

The potential protective effects of hypothermia that are lost during rewarming include both decreased release (Busto et al. 1989; Nakashima 1996) and accumulation (Thoresen et al. 1997) of excitatory amino acids. In animal models cooling increases both the threshold for induced seizures (Lundgren J 1994) and is associated with reduced numbers of seizures (Tooley et al. 2003). Removal of such protective effects by rewarming may increase

neuronal excitability. Additionally given that hyperthermia reduces the threshold for seizures (Lundgren J 1994) any overshoot in brain temperature could contribute to rebound seizures. Notwithstanding this the infant described in the case report had not reached neutral temperature let alone had overshoot when the seizure occurred.

In summary rebound seizures appear to be a real phenomena with several possible contributing pathways, which may not be mutually exclusive. Cooling may suppress the damaging processes or perhaps secondary deterioration may be delayed by the hypothermia. The lack of relationship between the rebound seizures in the first six hours and the subsequent degree of neuronal loss suggests that if seizures do occur this is not necessarily associated with an adverse long term outcome. Certainly the outcome of the clinical case was favourable and there was significantly less damage in the cooled animals suggesting protection. Moreover, in the sheep work the two cooled fetuses that demonstrated the most rebound activity were amongst the least damaged. However, in clinical practice it is clearly important to warn families that rebound seizures may occur. Such events are disturbing to a family that is already stressed.

6.3 Abnormal sagittal sinus blood flow in a term infant following a perinatal hypoxic ischaemic insult.

A pilot study of whole body hypothermia reported an unusual pattern of findings including sinus thrombosis on follow-up MRI in three of the 16 infants cooled (Azzopardi et al. 2000). The exact causation of the sinus thrombosis in

these cooled infants is unclear but this report describes abnormally sluggish or absent superior sagittal sinus (SSS) flow, diagnosed with ultrasound, in three term infants following HI insult and postulates a link between these findings and sinus thrombosis.

Case one

A 2320 gram growth restricted female infant was delivered by emergency LSCS for fetal distress and meconium staining of the liquor. The mother was a healthy 31-year-old Caucasian woman. The pregnancy had been considered unremarkable until 40 weeks gestation when spontaneous labour occurred. At delivery the infant was noted as growth restricted but otherwise stable and required no resuscitation. Cord arterial blood gases revealed a pH of 7.15 and a base deficit of 11.8 mmol/L. On review at four hours she had marked tachypnea and central cyanosis with an oxygen saturation of approximately sixty percent. She was given oxygen via a head box and required 80 % oxygen to achieve reasonable oxygenation. A decision was made to transfer her to the tertiary neonatal unit for further care. During the transport she deteriorated further. On arrival she was in poor condition and required immediate intubation and ventilation with high pressures and 100 percent oxygen. The arterial gases on arrival were pH 7.15 and base deficit of 10 mmol/L.

The neonatal course was complicated by an episode of pulmonary haemorrhage and persistent pulmonary hypertension. Echocardiography on day two demonstrated significant tricuspid regurgitation and confirmed

persistent pulmonary hypertension but cardiac contractility was judged to be satisfactory. She required ongoing ventilation with 100 percent oxygen, high ventilation pressures and nitric oxide and was eventually extubated on day eight and weaned into air on day 10. Inotropic support with dopamine and dobutamine was required from day two until day nine.

Cranial ultrasound scan performed on day two of life demonstrated normal anatomy with no evidence of haemorrhage but absent blood flow within the SSS as documented by both colour and pulsed Doppler ultrasonography (Figure 5). The RI in the anterior cerebral artery was 0.9. Follow-up ultrasonography at one week of age (Figure 6) showed normal appearance of the SSS with flow documented throughout its extent. The intracranial anatomy on ultrasonography remained normal and further imaging with CT showed no parenchymal abnormalities. On follow-up at 18 months physical examination and developmental assessment were within normal limits.

Case Two

A 5000g male infant was born to a 32-year-old Caucasian woman who was in good health. Her only previous child had been born by normal vaginal delivery two years previously and weighed 4600g at birth. Spontaneous labour occurred at 40 weeks gestation and, although the fetus was considered large, vaginal delivery was attempted. Delivery was complicated by shoulder dystocia. The infant received resuscitation with positive pressure ventilation but did not require cardiac massage. Apgar scores were given as 0 at 1, 3 at 5, 4 at 10 and 5 at 20 minutes respectively. Umbilical cord gases revealed a

pH of 7.18 and a base deficit of 6 mmol/l but the infant developed marked NE characterised by irritability and abnormal tone.

Cranial ultrasound scan performed at two hours of age demonstrated normal anatomy with no evidence of haemorrhage but absent blood flow within the anterior part of the SSS was documented by both colour and pulsed Doppler ultrasonography. There was also abnormal decreased flow documented in the posterior segment of the SSS. The RI in the anterior cerebral artery was 0.86. Selective head cooling with mild hypothermia was commenced at four hours and continued for 72 hours. The encephalopathy was complicated by seizures that required treatment with phenobarbitone, phenytoin and paraldehyde before abating. Follow-up ultrasonography at one week of age showed normal appearance of the sagittal sinus with flow documented throughout its extent.

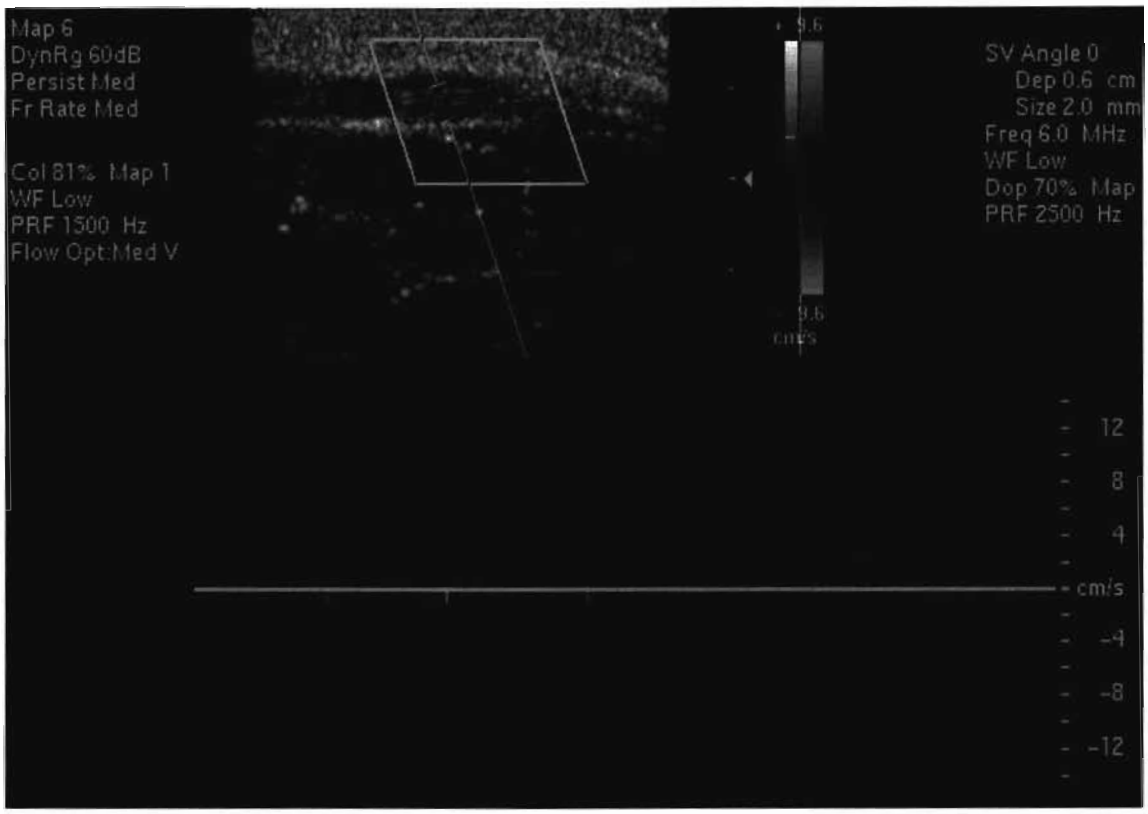


Figure 1. Initial examination of case one with Colour Doppler of the SSS in the sagittal plane. There is no demonstrable flow within the vessel lumen and absent venous waveform obtained on Pulsed Doppler of the SSS.

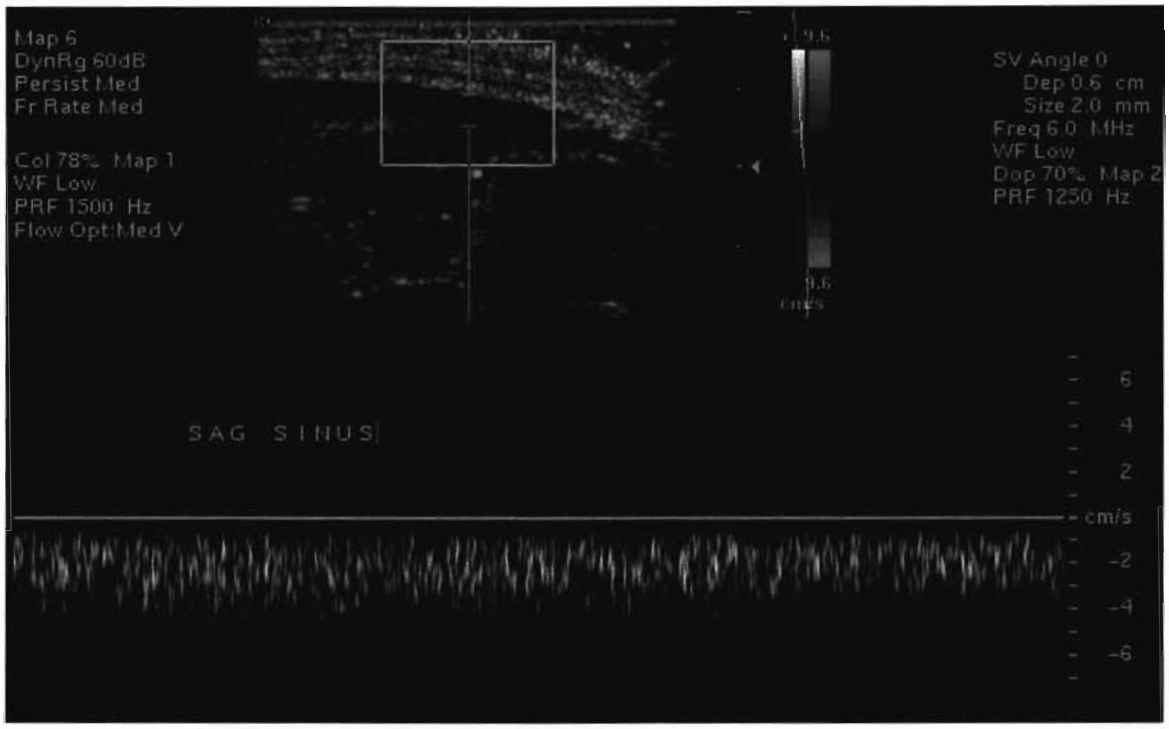


Figure 2. Interrogation of the SSS in the sagittal plane taken six days later. There is now flow within the lumen of the vessel and a normal pattern demonstrated on Pulsed Doppler.

CT scan at this stage showed a small amount of subdural blood but no parenchymal abnormalities and EEG showed mild abnormality but no seizures. At 18 months the physical examination and formal developmental assessment were within normal limits.

Case Three

A 4500g male infant was born to a 34-year-old, gravida three, woman who was in good health and booked with an independent midwife. The obstetric history was complicated by a previous child who required neonatal intensive care following a forceps delivery. At 29 weeks gestation a glucose tolerance test was performed to exclude gestational diabetes and, in view of the suspected large size, labour was induced at 38 weeks. The delivery was complicated by shoulder dystocia with the head delivering some 20 minutes prior to the shoulders. At birth he was noted to be pale and floppy with no signs of respiratory effort or heart rate. Resuscitation included intermittent positive pressure ventilation, prolonged cardiac massage, endotracheal and intravenous adrenaline plus intravenous sodium bicarbonate and dextrose via an emergency umbilical venous catheter. There was no sign of a cardiac output until 23 minutes of age when a heart rate was detected and at half an hour of age he was noted to have irregular gasping respirations. Umbilical cord gases demonstrated a marked metabolic acidosis with a pH of 6.75 and base deficit of 26 mmol/L.

On admission to the neonatal unit he was in very poor condition with poor perfusion, absent tone and irregular gasping respiration. He was ventilated in 100% oxygen and loaded with 20mg/kg of phenobarbitone for probable seizure activity. Heart rate and initial blood pressure measured by a cuff were within the normal range. Following umbilical artery catheterisation an invasive blood pressure was measured and found to be very low at 23 mmHg. Despite extensive fluid and pressor support including normal saline, whole blood, dopamine and adrenaline infusions his blood pressure remained intractably low.

At three hours of age a cranial ultrasound examination was performed which demonstrated normal anatomy, no evidence of haemorrhage and normal resistive index but no demonstrable flow in the superior sagittal sinus. At the time of scan the mean invasive blood pressure was 27 mm Hg and echocardiogram performed soon after demonstrated poor contractility of the myocardium with very low cardiac output. Physical examination revealed profound hypotension, poor capillary refill and a lack of any responsive or spontaneous movements. Tendon jerks were depressed and the pupils were fixed but mid sized. These appearances were consistent with severe hypoxic ischaemic encephalopathy with multi-system involvement particularly hypotension, and disseminated intravascular coagulation (DIC) (INR > 10, fibrinogen < 0.4) with spontaneous bleeding. In view of the very poor prognosis and deteriorating condition that had not responded to maximal therapy the supportive care was discontinued and he died soon thereafter. The family did not consent to post mortem examination.

6.3.1 Discussion

These cases demonstrate that absent or very low flow in the SSS may occur in an asphyxiated term newborn infant early after hypoxic ischaemic insult. Although sagittal sinus thrombosis has been reported on imaging in association with apparent perinatal asphyxia (Shevell MI 1989; Voorhies TM 1984; Wong et al. 1987) demonstration of initially absent flow without clot formation followed by return of normal flow, at one week of age, is unusual.

One possible implication of this finding is that the unusual pattern of MRI abnormalities with cerebral sinus thrombosis in three of the 16 infants, in a pilot study of whole body hypothermia (Azzopardi et al. 2000) was secondary to asphyxia rather than the cooling. In the infants described no evidence of thrombosis was seen on ultrasonography and the CT scan did not demonstrate signs of subcortical, thalamic, or basal ganglia haemorrhage. These imaging findings together with the good long-term outcome are in contrast to the described outcome of SSS thrombosis. Although thrombosis followed by recanulation has been described (Govaert P 1992) this does not appear to be the case with either of the surviving infants as no clot seen was seen in either the early or one week ultrasound scans. Manipulation of the neck (Cowan and Thoresen 1985) and calvarial moulding (Newton and Gooding 1975) are also excluded as causes of low flow due to the manner in which the scan was performed. In animal models blood flow within the SSS has been shown to correlate well with blood flow into the brain and to rapidly reflect changes in this blood flow (Grant DA 1995). In the current series of infants it is probable that the abnormal pattern seen was the result of

decreased cerebral blood flow either at the time of the insult or in the period following it. Indeed one infant was profoundly hypotensive at the time of investigation. It is interesting to note that although the infant was sick the RI obtained from the anterior cerebral artery was normal at the time of ultrasonography. RI has previously been used to assist in prediction of outcome following significant asphyxia in term infants. After an asphyxial insult there is a lag period that is followed by impaired cerebral artery autoregulation and increased cerebral blood flow. Specifically there is vasodilatation resulting in a relatively high diastolic flow relative to systolic flow giving a decrease in RI. Typically in the studies using this technique to predict longer-term outcome, assessment has been performed at 24-48 hours and it is likely that the very early assessment in our infant was prior to any changes in RI developing.

The normal patterns of blood flow within the SSS of a newborn infant have been established using ultrasound and Doppler (Taylor 1992) (Bezinque SL 1995). Ultrasonography may also be used to diagnose sagittal sinus thrombosis in neonatal period (Govaert P 1992) (Bezinque SL 1995). MRI is a sensitive non-invasive method of detecting cerebral venous thrombosis but may be impractical to perform on a sick newborn infant. CT scan is unreliable in making the diagnosis of sagittal sinus thrombosis in infants with a high false positive rate for hyperdensity in the posterior falx (Kriss VM 1998). Thus, in sick infants with multi-system effects of HI insult ultrasonography, performed at the bedside, may be the modality of choice for the interrogation of SSS blood flow.

Although the relationship of the abnormal flow characteristics to subsequent thrombosis is unclear, it is true that venous stasis contributes to thrombosis. Studies of six severely asphyxiated infants using digital intravenous angiography have demonstrated venous thrombosis indicating that occlusive vascular disease was a feature of severe perinatal asphyxia (Lee BC 1984; Voorhies TM 1984). Given these data and the report of unusual venous thrombosis following whole body hypothermia there may be a role for an early ultrasound assessment of the SSS in infants with perinatal asphyxia particularly if hypothermia is being considered. However, it is conceded that data on this is very limited and further study would be needed to document the effect in a larger numbers of babies.

It is not possible on the data available to comment on the implications of an abnormal or absent flow pattern in the SSS. This investigation was performed on an ad hoc basis and not systematically on all infants recruited to cooling studies. Data are available for only nine infants of whom five were studied within the first 24 hours after birth. Although abnormal patterns were described in three infants it is not clear whether there was a degree of selection involved and what proportion of infants would have an abnormal pattern if a larger series was performed. All studies performed outside of the first 24 hours (range 2-4 days) were normal. It is also not clear if early studies of SSS flow would have any role in prognostication. Certainly no clear link is established on the current data.

6.4 Sclerema neonatorum

Sclerema neonatorum is an uncommon disorder that may be associated with hypothermia. It is characterised by firm subcutaneous oedema in newborn infants. There is an abrupt onset with diffuse and generalised hardening of the skin, which becomes stony in consistency, cold and non-pitting. The extremities can be involved at first but generalised involvement occurs within four days and the appearance resolves within two weeks. Low environmental temperature alone can produce the injury (Margileth AM 1994) but it has also been associated with sepsis and other serious illness without hypothermia (Brescia and Tartaglione 1954; Hughes WE 1948; Lindenberg et al. 1987; Sato et al. 1977).

A 37 week gestation 3080 gram infant developed sclerema neonatorum following hypothermia. The mother was a 30 year old primigravida in good health and pregnancy was uneventful until fetal movements decreased 24 hours prior to delivery. The infant was born by emergency LSCS for 24 hours of decreased fetal movements and a non reassuring CTG. Resuscitation included bag and mask positive pressure ventilation but not cardiac massage or drugs. Apgar scores were 2 at 1, 4 at 5 and 7 at 10 minutes respectively. Cord gases revealed a pH of 7.07 and base excess of –16 mmol/l.

Because hypothermia is a proposed neuroprotective therapy that is undergoing assessment in several clinical trials, one of the transport team decided to keep the infant cool by turning off the transport incubator. On

arrival at the neonatal unit, age three hours five minutes, his core temperature was 34.2 °C. He was judged not to be encephalopathic and did not qualify for a clinical trial of cooling. However, his subsequent clinical course was complicated by marked generalised sclerema and mild thrombocytopenia persisting for 10 days.

6.4.1 Discussion

Sclerema neonatorum (SN) is a disorder of the subcutaneous fat in sick neonates. In an infant, fat has a higher saturated-to-unsaturated fatty acid ratio compared to adult fat. Prematurity, hypothermia, shock, and metabolic abnormalities have been postulated to further increase this ratio, possibly due to precipitation of fatty acid crystals within the lipocytes. SN is well recognised to occur in association with hypothermia but may also be secondary to serious illness including sepsis. Because SN is associated with serious underlying disease, the mortality in historical series is high, often over 70 % (Hughes WE 1948). If the underlying disease is treated successfully, the skin softens and returns to normal within a couple of weeks.

Subcutaneous fat necrosis of the newborn (SCFN) is the major differential diagnoses of SN. It is an uncommon and transient disorder in which focal areas of fat necrosis cause nodular skin lesions. They are initially discrete but may fuse to form large plaques and the overlying skin is often red or bluish-red. The onset is usually within the first three weeks of life and new nodules may continue to develop for a week or more. The exact pathophysiology is not known and few epidemiological data are available. However, the infants who develop SCFN are generally full-term or post-term and there is an association

with both asphyxia (Schulzke et al. 2005) and exposure to cold. Examples of the latter include induced hypothermia for cardiac surgery (Chuang et al. 1995) and ice pack exposure for treatment of supra- ventricular tachycardia (Craig JE 1998). It is a self limiting disease in which the lesions appear quickly and disappear by several weeks of life. Usually, in contrast to SN the infant does not have serious underlying illness. However, the condition has occasionally been fatal, particularly when visceral fat has been involved (Hicks et al. 1993). Clinically the cutanaeous symptoms are associated with thrombocytopenia and hypercalcaemia that may present late and be associated with seizures. Skin biopsy is the best way to differentiate from SN (Schulzke et al. 2005).

In one report (Wiadrowski TP 2001), a term female infant with severe meconium aspiration and perinatal asphyxia, developed early woody erythema in the first 24 hours followed by red-purple nodules on day six. The infant had under gone surface cooling for intended neuroprotective purposes. Although clinical findings were initially suggestive of cold panniculitis, subsequently clinical course and histological findings suggested subcutaneous fat necrosis of the newborn. Interestingly, the phenomenon was also reported in two infants with perinatal asphyxia treated with ice cold water immersion in the 1960's (Duhn et al. 1968). However, it is uncertain in both of these reports whether it was the initial asphyxia or the subsequent treatment that results in the condition. Moreover, given the rare occurrence, considerable numbers would be needed to answer this question. As the outcome is usually good it should not preclude a sensible program of

treatment with hypothermia provided appropriate research and audit is performed. The purpose of reviewing the current case is as a reminder that hypothermia does have potential side effects. In addition to dermatological problems these may also include hypoxia, altered coagulation profile and metabolic disturbance.

7 Review of Infants Not Recruited To The Coolcap Trial

7.1 Background

The utility of clinical scoring systems and associated investigations to predict outcome and aid the selection of infants for intervention with hypothermia was appraised in the literature review. However, there are some unresolved issues with regard to the identification of those infants who would benefit from the intervention. An assessment of the balance between potential harms and benefits is essential in the process of translating research data into routine clinical practice. Vis-à-vis selective cerebral hypothermia, previous work has demonstrated that this is well tolerated (Battin et al. 2003; Gunn et al. 1998a), thus if there was improvement in neurodevelopmental outcome this should outweigh potential systemic adverse effects. The CoolCap trial (Gluckman et al. 2005) further supports this, in that no excess adverse effects were reported and there was a significantly improved outcome on selected sub-group analysis. However, infants who do not satisfy the aEEG selection criteria may be disadvantaged if they are excluded from possible intervention yet still have a risk of adverse outcome.

In a controlled trial it is logical to recruit a narrow group of infants with the greatest chance of demonstrating the desired treatment effect. Although for clinical purposes it may be preferable to select a wider group of infants with *potential* to benefit from the intervention, particularly if the intervention was well tolerated or had minimal risk of adverse side effects.

Although aEEG may provide useful prognostic data before six hours of age (al Naqeeb et al. 1999; Hellstrom-Westas et al. 1995; Toet et al. 1999) there are only limited data on the negative predictive value when used at this early stage and some trials of hypothermia use solely clinical selection criteria (Shankaran S 2005). In order to evaluate further the merits of selection using aEEG criteria, particularly with reference to extension into routine clinical use, it seems appropriate to review the cohort of term infants from NWH who presented during the period of the CoolCap trial (Gluckman et al. 2005) with clinical features of NE but had a normal or only mildly abnormal aEEG and thus were not recruited into the trial. There are two aims to this review. Firstly, to compare the demographic and initial presenting clinical data from this cohort with similar data obtained from infants entered locally into the randomised trial, to determine if differences between the two groups were solely in aEEG criteria or whether clinical and demographic data also differed. Secondly, to describe the clinical course and determine the rate of poor outcome in this cohort who were assessed but did not fulfil the aEEG criteria. From this it would then be possible to calculate the negative predictive value (NPV) for the aEEG and to determine how many, if any, infants would be

denied a possibly helpful treatment if the trial inclusion criteria were applied to routine clinical use.

7.2 Method

Recruitment to the CoolCap trial at NWH proceeded from July 1999 until December 2001. During this period, term infants with a history suggestive of perinatal asphyxia were prospectively evaluated for signs of NE following admission to the neonatal unit. Infants were assessed for entry in to the trial against clinical and aEEG criteria. The clinical entry criteria were the presence of an encephalopathy in an infant greater than or equal to 36 weeks gestation in combination with a clinical history consistent with perinatal asphyxia plus one of the following 1) 10 minute Apgar score below 5; 2) requirement for ongoing resuscitation at 10 minutes; 3) cord/1st arterial pH < 7.00. Infants with obvious major congenital abnormalities or those who presented to NWH after six hours of age were excluded.

Infants who fulfilled the clinical entry criteria underwent aEEG. This was classified as normal, moderately or severely abnormal (al Naqeeb et al. 1999). Normal was defined by an upper margin of band of aEEG greater than 10 µvolts and lower margin greater than 5 µvolts; moderately abnormal by upper margin greater than 10 µvolts and lower margin equal to or less than 5 µvolts; and suppressed (severely abnormal) by upper margin less than 10 µvolts and lower margin equal to or less than 5 µvolts. In addition the trace was characterised by the presence or absence of seizures based on reported criteria (al Naqeeb et al. 1999) describing changes in the upper and lower trace margin.

The infants were classified by the most severely abnormal part of the aEEG collected. Infants classified as having a moderately abnormal or worse baseline or with manifest seizures on aEEG were entered into the randomised trial. The trial entry criteria stated that infants should have at least a moderate encephalopathy. It is recognised that in the initial stages clinical features may still be evolving; therefore some infants with features more consistent with mild encephalopathy but early in their clinical course or with borderline mild to moderate encephalopathy were considered to be potentially eligible so underwent observation and aEEG assessment. Infants who demonstrated only normal or mildly abnormal aEEG were observed for any deterioration in aEEG until the five and half hour cut-off. If they did not qualify within the prescribed time frame they received standard neonatal care. As these infants were not entered into the trial the subsequent management and follow-up was determined solely by the clinical team. Outcome and morbidity data were collected from review of the clinical records. It was not intended to use these morbidity data as a comparator with the trial infants; the groups were selected and treated differently. Therefore, comparison between both groups was limited to initial presentation and demographic data. Outcome and morbidity data for trial infants are reported elsewhere (Gluckman et al. 2005).

7.3 Results

Sixteen infants with encephalopathy who underwent aEEG but did not qualify for cooling were identified. In the same time period 11 infants were entered into the CoolCap trial.

7.3.1 Demographic data and presentation

There were no significant differences in either demographic data or presenting clinical data including Apgar scores, cord gases or resuscitation requirements between the two groups of infants (Table 15).

Table 15. Demographic and presenting clinical data for the two groups of infants.

	NWH cohort n=16	CoolCap trial n=11	
Gestation (weeks)	39 (36-41)	38 (36-41)	P = 0.67
BW (g)	3127 (633)	3405 (700)	P = 0.30
Maternal age (yrs)	30.12 (6.19)	31.81 (5.63)	P = 0.48
Male : Female	10:6	6:5	P= 0.17
Outborn	7 (44%)	6 (55%)	P = 0.30
LSCS	6 (37.5 %).	5 (45%)	P = 0.15
Base deficit (mmol/l)	12.4 (5.8) (n=9).	20 (3.4) (n=10)	P = 0.14
Apgar @ 1 min.	2 (0-4)	1 (0-2)	P = 0.79
Apgar @ 5 min.	4 (0-7)	4 (0-5)	-
Apgar @ 10 min.	6 (4-8)	4 (0-6)	P < 0.05
IPPV in resuscitation	16 (100%)	11 (100%)	-
Cardiac massage	1 (6 %)	2 (18%)	P= 0.33
Drugs in resuscitation	0	0	-

NWH = National Women's Hospital; LSCS = lower segment caesarean section; IPPV = intermittent positive pressure ventilation

Although there was a subsequent difference in staging of NE between the two groups this was allocated retrospectively after 72 hours and there was sufficient concern about the infants at the time of presentation for them all to be considered worth evaluating.

7.3.2 Neonatal course and neurodevelopmental outcome

7.3.2.1 Duration and timing of aEEG

For the cohort of infants who did not qualify for trial entry based on aEEG, the median (range) time delay between admission to the neonatal unit and starting to collect aEEG data was 128 (15-150) minutes. The median (range) duration of aEEG recording was 1.58 (0.66-3.8) hours. This relatively long period of recording reflects the intention to detect any deterioration in status consistent with becoming eligible for trial entry. Therefore, it substantially exceeds the minimum recording time required for trial entry of twenty minutes.

7.3.2.2 Neonatal morbidity and outcome

In the infants who were not recruited to the trial, the subsequent neonatal course was complicated by significant morbidity in the majority of cases. Respiratory support with ventilation was required for 9/16 (56%) infants for a median of two (1-8) days and a further two (12.5%) received CPAP for one day. In addition, 14/16 (87.5%) infants received supplementary oxygen for a median of two (1-77) days. Four infants (25 %) were diagnosed with meconium aspiration syndrome and pulmonary hypertension and of these three (19%) were treated with nitric oxide. Hypotension occurred in six infants (37%) and required inotropic support in four (25 %) cases. Oliguria (defined as <1ml/kg/hr) occurred in 15 (94%) of the infants with a mean (SD) creatinine of 0.10 (0.06) mmol/l. Clinical seizures occurred in five (31 %) of infants and clinical coagulopathy in only one (6 %) of the infants.

Thirteen infants (81 %) survived to discharge and three infants (19%) died. The neonatal encephalopathy was graded as mild in 11 infants, moderate in three and severe in two. Both of the infants with a severe encephalopathy and one infant with a moderate encephalopathy died. Of the two deaths in infants with a severe encephalopathy, one was *in extremis* and died from multi-system failure on day one so was not enrolled. The other infant had an encephalopathy that worsened over time. Between three and five hours the aEEG was normal with no seizures (Figure 7) but by seven hours there were overt clinical seizures. This infant had a severely abnormal CT scan and EEG at a week and died at five weeks of age.

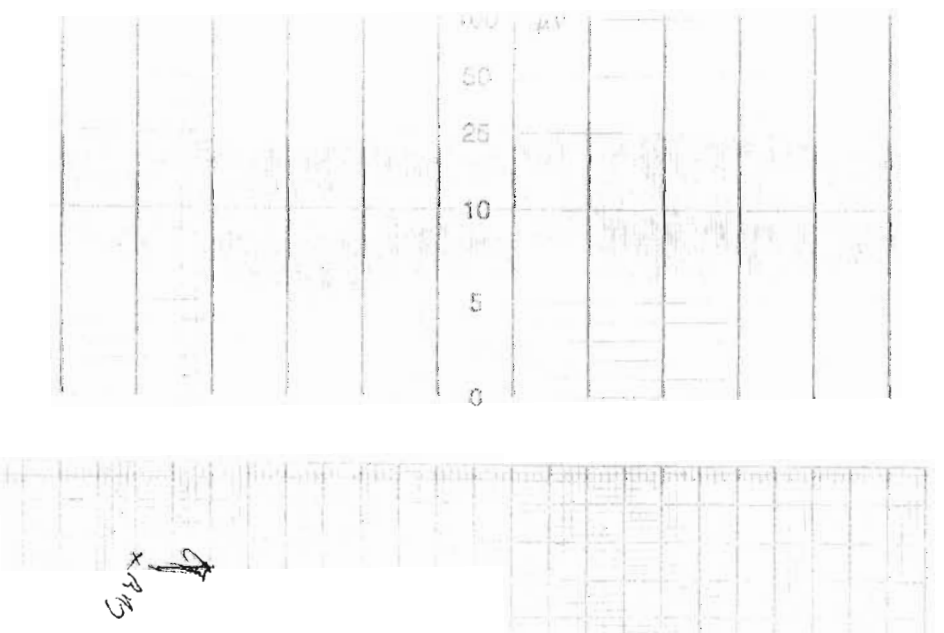


Figure 7. The aEEG trace obtained in the infant who deteriorated. It shows a good base line with minimum levels well above 5 microvolts and no overt seizures. One period of movement artefact was associated with handling for chest x-ray.

The death in an infant with only moderate encephalopathy occurred on day four and was due to an acute respiratory deterioration associated with

pulmonary hypertension and meconium aspiration syndrome. Consent was not given for post mortem examination in any of these three infants who died. Of the two infants with a moderate encephalopathy who survived, one had a normal CT scan and EEG at one week of age and had normal clinical examination findings. Although he was lost to follow-up hence not formally assessed at 18 months there were no overt neurodevelopmental problems when reviewed at three years of age, at the local paediatric hospital, for respiratory problems. The other infant had an abnormal CT scan with areas of low attenuation seen in the white matter consistent with watershed type injury bilaterally. The EEG was also mildly abnormal with an excess of sharp transients. On clinical follow at 18 months there was mild speech delay but no sign of motor impairment. All infants with mild encephalopathy had a normal examination at the time of NICU discharge, reassuring investigation with EEG and/or imaging and were deemed normal on follow-up clinical examination although some were discharged before 18 months due to good clinical course (Table 16).

Table 16. Clinical outcome details for the five infants with moderate or severe encephalopathy.

HIE stage	CT	EEG	Alive/dead	Cause of death	Impairment
3	Not done	Not done	Died day 1	Multi-system failure	N/A
3	Abnormal	Abnormal	Died day 35	Neurological	N/A
2	Not done	Not done	Died day 4	Respiratory	N/A
2	Normal	Normal	Alive	N/A	No
2	Abnormal	Mildly abnormal	Alive	N/A	Speech delay

If the infant who presented in extremis and the infant who died from a respiratory death are excluded then the NPV for predicting adverse neurological outcome or death following NE using clinical criteria plus aEGG is 93%.

7.4 Discussion

The correct selection of infants for intervention following perinatal HI insult is a major challenge. The use of clinical assessment in combination with aEEG before five and a half hours of age, as per the CoolCap trial, appeared to perform well with an acceptably low false negative rate. Three infants who were not selected for trial entry subsequently died. One was in extremis at presentation and had a limited period of aEEG recording hence would be excluded on the basis of futility and incomplete assessment. One other died from respiratory causes after a moderate encephalopathy. Thus when the clinical condition permitted a complete period of assessment, only one infant who died secondary to neurological compromise and one with mild neurodevelopmental impairment were not selected.

Although the neurodevelopmental outcome for the infants not selected for the trial was reassuring it is important to note that these infants did have overt neurological and multi-system morbidities in neonatal period. Specifically nearly a third developed clinical seizures during the neonatal course, over a half were sick enough to require ventilation and several required blood pressure support or treatment with nitric oxide at some time during their admission. Therefore, it is evident that the utmost care must be taken when counselling parents, as short term aEEG monitoring in combination with

clinical criteria does not exclude significant neonatal morbidity. Moreover, a small number of infants who do not exhibit major aEEG abnormality prior to six hours of age will subsequently die or have severely abnormal outcome secondary to neurological compromise. In this aspect our results are consistent with other published data that suggest a negative predictive value of circa 90 to 95 percent (al Naqeeb et al. 1999; Hellstrom-Westas et al. 1995; Toet et al. 1999).

Of note, it was not possible to differentiate the two groups of infants solely on the basis of the presenting clinical data without aEEG. Although there was a likely difference in degree of insult as demonstrated on pH and base deficit there was overlap between the two groups. This review includes some infants who subsequently only had a mild encephalopathy, hence would be expected to have a good outcome. However, it equates to how the technique could be used in practice to back up clinical selection, particularly in borderline cases or where there are possible seizures clinically and the aEEG trace confirms or excludes this.

The false negative cases, defined as those with reassuring aEEG but adverse neurological outcome, may be due to a variety of causes. The most pertinent of which is the evolving nature of the encephalopathy whereby ongoing deterioration occurs outside of the first six hours. This appears to be the most likely explanation for the infant we describe who deteriorated after the six hour cut off and died. Alternative explanations are that the injury occurred earlier in time, probably prior to delivery, and that recovery of the aEEG had occurred

by the time it was sampled or that focal, primarily subcortical injury occurred. While the former are unlikely to benefit from intervention it is possible that primarily subcortical injury could have an improved outcome if cooling is used.

There are some limitations of this report. Firstly, the neurodevelopmental follow-up was limited in some of the infants with mild encephalopathy. However, the outcome for such infants is consistently reported to be good (Amiel-Tison 1978; Levene et al. 1986; Volpe 2001); hence further follow-up visits that were not clinically indicated could not be justified for infants not enrolled in a clinical trial. Unfortunately due to this limitation we are unable to make comment on the presence or absence of subtle cognitive impairment on follow-up. Secondly, for access reasons CT was the first line imaging modality rather than MRI, which may have been more sensitive in detection of subtle injury or more accurately time the insult. However, it is our experience that CT does not miss major lesions and has performed well in assisting in the prediction of outcome following NE (Battin MR 2001). Thirdly, despite the fact that interpretation of the aEEG may be learnt quickly and has a high interobserver agreement it is apparent from a recent study that seizures that are focal, less than one minute duration or low amplitude may be missed by this technique (Rennie et al. 2004). Although seizures on aEEG would have satisfied the trial entry criteria there are no data on the importance of missing this type of seizure apropos selection for intervention. This may require further investigation, as there is currently no published evidence of a proven benefit from intervention in infants with mild NE. Cognitive impairments may occur in the absence of motor impairments following moderate or severe NE (Cowan

et al. 2004; Marlow N 2005) but further work would be required before comment can be made on the role of hypothermia in preventing these impairments.

In conclusion, selection for clinical and trial purposes may differ. Nevertheless, the approach using assessment by clinical staging and aEEG before five and half hours of age appears to be feasible. Data can certainly be collected and interpreted in the appropriate time period. Furthermore it selects infants who have NE plus significant systemic morbidity. Although the NPV is not perfect it performs considerably better than clinical estimate alone. However, infants who are not selected by this process still have significant neonatal morbidity in terms of requirement for neonatal intensive care and a small number of infants will have serious morbidity or mortality despite a reassuring aEEG. This is important particularly when counselling parents. Finally, on currently available data it is not possible to comment on the potential for long-term benefit from selective hypothermia in this small number of infants, who are excluded from intervention by the aEEG criteria but then manifest an adverse neurodevelopmental outcome.

8 Summary of Findings, Discussion and Future Research Direction

It was established in the literature review that although attempts to improve outcome by altered obstetric practice have been laudable the results are disappointing (Grant et al. 1989). Therefore, in order to pursue the definitive aim of no pregnancy having an adverse outcome, either death or impairment, secondary to perinatal HI injury it is necessary to pursue the alternative approach of postnatal intervention to salvage high risk infants.

" That it will ever come into general use, notwithstanding its value, is extremely doubtful; because its beneficial application requires much time and gives a good bit of trouble both to the patient and the practitioner; because its hue and character are foreign and opposed to all our habits and associations"

London Times 1834

The quotation above refers to the invention of the stethoscope but could until relatively recently have been applied to the novel technique of selective cooling. Although the technique is in stark contrast to our previous teaching of keeping infants warm it can no longer be considered "foreign and opposed". This thesis reports a series of studies centred on its use as an intervention following HI injury in the term infant. The chapters examine key steps toward the eventual aim of intervention to improve clinical outcome for affected infants with this early clinical experience fulfilling an important role in bridging

the gap between basic science and adequately powered randomised controlled trial. Naturally, other trials have followed (Shankaran S 2005) and these have now been the subject of meta-analyses (Jacobs et al. 2007; Schulzke et al. 2007; Shah et al. 2007), which confirm benefit both in terms of survival and prevention of neurological handicap. The current chapter will summarise the important findings of the previous chapters, discuss the implications and offer future research directions.

8.1 Summary Of Important Findings And Their Implications

An important first step towards the utilization of selective hypothermia as a clinical intervention was to show that the technique was both feasible and well tolerated. This was accomplished by the work described in Chapter 3. Although previous safety data were available (Gunn et al. 1998a) the level of cooling had been very cautious and considered unlikely to provide effective neuro-protection. Chapter 3 reports that a rectal temperature of 35 ^{o}C and 34.5 ^{o}C in term infants with encephalopathy was not associated with an excessive requirement for respiratory support in the cooled infants compared with the controls. Although infants demonstrated a decrease in heart rate during induced hypothermia, this was expected and there was no significant change in blood pressure during either the cooling or rewarming phase. Nonetheless, prolongation of the QT interval is reported in hypothermic infants (Debillon et al. 2003; Gunn et al. 1999b) so it has been considered prudent to perform continuous close cardiac monitoring during hypothermia, with formal ECG if the heart rate was below 70 bpm. Additional reassuring findings were that neither major electrolyte disturbance; hypoglycaemia or haematological

changes, including excessive haemorrhage were observed during hypothermia. However, the small number of infants studied meant that a type two error was possible with potential for uncommon sequelae to be missed. Thus it was important that the two large multicentre studies, CoolCap trial (Gluckman et al. 2005) and NICHD (Shankaran S 2005), also reported no significant difference in these or other morbidities between the cooled and control infants. Lastly in small pilot series, the severity of encephalopathy or other early outcome measures may vary between the groups by chance. Indeed, this was likely the explanation for the trend towards a discrepancy in seizure frequency between control and cooled infants seen in data reported in pilot data but not evident in the larger CoolCap trial (Gluckman et al. 2005).

The second step towards making selective hypothermia a clinically viable intervention was to collect appropriate follow-up data. Chapter 4 describes the first follow-up study of neurodevelopmental status in infants who had received selective cerebral hypothermia (Battin et al. 2001). Importantly, there was no evidence of any unexpected late adverse effects. The major adverse outcomes reported were cerebral palsy, developmental delay, microcephaly, hearing impairment and seizure disorders, consistent with the well known effects of perinatal hypoxia-ischaemia (Robertson and Finer 1985; Robertson and Finer 1993). Moreover, the outcomes of perinatal encephalopathy were closely related to the severity of the encephalopathy, with favourable outcomes in infants with mild encephalopathy (Levene et al. 1986; Robertson and Finer 1993). In infants with moderate to severe encephalopathy, the combined mildly cooled groups demonstrated a trend

towards better outcome. These results provided valuable safety data and confirmed the potential for selective cerebral hypothermia to provide a degree of neuroprotection following perinatal asphyxia. However, the data should not be over interpreted as it did not have adequate power to look at efficacy. A further caveat is that the assessment was performed at a maximum of two years of age. In due course, longer term outcome will be studied but at present there are no data on school age outcome in children who have undergone intervention with hypothermia in the neonatal period. Whilst recognising the need for such data, there is a trade off between the effect of family environment including socio-economic status and any effect of neonatal intervention. The family effect becomes more evident with increasing interval. Thus overall, favourable outcome at 18 months of age can be taken to be reasonably reassuring with regard to major motor handicap although alternative manifestations including cognitive deficit or more subtle motor impairment may arise as more complex tasks can be tested.

The third contribution to the research field was the examination of the utility of CT imaging in term infants with perinatal HI injury. The data presented in Chapter 5 suggest that CT scan performed at one week was a helpful adjunct to clinical staging in predicting neurodevelopmental outcome. This was important as imaging is often used to assist with prognostication but also may be used as a proxy outcome in clinical studies. A normal or only mildly abnormal CT scan at one week appeared to be very predictive of a favourable neurodevelopmental outcome. This high negative predictive value was useful for reassuring parents. However, the study was limited in what it tells us about

mechanism of injury protection or proclivity for injury in specific regions of the brain. In future studies, the use of MRI with its greater precision may have a greater role in identifying the regional patterns of brain injury. MRI may also be used as a proxy outcome in studies that compare efficacy of different methods of induced hypothermia and may also, if combined with MR spectroscopy, provide further insights into the neuroprotective mechanisms underlying these approaches.

The clinical experience, described in Chapter 6, identified three important areas that need exploration in future studies namely rebound seizures, skin problems associated with hypothermia and abnormal flow in SSS. The presence of rebound seizures appears to be a real phenomenon but it is not possible to comment on the frequency of this occurrence based on currently available data. Certainly it will be important to continue to examine this when other multicentre trials are published. Although each trial mandates slow rewarming it is possible that the actual warming regimen will vary due to differences in cooling method. Risk of seizure may also differ due to patterns of remaining injury and variation in inclusion criteria. The role of reinitiating cooling if a seizure occurs on warming also needs to be explored. The skin manifestations of hypothermia including sclerema neonatorum and subcutaneous fat necrosis of the newborn are recognised adverse effects from hypothermia and will be observed in some infants. Indeed one infant with sclerema was reported in the CoolCap trial (Gluckman et al. 2005) and one with subcutaneous fat necrosis in pilot whole body cooling work (Debillon et al. 2003). However, the conditions are both uncommon and transient.

Furthermore they may occur in sick infants that do not undergo hypothermia and it is likely that the long term outcome and mortality would be better than that historically reported. Further comment on their importance would require that data be available from larger numbers of infants when the other trials are published. The final phenomenon that requires further investigation before the implications are clear is that of abnormal flow in the SSS. The normal patterns of blood flow in a well newborn infant are well established (Taylor 1992) (Bezinque SL 1995) but they have not been widely studied in term infants with perinatal HI injury and the clinical implications of abnormal findings are not well elucidated. It is likely that poor SSS flow reflects a low output state, although the relationship of the abnormal flow patterns to subsequent thrombosis is unclear. Additional work is required before a recommendation can be made about the importance of Doppler assessment of SSS blood flow patterns.

Chapter 7 reviewed the outcome of those infants assessed but not recruited to the CoolCap trial. This was a valuable undertaking because the trial entry criteria could potentially be applied to future clinical use. Accordingly, it was considered important to ensure that these criteria would not exclude large numbers of infants with potential to benefit from intervention. With regard to selection of infants, one approach would be to accept that hypothermia was well tolerated hence loose selection criteria and a wide sample would suit. However, there are both resource implications and issues around over treatment of infants with this approach. An alternative approach is that used in the CoolCap trial, where aEEG was used to narrow down the group of infants

selected for intervention. An abnormal aEEG obtained before six hours of age is highly predictive of adverse outcome (al Naqeeb et al. 1999; Toet et al. 1999). Using aEEG may reduce the number of infants with a good prognosis who would be unnecessarily cooled; reduce the number of infants needed to be recruited to a trial, enabling smaller, faster trials; and make it feasible to evaluate different cooling strategies with smaller samples. Conversely, some infants would not be selected who would benefit from cooling and use of an EEG technique even at the cotside increases the complexity of recruitment and may lead to delay in initiation or impaired access to cooling for infants born at peripheral centres. In Chapter 7 it was demonstrated that the aEEG criteria used effectively differentiated between infant groups despite major overlap in presenting clinical features. Using crude assessment of neurodevelopmental status the work suggested that outcome for this cohort, excluded on the basis of their aEEG, was largely favourable but a small number deteriorated and had adverse neurodevelopmental outcome or died and the majority had short term morbidity.

There are other advantages associated with the use of aEEG in selection. Recent advances in cotside EEG technology and the wish to learn more about the condition of the infant's brain, particularly the need to identify infants at high risk of adverse outcome prior to clinical intervention has driven an increase in the clinical use of EEG in neonatology over the last few years. This has two advantages with regard to cooling. Firstly cotside aEEG allows impartial comparison of infants recruited from different centres. Secondly, inexperienced clinical staff with limited extra training may assess infants and

allocate them appropriate treatment. Further research is still required into the relationship between electrophysiological changes and the evolution of NE, particularly with regard to identifying the end of the latent period and the onset of secondary injury. Indeed an alternative role for aEEG could be to exclude some infants from intervention with cooling. In the CoolCap trial (Gluckman et al. 2005) infants with the most serious aEEG abnormality were unlikely to benefit from intervention. At present, local practice in New Zealand and Australia is quite variable, most centres collect some aEEG data but are not routinely using aEEG criteria to select infants for cooling (personal communication M Battin).

8.2 Outstanding Issues

Having outlined the important advances in selective hypothermia following perinatal HI injury it is important to review some aspects of research in this area that are not optimal. One issue is the lack of precision in the terminology used for the degree of hypothermia, terms such as mild, moderate hypothermia are defined in the literature with regard to accidental hypothermia (Danzl DF 1994) but there is no consensus on use for induced hypothermia. In some ways this is similar to the issue raised in the literature review with imprecision in use of terms such as *asphyxia* and *perinatal depression*. This imprecision in combination with differing selection criteria impairs the ability to critically compare outcomes between studies. These issues need to be addressed when defining which population of infants would benefit from intervention.

A second and more vexing issue is the limited ability to detect and document any putative insult. It is well recognised that HI insults vary in severity, duration and timing, in addition the infant or fetus may have varying degrees of resilience, dependent on substrate reserves and previous clinical condition. Such a heterogeneous population presents a significant challenge for both future clinical trials and clinical use. Linked with this is the fact that in clinical paradigm exact timing of the insult may not be clear. Just a quarter of infants with post-asphyxial encephalopathy are exposed to a sentinel event (West C 2005a), and a further 10-25 % have evidence of chronic, antenatal hypoxia (West C 2005a; Westgate et al. 1999). In the remainder an evolving process during labour is probable and the precise timing of injury unclear. Hypothermia is recognised to be most effective if started soon after the insult during the latent phase of injury. At present we are unable to be certain of time of HI injury and take birth as time zero. Minimizing the elapsed time before initiation of cooling requires a proactive approach with education of staff at peripheral hospitals, including making information sheets for parents available; rapid transfer between units; and having staff on call for the assessment and initiation of cooling. It may also mean that methods of employing cooling during transit should be explored for some circumstances. However, it should be recognised that this is a difficult time for parents and entry to a study is a potential source of stress thus adequate time for explanation and discussion is required.

8.3 Future Research Directions

The use of hypothermia following perinatal asphyxia will provide research questions for some time. Some questions will need to be addressed by basic science and animal models but there is much that can only be assessed in clinical trials and their subsequent meta-analysis.

Currently, there are four large clinical trials and several smaller trials that are either underway or recently completed: The CoolCap trial, which uses selective head cooling and has aEEG entry criteria; the Toby trial, which uses whole body cooling and has the same aEEG entry criteria; the NIHCD trial (Shankaran S 2005), which uses whole body cooling and selects on clinical criteria and; the ICE trial that uses whole body cooling and selects on clinical criteria alone. Results from the CoolCap (Gluckman et al. 2005) and NIHCD (Shankaran S 2005) trials have been published but the other two trials have not yet been published. There has been some debate around the need for ongoing cooling trials (Kirpalani et al. 2007) but the current position, in Australasia, appears to be that cooling should be made available outside of placebo controlled trials but should not yet be considered the standard of care (personal communication M Battin). This is likely to be reviewed as the other trials report but is based on the data available from the first two trials totalling over 500 infants. There are some parallels with adult work where two trials of cooling following out of hospital cardiac arrest (Bernard et al. 2002; Hypothermia after Cardiac Arrest Study 2002), reporting less than 400 cases, were sufficient to change clinical guidelines (Nolan JP 2003). On the other hand two major improvements in perinatal care, antenatal steroids (Crowley P

2000) and surfactant use (Soll RF 2000a; Soll RF 2000b), each took several trials and nearly 2500 and 4000 infants respectively before being widely adopted in clinical practice.

There are several important questions to address future work. Some questions such as whether there is variation in the efficacy of therapeutic hypothermia in relation to the specific cause of the HI insult may be possible to address following publication of data from the four aforementioned trials. Specifically it would be useful to compare the size of neuroprotective effect following an acute event such as placental abruption or cord accident with that following a chronic low grade insult.

Other major issues that may be explored in future trials include the optimal degree and duration of cooling for maximum neuroprotection without side effects, the optimal mode of brain cooling (selective head versus whole body), and the applicability of hypothermia in countries with limited resources where NE is a major problem (Cooke R 2005). However, such clinical trials involve recruitment of large numbers of infants hence require much time and money with trials typically studying more than a hundred infants. Some local "add on studies" may also add to knowledge by publishing further clinical data on aspects of care such as the effects of therapeutic hypothermia on drug interactions and drug kinetics or imaging with MRI (Inder et al. 2004; Rutherford MA 2005). Sedation, anticonvulsants and paracetamol all have the potential to interact with temperature and cause excessive cooling. Additionally, there is need to explore bedside markers NE evolution,

particularly identifying the end of the latent period and onset of secondary deterioration.

The role of temperature during the resuscitation and immediate period after birth also needs further work. There is potential for excessive warming during stabilization or transport to modify the injury cascade and subsequent response to hypothermia. Non invasive measurement of brain temperature may allow measurement of regional brain temperature from a baseline obtained prior to birth until after secondary injury ceases. These data would enable studies on the role of maternal temperature and warming during resuscitation to be performed.

Finally, there are two broad areas that need further basic science work before they can be considered ready for clinical trials in human infants. The first of these is further examination of the role of drugs to augment the protective effect of hypothermia or as a means to replicate the protective mechanisms of cooling. It is well accepted in the neonatal literature that hypothermia may modify the adverse effects of HI injury at more than one point, which actually makes it an attractive therapy for clinical use. However, the exact contribution of the different proposed protective mechanisms at different points in the injury cascade are difficult to quantify from the literature on the newborn infant so it is not clear which actions are the most clinically important.

It is likely that future work will focus on either replicating the benefits of cooling or augmenting them using drugs. Calcuim channel blockers have hitherto

been both dangerous and disappointing but the search for other NMDA blockers or drugs that either decrease the effect or reduce the concentration of the excitatory amino acid glutamate is likely to continue. Similarly there should be ongoing research into the use of agents such as allopurinol or other agents that decrease free radical mediated damage and anticonvulsant drugs that decrease ongoing substrate consumption by reducing seizure frequency and/or duration. Attention should also be paid to preserving the blood brain barrier, the use of erythropoietin and inhibition of NOS as other potential treatments that may decrease neurological injury; although there are still considerable obstacles to the use of NOS inhibition. Specifically, the experimental effects of NOS inhibition are not consistent across animal species and vary with agent used, dose, and timing. So currently there is little chance of this being used soon in human trials. Another intervention that is not yet ready for human trials but has much potential is the use of growth factors that may influence apoptosis and/or trigger long term repair via brain plasticity (Sizonenko et al. 2007).

The other area that needs further basic research is the critical examination of the role of hypothermia in immature animal models. Indeed such work is emerging and a recent paper by Tooley (Tooley JR 2005) reported that it was possible to maintain systemic normothermia in piglets while significantly cooling the deeper structures of the brain. Such an approach may curtail side effects associated with systemic hypothermia and may be feasible for premature infants. However, there is considerable work to be done before this is likely to be transferred to clinical studies in preterm infants particularly in the

areas of safety, efficacy and timing of injury. Although previous studies have reported that term infants tolerate cooling, recent randomised pilot data (Eicher DJ 2005) from slightly smaller and less mature infants reports some side effects. Specifically, in infants greater than 2000g and 35 weeks gestation cooled using a blanket to 33 °C rectal for 48 hours an increased rate of bradycardia, inotrope use, plasma and platelet transfusion and pulmonary hypertension treated with nitric oxide occurred in the cooled group. Although pilot data this may represent a threshold for increased side effects with regard to either decreasing gestation or temperature range. However, there were only a small number of immature infants and it is more likely the temperature is responsible with 33 °C being somewhat lower end of range for published studies. Either way a cautious approach to study in premature infants is mandated.

In order to make the most effective progress on these issues there is a need for cooperation in ongoing development of cooling as a clinical intervention. A recent meeting on this subject established a cadre of like minded researchers with common priorities and a cohesive approach. The recommendation of the meeting was that ongoing trials on hypothermia need to be continued as planned; longer-term follow-up of infants who participated in the completed, ongoing, and future hypothermia trials need to be strongly encouraged; institutions who offer therapeutic hypothermia should collect clinical data; future research should consider utilising the existing paradigms or consider data sharing with other researchers to facilitate systematic reviews. Furthermore, national and international registries need to be created that facilitate ongoing assessment of the burden of NE, its treatment and

outcomes. Finally international interest groups of scientists, practitioners and other persons involved in public policy need to be formed for continued evaluation of accumulating evidence in this field.

In conclusion, selective cerebral hypothermia or "head cooling" is a feasible and well tolerated way of providing this intervention. Although further work is required in a number of areas to improve selection of infants and optimise cooling delivery, neonatal care can now offer intervention rather than just supportive care and hope for infants with NE.

9 Appendicies

9.1 Appendix 1: Nursing Guideline For Care Of Infant Undergoing Selective Cerebral Cooling

This document covers the following topics relating to Head Cooling

Overview

A safety study performed from January 1996 to October 1997 of 22 infants at National Women's Hospital, Auckland concluded that mild head cooling (with rectal temperature 36 – 36[5]) of term infants following perinatal asphyxia reduced cerebral temperature and may improve cerebral outcome. Subsequent study using head cooling with rectal temperatures 34.5°-35.5°C has not shown an increased incidence of adverse side effects.

A multi-centred therapeutic trial of prolonged selective head cooling combined with mild systemic hypothermia following hypoxia ischaemia is now planned. It will be a **randomised study** in term infants with encephalopathy following perinatal asphyxia. Infants undergoing selective head cooling (plus mild systemic hypothermia) for 72 hours will be compared with control infants who will have rectal temperatures maintained at 37.0.

The study will be open because for safety reasons the clinical staff must know the rectal temperature of the control and cooled infants to adjust the cooling and heating appropriately. The non-cooled (control) infants will not wear a cap because this might heat the head.

Criteria for entry to Study	Newborn infants: ≥ 36 weeks gestation. With evidence of Encephalopathy **Plus** Umbilical artery pH ≤ 7.09 **or** Apgars ≤ 6 at 5 minutes Infants with major congenital abnormalities are excluded.
Consent for Study	Informed written consent must be obtained (by one of the Investigators) from a parent after a full verbal and written explanation of the study. **An interpreter may be required.**
The age by which baby must enter study	Ideally the infant should be entered as soon as possible and cooling should commence **no later than 6 hours of age.** There is rapid attenuation of neuroprotection with delay in the start of cooling.

The overall responsibility for the clinical care of the infant rests with the attending Neonatologist.
The Investigators will be available at all times (day or night) to discuss specific cooling issues.

Baseline bloods prior to cooling should include gas, glucose, FBC, electrolytes, urea, creatinine, Ca^{2+} albumin, AST and blood cultures. Blood gases and glucose levels required 4 hourly during the initial 12 hours of cooling. Further blood tests as per medical orders.

Ultrasound of infant's head is performed prior to colling by the Investigators. A formal scan will be performed by the Radiologist at the next opportunity.

Anticonvulsant therapy initially with phenobarbitone may be used as clinical indicated

Process Title

Purpose

The following Policy/Recommended Best Practice outlines the way in which Head Cooling is administered in Newborn Services to ensure safety to the baby.

Scope

Applies to all nurses in Newborn Services who are involved in caring for infant receiving head cooling.

Associated documents

The table below indicates other documents associated with this policy.

Type	Document Title(s)
Gunn AJ Gluckman PD Gunn TR Pediatrics Oct 98 102: 885-892	Selective Head Cooling in Newborn Infants following Perinatal Asphyxia; A Safety Study
Newborn Services 1998	Umbilical Arterial Catheterisation
Newborn Services 1998	Peripheral Arterial Catheterisation
Newborn Services 1998	Safety of the Baby

Information

Equipment Required

Radiant Heat Table - Servo controlled to the abdominal skin of the infant.

Cardiac, respiratory, blood pressure, $Sp0_2$ monitor.

Ivac pump for intravenous fluids

Sheepskin for baby to lie on.

Sufficient space each side of Radiant Heat Table for the following:
- water cooler with water temp read out
- monitor for continual recording of nasopharyngeal temperature
  and rectal temperature from temperature probes.

Temperature probes; - nasopharyngeal and rectal

Special headbox which has cool water circulating around it continuously.

Padding for chin strap typing cooling cap.

Respiratory support as needed i.e. Hudson CPAP or IPPV

Recommended Best Practice: Nursing care of baby receiving head cooling

Process

Follow the steps below to ensure nursing care is delivered safely.

Step	Action
1	Consultant applies the cap and fixes the probes in place initially. (The cup is cooled first before being applied to the baby.)
2	If there are any concerns or questions regarding infant cooling during a run, call the head cooling consultant at any time of day or night.
3	Consultant in charge will discuss the variations/limits which they wish to be notified for the following:- heart rate temperatures- rectal/nasopharyngeal probes (usually an increase, or decrease of 0.2) cooling cap temperature (usually kept between 17.2-17.5)
4	Keep the area as quiet as possible and reduce lighting around baby's area.
5	Baby will be NBM for 2-3 days. If baby becoming more alert and hungry on day 2 or 3, the Consultant may allow the cooling cap to be removed for 10 minutes and baby to have a breast feed (maximum of 10 minutes).
6	Intravenous fluids administered as prescribed by medical staff.
7	Arterial line in situ. Blood gases done 4hrly initially (as baby may be acidotic) then as per medical orders.
8	Rectal temp 4 hourly (may need to be 2hrly if having trouble cooling baby).

Continued

Recommended Best Practice: Nursing care of baby receiving head cooling continued:

Step	Action
9	Radiant heat table is adjusted to maintain rectal temp at 35°C (i.e. 35^{6}).
10	Ensure the nasopharyngeal temperature probe and rectal temperature probe remain in position (both are inserted 5cms).
11	Minimal handling as baby will be irritable. Baby's eyes will be shut and swollen due to increased cerebral oedema.
12	Use padding under chin ties to prevent pressure.
13	Ensure no kinks in the water cooling tube as this will interrupt the water flow.
14	No oral gastric/nasogastric tube to be inserted initially as this will aggravate the baby.
15	Ensure the tin foil covered special headbox remains over baby's head to reflect heat off baby's head.
16	Careful positioning and nesting of baby. Do not nurse baby prone.
17	Ultrasound of baby's head is done initially and then at 1½ days age.
18	Baby may need administration of Phenobarb if fitting occurs.
19	Urine weighs are done and urinalysis done 8 hrly. Monitor for oliguria (<0.5ml/hr for 24 hrs).

RBP: Safety of the Baby

Process

Follow the steps below to ensure safety of the baby is maintained.

Step	Action
1	Ensure alarm limited for heart rate, respiration, saturation and blood pressure are set appropriately.
2	Radiant heat table to be kept **flat** (overhead heater is most efficient when table is kept horizontal)
3	Consultant in charge of head cooling will advise nursing staff of variations allowable before consultant to be contacted re: - nasopharyngeal temperature probe - rectal temperature probe - blood pressure means - cardiac arrthymias (report immediately)
4	Ensure there is adequate room for equipment either side of bed space and that **all** monitors are easily visible to staff and equipment is earthed.
5	Ensure padding is put under chin strap ties to avoid pressure.
6	Consultant will take cooling cap off daily to check baby's head to ensure no pressure areas are developing.

RBP: Documentation is accurate

Process Follow the steps below.

Step	Action
1	Continuous monitoring and hourly recordings on nursing care sheet of the following: - heart rate - respirations - $Sp0_2$ - Rectal temperature probe - Nasopharyngeal temperature probe - Cap temperature
2	Rectal temperature is taken and recorded 4 hrly (more frequently if ordered by consultant). The overhead heater temperature is adjusted to maintain rectal temp at the allocated range of 35.0 to 35.5°C.
3	Blood pressure is recorded hourly for first 12 hours then 4 hourly. (Hypotension may occur.)
4	Blood gases done at entry to study then at 4, 8 and 12, 24, 48 and 72 hours and as clinically indicated.
5	Full blood count, haematrocrit and platelet count daily during study.
6	Record time and length of seizures and limbs involved.

RBP: Support of Parents

Process Follow the steps below to ensure parents are given support..

Step	Action
1	The nurse must be aware that this is an extremely stressful time for the parents i.e.: - they can't hold their baby - Mum can't breast feed - the outcome for their baby is unknown
2	Explain carefully to parents all the equipment and procedures you are doing to their baby.
3	Encourage Mum to express 3 hrly during her waking hours and that her milk will be stored for using later. If she has not been given an expressing pump in the ward, ensure she gets one, and tell her about expressing at her baby's bedside. Involve Lactation Consultant if necessary.
4	Ensure parents have frequent talks with medical staff/consultant re their baby.
5	Explain visiting policy re grandparents and siblings.
6	Tell parents they may bring their camera in and take photos of their baby.
7	Be there to listen to the parents re their concerns/worries.
8	Involve other multi-disciplinary team as required i.e.: - Maori advisor - Social worker - Minister/Priest - Lactation Consultant

RBP: On completion of cooling - slow rewarming baby

Process Follow the steps below for slow rewarming of baby.

Step	Action
1	When cooling is concluded (after 72 hrs) Consultant will remove the cap.
2	The special head box with tin foil reflector shield must remain in place for further 4 hours.
3	The rectal temperature should be allowed to rise by no more than 0.5°C per hour. (Adjust radiant heat table temperature half hourly in 0.25°C to rewarm baby.
4	Baby's rectal temperature should be carefully monitored to prevent rebound hyperthermia.
5	Nasopharyngeal probe and rectal probe monitored and recorded hourly for 4 hours after stopping cooling.
6	Blood pressure. Monitor and record hourly for 4 hours.

RBP: Followup of baby after head cooling completed

Process Follow the steps below.

Step	Action
1	**Followup Visits** **Review at 5-7 days of age** a) Neurological examination of the baby and head circumference, weight and length measurements b) Electroencephalogram and computerised tomography (CT) or magnetic resonance imaging (MRI) to be done when clinically possible c) Any tests that were abnormal at 72 hours, such as an elevated creatinine level should be repeated.
2	**Review at 3 months of age** At 3 months there should be followup clinic visits with the paediatrician with neurodevelopmental examination and measurements of head circumference, weight and length.
3	**Age one year** a) This will be the completion of the study participation for each subject with a neurodevelopmental examination by a paediatrician blind to the treatment and measurement of head circumferences, weight and length. b) Formal psychometric testing by a clinical psychologist blind to the treatment received c) CT or MRI d) Audiology assessment e) Ophthalmology examination f) Premature exit from the study. If premature exit from the study document the reasons. In cases where a decision is made by the attending paediatrician and parents not to pursue established medical therapy, the cooling cap should be removed 30 minutes before support is withdrawn. g) If the infant died state if support was withdrawn and give autopsy results if available.

9.2 Appendix 2 : Presentations And Publications Associated With The Research

Peer Reviewed Papers

Neurodevelopmental outcome of infants treated with head cooling and mild hypothermia following perinatal asphyxia. MR Battin, JA Dezoete, TR. Gunn, PD Gluckman, AJ Gunn. *Pediatrics* 2001;107 480-484

Treatment of term infants with head cooling and mild systemic hypothermia (35 and 34.5 degrees C) following perinatal asphyxia. MR Battin, J Penrice, TR. Gunn, AJ Gunn. *Pediatrics 2003;111:244-51*

Abnormal sagittal sinus blood flow in term infants following perinatal hyoxic ischaemic insult. MR Battin, RL Teele. *Pediatr Radiol 2003; 33: 559-62*

Selective head cooling with mild systemic hypothermia to improve neurodevelopmental outcome following neonatal encephalopathy. PD Gluckman, JS Wyatt, D Azzopardi et al. *Lancet 2005; 365: 663-70*

Epileptiform Activity During Rewarming From Moderate Cerebral Hypothermia In The Near-Term Fetal Sheep. LC. Gerrits, MR Battin, L Bennet, H Gonzalez, AJ Gunn. *Pediatric Research 2005;57(3):342-346*

Invited commentary and letters

Rebound seizures during rewarming. M Battin, L Bennett, A Gunn. *Pediatrics 2004;114(5):1369*

Sclerema neonatorum following hypothermia. M Battin, J Harding, A Gunn. *J Paediatr Child Health. 2002;38:533-4.*

Hypothermic Centralization: New Use for Old Knowledge? AJ Gunn, MR Battin. *Pediatrics 2000;106:133 -134*

Therapeutic Hypothermia: From Lab To NICU. Gunn AJ, Battin M, Gluckman PD, Gunn TR, Bennet L. *Perinatal Medicine 2005;33(4):340-6*

Selected Abstracts and Presentations

Selective Head Cooling with Mild Systemic Hypothermia To Improve Neurodevelopmental Outcome Following Neonatal Encephalopathy: The CoolCap Study. PD Gluckman, JS Wyatt, D Azzopardi et al. Society for Pediatric Research, San Francisco, USA 2004

Epileptiform activity during rewarming from moderate cerebral hypothermia In near-term fetal sheep. Gerrits LC, Battin MR, Bennet L, Gonzalez H, Gunn AJ. Annual

meeting of the Society for Pediatric Research, San Francisco, USA 2004

Therapeutic Hypothermia - from lab to NICU. 6th World Congress of Perinatal Medicine. Gunn AJ, Battin M, Bennet L, Gunn TR, on behalf of the CoolCap Investigators. J Perinat Med 31:50, 2003.

Safety of brain cooling with mild systemic hypothermia for the treatment of perinatal hypoxic ischaemic encephalopathy: The cool cap trial. AJ Gunn and Cool Cap investigators. Pediatric Research 2003;53:128A

What happened to the term infants with HIE who did not qualify for the trial of selective head cooling ? Battin MR, Armstrong DL West CR, Gunn AJ. *Proceedings of the Perinatal Society of Australia & New Zealand 6th Annual Congress 2003* A41

Abnormal sagittal sinus blood flow on cerebral ultrasound prior to treatment with hypothermia in term infants with hypoxic ischaemic insult. Battin MR, Teele RL. P153 *Proceedings of the Perinatal Society of Australia & New Zealand 6th Annual Congress 2002*

Does CT scan performed at one week of age help predict neurodevelop-mental outcome following perinatal hypoxic-ischaemic injury in term infants? Battin MR, Gunn M, O'Connor K, Teele RL, Hope JA. *Proceedings of the Perinatal Society of Australia & New Zealand 5th Annual Congress 2001 p.202.*

Why cool the head after perinatal asphyxia? Gunn AJ, Battin M, Bennet L, Gunn TR. *Teratology 2000;62(2):138*

Hypothermia – a multicentre trial. Gluckman PD, Gunn TR, Battin M, Gunn AJ. *XVII European Congress of Perinatal Medicine, Portugal, June 2000.*

Neurodevelopmental outcome of infants treated with selective head cooling following perinatal asphyxia. M Battin, A Dezoete, AJ Gunn. *Pediatric Research. 2000 ; 47 : 1783A*

Head cooling with mild (34-35ºC) systemic hypothermia following birth asphyxia: safe & feasible? Gunn TR, Penrice J, Battin M, Gunn AJ. *International Fetal Physiology Symposium*, Aspen, Colorado, June 1999, p47

Neuronal rescue with cerebral hypothermia: implications for the timing of irreversible programmed cell death? Gunn TR, Penrice J, Battin M, Gunn A J. *International Fetal Physiology Symposium*, Aspen, Colorado, June 1999, p48

Why cool the head after perinatal asphyxia? Gunn AJ, Battin M, Bennet L, Gunn TR. *Proceedings of the Birth Defects and Birth Injuries Symposium, Sydney, Nov 1999, p 15*

Head cooling with mild systemic hypothermia following birth asphyxia in term infants. J Penrice, M R Battin, TR Gunn. *Early Human Development 1999 ; 55 :183*

Neurodevelopmental follow-up in a group of infants treated with selective head cooling following perinatal asphyxia. M Battin, A Dezoete, T Gunn. *Proceedings Of The 3rd Annual Congress Of The Perinatal Society Of Australia And New Zealand 1999 page 58.*

Head cooling with mild systemic hypothermia following birth asphyxia: A safety study. T Gunn, J Penrice, M Battin, A Gunn. *Proceedings Of The 3rd Annual Congress Of The Perinatal Society Of Australia And New Zealand. 1999 page 151.*

Head cooling with mild systemic hypothermia (34.5 - 35° C) following birth asphyxia; A safety study. Penrice J, Battin M, Gunn T. *Hot Topics in Neonatology 1998.*

Developmental follow-up in a group of infants treated with selective head cooling following perinatal aspyxia. Battin M, JA Dezote, Gunn T. *Hot Topics in Neonatology 1998.*

Invited lectures

Cooling the brain to prevent damage after birth asphyxia at term. Westmead International Update on Controversies in Perinatal Care. Sydney, Australia, 2005

Keeping cool – the latest evidence on cerebral function after asphyxia. Hot topics in neonatal practice. Perinatal Society of Australia and New Zealand Congress. Adelaide, Australia 2005.

Hypothermia for Term Infants Born Following Perinatal Asphyxia. Keynote address : International Update in Neonatology. Vancouver, Canada 2003.

Interventions to prevent perinatal brain injury. Royal Australian New Zealand College of Obstetrics and Gynecology Annual Scientific Meeting, Auckland 2003.

Rescue strategies to ameliorate CNS damage following perinatal asphyxia in the term infant. 50 th Jubilee Academic Obstetrics and Gynaecology in Auckland 2001.

Selective Head Cooling In Perinatal Asphyxia : An Update. Keynote address : Perinatal Society of New Zealand, Wellington 1999

Bibliography

Abella BS, Rhee JW, Huang KN, Vanden Hoek TL, Becker LB. (2005) Induced hypothermia is underused after resuscitation from cardiac arrest: a current practice survey. *Resuscitation* 64:181-186

Abman SH. (1999) New developments in the pathogenesis and treatment of neonatal pulmonary hypertension. *Pediatric Pulmonology* 18:201-204

Ackermann-Liebrich U, Voegeli T, Gunter-Witt K, Kunz I, Zullig M, Schindler C, Maurer M. (1996) Home versus hospital deliveries: follow-up study of matched pairs for procedures and outcome. Zurich Study Team. *BMJ* 313:1313-1318

Adamson SJ, Alessandri LM, Badawi N, Burton PR, Pemberton PJ, Stanley F. (1995) Predictors of neonatal encephalopathy in full-term infants. *BMJ* 311:598-602

Adén U, Leverin AL, Hagberg H, Fredholm BB. (2001) Adenosine A(1) receptor agonism in the immature rat brain and heart. *Eur J Pharmacol.* 426:185-192

Adhikari M, Moodley M, Desai PK. (1990) Mannitol in neonatal cerebral oedema. *Brain & Development* 12:349-351

Adlard BP, De Souza SW. (1974) Influence of asphyxia and of dexamethasone on ATP concentrations in the immature rat brain. *Biology of the Neonate* 24:82-88

Adsett DB, Fitz CR, Hill A. (1985) Hypoxic-ischaemic cerebral injury in the term newborn: correlation of CT findings with neurological outcome. *Developmental Medicine & Child Neurology.* 27:155-160

Ahn MO, Korst LM, Phelan JP, Martin GI. (1998) Does the onset of neonatal seizures correlate with the timing of fetal neurologic injury? *Clinical Pediatrics.* 37:673-676

Airede AI. (1991) Birth asphyxia and hypoxic-ischaemic encephalopathy: incidence and severity. *Annals of Tropical Paediatrics.* 11:331-335

al Naqeeb N, Edwards AD, Cowan FM, Azzopardi D. (1999) Assessment of neonatal encephalopathy by amplitude-integrated electroencephalography. *Pediatrics.* 103:1263-1271

Altshuler G. (1993) Some placental considerations related to neurodevelopmental and other disorders. *Journal of Child Neurology.* 8:78-94

Altshuler G. (1996) Role of the placenta in perinatal pathology (revisited). *Pediatric Pathology & Laboratory Medicine.* 16:207-233

American College of Obstetricians and Gynecologists. (1992) *Fetal and neonatal injury.* Washington: American College of Obstetricians and Gynecologists

American College of Obstetricians and Gynecologists TFoNEaCP. (2002) Neonatal Encephalopathy and Cerebral Palsy, Washington

Amess PN, Penrice J, Cady EB, Lorek A, Wylezinska M, Cooper CE, D'Souza P, Tyszczuk L, Thoresen M, Edwards AD, Wyatt JS, Reynolds EO. (1997)

Mild hypothermia after severe transient hypoxia-ischemia reduces the delayed rise in cerebral lactate in the newborn piglet. *Pediatric Research.* 41:803-808

Amess PN, Penrice J, Wylezinska M, Lorek A, Townsend J, Wyatt JS, Amiel-Tison C, Cady EB, Stewart A. (1999) Early brain proton magnetic resonance spectroscopy and neonatal neurology related to neurodevelopmental outcome at 1 year in term infants after presumed hypoxic-ischaemic brain injury. *Developmental Medicine & Child Neurology.* 41:436-445

Amiel-Tison C. (1978) A method for neurological evaluation within the first year of life: experience with full-term newborn infants with birth injury. *Ciba Foundation Symposium.*:107-137

Amiel-Tison C, Ellison P. (1986) Birth asphyxia in the fullterm newborn: early assessment and outcome. *Developmental Medicine & Child Neurology.* 28:671-682

Anand SK. (1982) Acute renal failure in the neonate. *Pediatric Clinics of North America.* 29:791-800

Anand SK, Northway JD, Crussi FG. (1978) Acute renal failure in newborn infants. *Journal of Pediatrics.* 92:985-988

Andrew M, O'Brodovich H, Mitchell L. (1988) Fetal lamb coagulation system during birth asphyxia. *American Journal of Hematology.* 28:201-203

Anonymous. (1996) Collaborative survey of perinatal loss in planned and unplanned home births. Northern Region Perinatal Mortality Survey Coordinating Group. *BMJ.* 313:1306-1309

Anonymous. (2000) Part 11: Neonatal Resuscitation. *Resuscitation* 46:401-416

Apgar V. (1953) Proposal for new method of evaluation of newborn infant. *Anesth Analg.* 32:260-267

Araki H, Karasawa Y, Kawashima K, Hayashi M, Aihara H, Huang JH. (1989) The adenosine analogue and cerebral protecting agent, AMG-1, has no effect on delayed neuronal death following ischemia. *Methods & Findings in Experimental & Clinical Pharmacology* 11:731-736

Archer LN, Levene MI, Evans DH. (1986) Cerebral artery Doppler ultrasonography for prediction of outcome after perinatal asphyxia. *Lancet.* 2:1116-1118

Armstead WM. (1997) Role of activation of calcium-sensitive K+ channels in NO- and hypoxia-induced pial artery vasodilation. *Am J Physiol Heart Circ Physiol* 272:H1785-H1790

Armstrong DL, Battin MR. (2001) Pervasive seizures caused by hypoxic-ischemic encephalopathy: treatment with intravenous paraldehyde. *Journal of Child Neurology.* 16:915-917

Ashwal S, Dale PS, Longo LD. (1984) Regional cerebral blood flow: studies in the fetal lamb during hypoxia, hypercapnia, acidosis, and hypotension. *Pediatric Research* 18:1309-1316

Ashwal S, Majcher JS, Longo LD. (1981) Patterns of fetal lamb regional cerebral blood flow during and after prolonged hypoxia: studies during the

posthypoxic recovery period. *American Journal of Obstetrics & Gynecology* 139:365-372

Aso K, Scher MS, Barmada MA. (1989) Neonatal electroencephalography and neuropathology. *Journal of Clinical Neurophysiology* 6:103-123

Azzopardi D, Guarino I, Brayshaw C, Cowan F, Price-Williams D, Edwards AD, Acolet D. (1999) Prediction of neurological outcome after birth asphyxia from early continuous two-channel electroencephalography. *Early Human Development.* 55:113-123

Azzopardi D, Robertson NJ, Cowan FM, Rutherford MA, Rampling M, Edwards AD. (2000) Pilot study of treatment with whole body hypothermia for neonatal encephalopathy. *Pediatrics.* 106:684-694

Azzopardi D, Wyatt JS, Cady EB, Delpy DT, Baudin J, Stewart AL, Hope PL, Hamilton PA, Reynolds EO. (1989) Prognosis of newborn infants with hypoxic-ischemic brain injury assessed by phosphorus magnetic resonance spectroscopy. *Pediatric Research* 25:445-451

Babcock DS, Ball W, Jr. (1983) Postasphyxial encephalopathy in full-term infants: ultrasound diagnosis. *Radiology* 148:417-423

Badawi N, Kurinczuk JJ, Keogh JM, Alessandri LM, O'Sullivan F, Burton PR, Pemberton PJ, Stanley FJ. (1998a) Antepartum risk factors for newborn encephalopathy: the Western Australian case-control study. *BMJ.* 317:1549-1553

Badawi N, Kurinczuk JJ, Keogh JM, Alessandri LM, O'Sullivan F, Burton PR, Pemberton PJ, Stanley FJ. (1998b) Intrapartum risk factors for newborn encephalopathy: the Western Australian case-control study. *BMJ.* 317:1554-1558

Baena RC, Busto R, Dietrich WD, Globus MY, Ginsberg MD. (1997) Hyperthermia delayed by 24 hours aggravates neuronal damage in rat hippocampus following global ischemia. *Neurology* 48:768-773

Barberi I, Calabro MP, Cordaro S, Gitto E, Sottile A, Prudente D, Bertuccio G, Consolo S. (1999) Myocardial ischaemia in neonates with perinatal asphyxia. Electrocardiographic, echocardiographic and enzymatic correlations. *European Journal of Pediatrics.* 158:742-747

Barkovich AJ. (1997) The encephalopathic neonate: choosing the proper imaging technique. *AJNR: American Journal of Neuroradiology* 18:1816-1820

Barkovich AJ, Hajnal BL, Vigneron D, Sola A, Partridge JC, Allen F, Ferriero DM. (1998) Prediction of neuromotor outcome in perinatal asphyxia: evaluation of MR scoring systems. *AJNR: American Journal of Neuroradiology* 19:143-149

Barkovich AJ, Westmark K, Partridge C, Sola A, Ferriero DM. (1995) Perinatal asphyxia: MR findings in the first 10 days. *AJNR: American Journal of Neuroradiology* 16:427-438

Barnett A, Mercuri E, Rutherford M, Haataja L, Frisone MF, Henderson S, Cowan F, Dubowitz L. (2002) Neurological and perceptual-motor outcome at 5

- 6 years of age in children with neonatal encephalopathy: relationship with neonatal brain MRI. *Neuropediatrics.* 33:242-248

Barnett CP, Perlman M, Ekert PG. (1997) Clinicopathological correlations in postasphyxial organ damage: a donor organ perspective. *Pediatrics.* 99:797-799

Bartha AI F-BA, Miller SP, Vigneron DB, Glidden DV, Barkovich AJ, Ferriero DM. (2004) Neonatal encephalopathy: association of cytokines with MR spectroscopy and outcome. *Pediatric Research* 56:960-966

Bastian H, Keirse MJ, Lancaster PA. (1998) Perinatal death associated with planned home birth in Australia: population based study. *BMJ.* 317:384-388

Battin MR, Dezoete JA, Gunn TR, Gluckman PD, Gunn AJ. (2001) Neurodevelopmental outcome of infants treated with head cooling and mild hypothermia after perinatal asphyxia. *Pediatrics.* 107:480-484

Battin MR GM, O"connor K, Teele RL, Hope JA. (2001) Does CT scan performed at one week of age help predict neurodevelopmental outcome following perinatal hypoxic injury in term infants? In: *5th Annual Congress of Perinatal Society of Australia and New Zealand*, Canberra: Perinatal Society of Australia and New Zealand, p P117

Battin MR, Penrice J, Gunn TR, Gunn AJ. (2003) Treatment of term infants with head cooling and mild systemic hypothermia (35.0 degrees C and 34.5 degrees C) after perinatal asphyxia. *Pediatrics.* 111:244-251

Bayley N. (1993) *Bayley scales of infant development, Second Edition* :. San Antonio: The Psychological Corporation. Harcourt, Brace & Co

Beilharz EJ, Williams CE, Dragunow M, Sirimanne ES, Gluckman PD. (1995) Mechanisms of delayed cell death following hypoxic-ischemic injury in the immature rat: evidence for apoptosis during selective neuronal loss. *Brain Research. Molecular Brain Research* 29:1-14

Belai Y, Goodwin TM, Durand M, Greenspoon JS, Paul RH, Walther FJ. (1998) Umbilical arteriovenous PO2 and PCO2 differences and neonatal morbidity in term infants with severe acidosis. *Am J Obstet Gynecol.* 178:13-19

Bell AH, McClure BG, Hicks EM. (1990) Power spectral analysis of the EEG of term infants following birth asphyxia. *Developmental Medicine & Child Neurology* 32:990-998

Benders MB, AF. Rademaker, CM. Rijken, M. Torrance, HL. Groenendaal, F. van Bel, F. (2006) Early postnatal allopurinol does not improve short term outcome after severe birth asphyxia. *Arch Dis Child Fetal Neonatal Ed.* 91:F163-165

Benumof JL, Wahrenbrock EA. (1977) Dependency of hypoxic pulmonary vasoconstriction on temperature. *Journal of Applied Physiology: Respiratory* Environmental & Exercise Physiology. 42:56-58

Bernard SA. (2004) Therapeutic hypothermia after cardiac arrest. Hypothermia is now standard care for some types of cardiac arrest. *Medical Journal of Australia* 181:468-469

Bernard SA, Gray TW, Buist MD, Jones BM, Silvester W, Gutteridge G, Smith K. (2002) Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. *New England Journal of Medicine* 346:557-563

Berrington de Gonzalez A, Darby S. (2004) Risk of cancer from diagnostic X-rays: estimates for the UK and 14 other countries. *Lancet* 363:345-351

Bezinque SL ST, Touchette AS, Schave DM, Jarski RW, Bedard MP, Martino AM. (1995) Characterization of superior sagittal sinus blood flow velocity using color flow Doppler in neonates and infants. *Pediatr Radiol.* 25:175-179

Bhutani VK. (1997) Extrauterine adaptations in the newborn. *Seminars in Neonatology.* 2:1-12

Biagioni E, Bartalena L, Boldrini A, Pieri R, Cioni G. (1999) Constantly discontinuous EEG patterns in full-term neonates with hypoxic-ischaemic encephalopathy. *Clinical Neurophysiology* 110:1510-1515

Biagioni E, Mercuri E, Rutherford M, Cowan F, Azzopardi D, Frisone MF, Cioni G, Dubowitz L. (2001) Combined use of electroencephalogram and magnetic resonance imaging in full-term neonates with acute encephalopathy. *Pediatrics.* 107:461-468

Bifano EM, Pfannenstiel A. (1988) Duration of hyperventilation and outcome in infants with persistent pulmonary hypertension. *Pediatrics* 81:657-661

Bigelow WG LW, Harrison RE, Gordon RA, Greenwood WF. (1950) Oxygen transport and utilization in dogs at low body temperatures. *American Journal of Physiology* 160:125-137

Bilger A, Nehlig A. (1993) Regional cerebral blood flow response to acute hypoxia changes with postnatal age in the rat. *Brain Res Dev Brain Res* 76:197-205

Blair E. (1993) A research definition for 'birth asphyxia'? *Developmental Medicine & Child Neurology.* 35:449-452

Blair E, Stanley FJ. (1988) Intrapartum asphyxia: a rare cause of cerebral palsy. *Journal of Pediatrics.* 112:515-519

Blankenberg FG, Loh NN, Bracci P, D'Arceuil HE, Rhine WD, Norbash AM, Lane B, Berg A, Person B, Coutant M, Enzmann DR. (2000) Sonography, CT, and MR imaging: a prospective comparison of neonates with suspected intracranial ischemia and hemorrhage. *Ajnr: American Journal of Neuroradiology* 21:213-218

Blennow M, Hagberg H, Rosengren L. (1995a) Glial fibrillary acidic protein in the cerebrospinal fluid: a possible indicator of prognosis in full-term asphyxiated newborn infants? *Pediatric Research* 37:260-264

Blennow M, Ingvar M, Lagercrantz H, Stone-Elander S, Eriksson L, Forssberg H, Ericson K, Flodmark O. (1995b) Early [18F]FDG positron emission tomography in infants with hypoxic-ischaemic encephalopathy shows hypermetabolism during the postasphyctic period. *Acta Paediatrica* 84:1289-1295

Blennow M, Savman K, Ilves P, Thoresen M, Rosengren L. (2001) Brain-specific proteins in the cerebrospinal fluid of severely asphyxiated newborn infants. *Acta Paediatrica* 90:1171-1175

Blennow M, Zeman J, Dahlin I, Lagercrantz H. (1995c) Monoamine neurotransmitters and metabolites in the cerebrospinal fluid following perinatal asphyxia.[see comment]. *Biology of the Neonate* 67:407-413

Blood AB HC, Power GG. (2002) The role of adenosine in regulation of cerebral blood flow during hypoxia in the near-term fetal sheep. *J Physiol.* 15:1015-1023

Blood AB HC, Power GG. (2003) Adenosine mediates decreased cerebral metabolic rate and increased cerebral blood flow during acute moderate hypoxia in the near-term fetal sheep.. *J Physiol.* 553:935-945

Blumberg RM, Cady EB, Wigglesworth JS, McKenzie JE, Edwards AD. (1997) Relation between delayed impairment of cerebral energy metabolism and infarction following transient focal hypoxia-ischaemia in the developing brain. *Experimental Brain Research.* 113:130-137

Boddy K, Mantell C. (1973) Human foetal breathing in utero. *Journal of Physiology* 231:105P-106P

Boddy K, Mantell CD. (1972) Observations of fetal breathing movements transmitted through maternal abdominal wall. *Lancet* 2:1219-1220

Bona E, Aden U, Gilland E, Fredholm BB, Hagberg H. (1997) Neonatal cerebral hypoxia-ischemia: the effect of adenosine receptor antagonists. *Neuropharmacology.* 36:1327-1338

Bona E, Hagberg H, Loberg EM, Bagenholm R, Thoresen M. (1998) Protective effects of moderate hypothermia after neonatal hypoxia-ischemia: short- and long-term outcome. *Pediatric Research.* 43:738-745

Booth D, Evans D. (2004) Anticonvulsants for neonates with seizures. *Cochrane Database of Systematic Reviews* 4:CD004218

Borg E. (1997) Perinatal asphyxia, hypoxia, ischemia and hearing loss. An overview. *Scandinavian Audiology.* 26:77-91

Bourchier D. (1991) Birth asphyxia associated oliguria: relationship to outcome at 1 year. *Journal of Paediatrics & Child Health.* 27:302-303

Boutilier R. (2001) Mechanisms of metabolic defense against hypoxia in hibernating frogs. *Respir Physiol.* 128:365-377

Boylan GB, Young K, Panerai RB, Rennie JM, Evans DH. (2000) Dynamic cerebral autoregulation in sick newborn infants. *Pediatric Research.* 48:12-17

Brescia MA, Tartaglione EF. (1954) Sclerema neonatorum. *Journal of Pediatrics* 45:720-723

Bucciarelli RL, Nelson RM, Egan EA, Eitzman DV, Gessner IH. (1977) Transient tricuspid insufficiency of the newborn: a form of myocardial dysfunction in stressed newborns. *Pediatrics.* 59:330-337

Buchmann EJ, Pattinson RC, Nyathikazi N. (2002) Intrapartum-related birth asphyxia in South Africa--lessons from the first national perinatal care survey. *South African Medical Journal.* 92:897-901

Buetow KC, Klein SW. (1964) Effect of maintenance of "normal" skin temperature on survival of infants of low birth. *Pediatrics* 34:163-170

Burnard ED, Cross KW. (1958) Rectal temperature in the newborn after birth asphyxia. *BMJ.* 14:1197-1199

Busto R, Dietrich WD, Globus MY, Valdes I, Scheinberg P, Ginsberg MD. (1987) Small differences in intraischemic brain temperature critically determine the extent of ischemic neuronal injury. *Journal of Cerebral Blood Flow & Metabolism* 7:729-738

Busto R, Globus MY, Dietrich WD, Martinez E, Valdes I, Ginsberg MD. (1989) Effect of mild hypothermia on ischemia-induced release of neurotransmitters and free fatty acids in rat brain. *Stroke* 20:904-910

Bydder GM, Rutherford MA, Cowan FM. (2001) Diffusion-weighted imaging in neonates. *Childs Nervous System* 17:190-194

Cabal LA, Devaskar U, Siassi B, Hodgman JE, Emmanouilides G. (1980) Cardiogenic shock associated with perinatal asphyxia in preterm infants. *Journal of Pediatrics* 96:705-710

Calabrese V MC, Calvani M, Rizzarelli E, Butterfield DA, Stella AM. (2007) Nitric oxide in the central nervous system: neuroprotection versus neurotoxicity. *Nature Reviews Neuroscience* 8:766-775

Campistol J, Poo P, Fernandez Alvarez E, Carratala F. (1999) Parasagittal cerebral injury: magnetic resonance findings.[see comment]. *Journal of Child Neurology* 14:683-685

Carey H, Andrews M, Martin S. (2003) Mammalian hibernation: cellular and molecular responses to depressed metabolism and low temperature. *Physiol Rev.* 83:1153-1181

Carter AM. (1999) Placental oxygen transfer and the oxygen supply to the fetus. *Fetal and Maternal Medicine Review* 11:151-161

Carter BS, McNabb F, Merenstein GB. (1998) Prospective validation of a scoring system for predicting neonatal morbidity after acute perinatal asphyxia. *Journal of Pediatrics.* 132:619-623

Casalaz DM, Marlow N, Speidel BD. (1998) Outcome of resuscitation following unexpected apparent stillbirth. *Archives of Disease in Childhood Fetal & Neonatal Edition.* 78:F112-115

Casey BM, McIntire DD, Leveno KJ. (2001) The continuing value of the Apgar score for the assessment of newborn infants. *New England Journal of Medicine* 344:467-471

Castle V, Andrew M, Kelton J, Giron D, Johnston M, Carter C. (1986) Frequency and mechanism of neonatal thrombocytopenia. *Journal of Pediatrics.* 108:749-755

Castle V, Coates G, Mitchell LG, O'Brodovich H, Andrew M. (1988) The effect of hypoxia on platelet survival and site of sequestration in the newborn rabbit. *Thrombosis & Haemostasis.* 59:45-48

Chadd MA, Elwood PC, Gray OP, Muxworthy SM. (1971) Coagulation defects in hypoxic full-term newborn infants. *BMJ.* 4:516-518

Chadd MA, Gray OP. (1972) Hypothermia and coagulation defects in the newborn. *Archives of Disease in Childhood* 47:819-821

Charpie JR, Dekeon MK, Goldberg CS, Mosca RS, Bove EL, Kulik TJ. (2000) Serial blood lactate measurements predict early outcome after neonatal repair or palliation for complex congenital heart disease. *Journal of Thoracic & Cardiovascular Surgery* 120:73-80

Chauhan SP, Sanderson M, Hendrix NW, Magann EF, Devoe LD. (1999) Perinatal outcome and amniotic fluid index in the antepartum and intrapartum periods: A meta-analysis. *American Journal of Obstetrics & Gynecology* 181:1473-1478

Chernick V, Manfreda J, De Booy V, Davi M, Rigatto H, Seshia M. (1988) Clinical trial of naloxone in birth asphyxia. *Journal of Pediatrics* 113:519-525

Chessells JM, Wigglesworth JS. (1971) Coagulation studies in severe birth asphyxia. *Archives of Disease in Childhood.* 46:253-256

Choi DW. (1988) Calcium-mediated neurotoxicity: relationship to specific channel types and role in ischemic damage. *Trends in Neurosciences* 11:465-469

Chou YH, Tsou Yau KI, Wang PJ. (1998) Clinical application of the measurement of cord plasma lactate and pyruvate in the assessment of high-risk neonates. *Acta Paediatrica* 87:764-768

Christophe C, Clercx A, Blum D, Hasaerts D, Segebarth C, Perlmutter N. (1994) Early MR detection of cortical and subcortical hypoxic-ischemic encephalopathy in full-term-infants. *Pediatric Radiology* 24:581-584

Chuang SD, Chiu HC, Chang CC. (1995) Subcutaneous fat necrosis of the newborn complicating hypothermic cardiac surgery. *British Journal of Dermatology* 132:805-810

Clardy CW, Edwards KM, Gay JC. (1985) Increased susceptibility to infection in hypothermic children: possible role of acquired neutrophil dysfunction. *Pediatric Infectious Disease* 4:379-382

Clark SJ, Newland P, Yoxall CW, Subhedar NV. (2001) Cardiac troponin T in cord blood. *Archives of Disease in Childhood Fetal & Neonatal Edition.* 84:F34-37

Clark W, O'Donovan D. (2001) Transient hyperinsulinism in an asphyxiated newborn infant with hypoglycemia. *American Journal of Perinatology.* 18:175-178

Cleary GM, Wiswell TE. (1998) Meconium-stained amniotic fluid and the meconium aspiration syndrome. An update. *Pediatric Clinics of North America.* 45:511-529

Clifton GL. (2004) Is cooling still hot? *Current Opinion in Critical Care* 10:116-119

Cohn HE, Sacks EJ, Heymann MA, Rudolph AM. (1974) Cardiovascular responses to hypoxemia and acidemia in fetal lambs. *American Journal of Obstetrics & Gynecology.* 120:817-824

Colbourne F, Sutherland GR, Auer RN. (1999) Electron microscopic evidence against apoptosis as the mechanism of neuronal death in global ischemia. *Journal of Neuroscience* 19:4200-4210

Collins JE, Leonard JV. (1984) Hyperinsulinism in asphyxiated and small-for-dates infants with hypoglycaemia. *Lancet.* 2:311-313

COMLINE RS, SILVER IA, M. S. (1965) FACTORS RESPONSIBLE FOR THE STIMULATION OF THE ADRENAL MEDULLA DURING ASPHYXIA IN THE FOETAL LAMB. *J Physiol.* 178:211-238

Cooke R. (2005) Head cooling in neonatal hypoxic-ischaemic encephalopathy. *Lancet* 365:632-634

Cordes I, Roland EH, Lupton BA, Hill A. (1994) Early prediction of the development of microcephaly after hypoxic-ischemic encephalopathy in the full-term newborn. *Pediatrics.* 93:703-707

Cordey R, Chiolero R, Miller JA, Jr. (1973) Resuscitation of neonates by hypothermia: report on 20 cases with acid-base determination on 10 cases and the long-term development of 33 cases. *Resuscitation* 2:169-181

Cornfield DN, Stevens T, McMurtry IF, Abman SH, Rodman DM. (1994) Acute hypoxia causes membrane depolarization and calcium influx in fetal

pulmonary artery smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 266:L469-L475

Correia M, Silva M, Veloso M. (2000) Cooling therapy for acute stroke. *Cochrane Database of Systematic Reviews* 2:CD001247

Coughtrey H, Jeffery HE, Henderson-Smart DJ, Storey B, Poulos V. (1991) Possible causes linking asphyxia, thick meconium and respiratory distress. *Australian & New Zealand Journal of Obstetrics & Gynaecology.* 31:97-102

Coumans AB, Garnier Y, Supçun S, Jensen A, Hasaart TH, Berger R. (2003) The role of nitric oxide on fetal cardiovascular control during normoxia and acute hypoxia in 0.75 gestation sheep. *J Soc Gynecol Investig.* 10:275-282

Cowan F. (2000) Outcome after intrapartum asphyxia in term infants. *Seminars in Neonatology* 5:127-140

Cowan F, Dubowitz L, Mercuri E, Counsell S, Barnett A, Rutherford M. (2004) Cognitive deficits without major white matter difficulties following perinatal asphyxia and early encephalopathy: A consequence of white matter injury. *Pediatric Research* 55:416A

Cowan F, Rutherford M, Groenendaal F, Eken P, Mercuri E, Bydder GM, Meiners LC, Dubowitz LM, de Vries LS. (2003) Origin and timing of brain lesions in term infants with neonatal encephalopathy. *Lancet.* 361:736-742

Cowan F, Thoresen M. (1985) Changes in superior sagittal sinus blood velocities due to postural alterations and pressure on the head of the newborn infant. *Pediatrics* 75:1038-1047

Craig JE ST, Vanderhooft SL, Etheridge SP. (1998) Fat necrosis after ice application for supraventricular tachycardia termination. *J Pediatr.* 133:727

Crowley P. (2000) Prophylactic corticosteroids for preterm birth. *Cochrane Database Syst Rev.* 2:CD000065

da Silva S, Hennebert N, Denis R, Wayenberg JL. (2000) Clinical value of a single postnatal lactate measurement after intrapartum asphyxia. *Acta Paediatrica* 89:320-323

Daga SR, Gosavi DV, Kinikar NA, Deshapnde N, Hendre S. (1998) Management of cardiogenic shock in asphyxiated babies using a clinical scoring system. *Journal of Tropical Pediatrics* 44:249-250

Dagbjartsson A, Karlsson K, Kjellmer I, Rosen KG. (1985) Maternal treatment with a cardioselective beta-blocking agent--consequences for the ovine fetus during intermittent asphyxia. *Journal of Developmental Physiology* 7:387-396

Danzl DF PR. (1994) Accidental Hypothermia. *N Engl J Med.* 331:1756-1760

Daven JR, Milstein JM, Guthrie RD. (1983) Cerebral vascular resistance in premature infants. *Am J Dis Child* 137:328-331

Davignon A RP, Boiselle E, Soumis F, Magelas M, Choquette A. (1979) Normal ECG standards for infants and children. *Paediatr Cardiol* 1:133-152

Davis DJ, Creery WD, Radziuk J. (1999) Inappropriately high plasma insulin levels in suspected perinatal asphyxia. *Acta Paediatrica.* 88:76-81

Dawes GS, Fox HE, Leduc BM, Liggins GC, Richards RT. (1970) Respiratory movements and paradoxical sleep in the foetal lamb. *Journal of Physiology* 210:47P-48P

Dawes GS, Fox HE, Leduc BM, Liggins GC, Richards RT. (1972) Respiratory movements and rapid eye movement sleep in the foetal lamb. *Journal of Physiology* 220:119-143

Day RL, Caliguiri L, Kamenski C, Ehrlich F. (1964) Body temperature and survival of premature infants. *Pediatrics* 34:171-181

de Haan HH, Gunn AJ, Williams CE, Heymann MA, Gluckman PD. (1997) Magnesium sulfate therapy during asphyxia in near-term fetal lambs does not compromise the fetus but does not reduce cerebral injury. *American Journal of Obstetrics & Gynecology.* 176:18-27

De Praeter C, Vanhaesebrouck P, Govaert P, Delanghe J, Leroy J. (1991) Creatine kinase isoenzyme BB concentrations in the cerebrospinal fluid of newborns: relationship to short-term outcome. *Pediatrics* 88:1204-1210

De Vries LS, Pierrat V, Eken P, Minami T, Daniels H, Casaer P. (1991) Prognostic value of early somatosensory evoked potentials for adverse outcome in full-term infants with birth asphyxia. *Brain & Development* 13:320-325

Dear P, Newell S. (2000) Establishing probable cause in cerebral palsy. How much certainty is enough? *BMJ.* 320:1075-1076

Debillon T, Daoud P, Durand P, Cantagrel S, Jouvet P, Saizou C, Zupan V. (2003) Whole-body cooling after perinatal asphyxia: a pilot study in term neonates. *Developmental Medicine & Child Neurology.* 45:17-23

Delivoria-Papadopoulos M, Mishra OP. (1998) Mechanisms of cerebral injury in perinatal asphyxia and strategies for prevention. *Journal of Pediatrics* 132

Dempsey RJ CD, Maley ME, Cowen DE, Roy MW, Donaldson DL. (1987) Moderate hypothermia reduces postischemic edema development and leukotriene production. *Neurosurgery.* 21.:177-181

Dietrich WD, Busto R, Halley M, Valdes I. (1990) The importance of brain temperature in alterations of the blood-brain barrier following cerebral ischemia. *Journal of Neuropathology & Experimental Neurology* 49:486-497

DiSessa TG, Leitner M, Ti CC, Gluck L, Coen R, Friedman WF. (1981) The cardiovascular effects of dopamine in the severely asphyxiated neonate. *Journal of Pediatrics* 99:772-776

Donnelly WH, Bucciarelli RL, Nelson RM. (1980) Ischemic papillary muscle necrosis in stressed newborn infants. *Journal of Pediatrics.* 96:295-300

Drage J, Berendes H. (1966a) Apgar scores and outcome of the newborn. *Pediatr Clin North Am.* 13:637-643

Drage JS, Berendes H. (1966b) Apgar scores and outcome of the newborn. *Pediatric Clinics of North America* 13:637-643

Drago JR, Rohner TJ, Jr., Sanford EJ, Maisels MJ. (1977) Perinatal asphyxia and renal failure in neonatal patients. *Journal of Urology.* 118:80-82

Dragunow M, Faull RL. (1988) Neuroprotective effects of adenosine. *Trends in Pharmacological Sciences* 9:193-194

Draper ES, Kurinczuk JJ, Lamming CR, Clarke M, James D, Field D. (2002) A confidential enquiry into cases of neonatal encephalopathy. *Archives of Disease in Childhood Fetal & Neonatal Edition.* 87:F176-180

D'Souza SW, McCartney E, Nolan M, Taylor IG. (1981) Hearing, speech, and language in survivors of severe perinatal asphyxia. *Archives of Disease in Childhood.* 56:245-252

Duhn R, Schoen EJ, Siu M. (1968) Subcutaneous fat necrosis with extensive calcification after hypothermia in two newborn infants. *Pediatrics* 41:661-664

Dunwiddie T, Masino S. (2001) The role and regulation of adenosine in the central nervous system. *Annu Rev Neurosci.* 24:31-55

Edwards AD, Nelson KB. (1998) Neonatal encephalopathies. Time to reconsider the cause of encephalopathies. *BMJ.* 317:1537-1538

Edwards AD, Yue X, Squier MV, Thoresen M, Cady EB, Penrice J, Cooper CE, Wyatt JS, Reynolds EO, Mehmet H. (1995) Specific inhibition of apoptosis after cerebral hypoxia-ischaemia by moderate post-insult hypothermia. *Biochemical & Biophysical Research Communications.* 217:1193-1199

Edwards D. (2004) Brain protection for girls and boys. *J Pediatr.* 145:723-724.

Eicher D, Wagner C, Katikaneni L, Hulsey T, Bass W, Kaufman D, Horgan M, Languani S, Bhatia J, Givelichian L, Sankaran KY, JY. (2005) Moderate

hypothermia in neonatal encephalopathy: safety outcomes. *Pediatr Neurol.* 32:18-24

Eicher DJ WC, Katikaneni LP, Hulsey TC, Bass WT, Kaufman DA, Horgan MJ, Languani S, Bhatia JJ, Givelichian LM, Sankaran K, Yager JY. (2005) Moderate hypothermia in neonatal encephalopathy: safety outcomes. *Pediatr Neurol.* 32:18-24

Eken P, Jansen GH, Groenendaal F, Rademaker KJ, de Vries LS. (1994) Intracranial lesions in the fullterm infant with hypoxic ischaemic encephalopathy: ultrasound and autopsy correlation. *Neuropediatrics* 25:301-307

Eken P, Toet MC, Groenendaal F, de Vries LS. (1995) Predictive value of early neuroimaging, pulsed Doppler and neurophysiology in full term infants with hypoxic-ischaemic encephalopathy. *Archives of Disease in Childhood Fetal & Neonatal Edition* 73

Ekert P, Perlman M, Steinlin M, Hao Y. (1997) Predicting the outcome of postasphyxial hypoxic-ischemic encephalopathy within 4 hours of birth. *Journal of Pediatrics.* 131:613-617

Ellis M, Manandhar N, Shrestha PS, Shrestha L, Manandhar DS, Costello AM. (1999) Outcome at 1 year of neonatal encephalopathy in Kathmandu, Nepal. *Developmental Medicine & Child Neurology.* 41:689-695

Engle WD, Laptook AR, Perlman JM. (1999) Acute changes in arterial carbon dioxide tension and acid-base status and early neurologic characteristics in term infants following perinatal asphyxia. *Resuscitation.* 42:11-17

Erecinska M TM, Silver IA. (2003) Effects of hypothermia on energy metabolism in Mammalian central nervous system. *J Cereb Blood Flow Metab* 23

Ergander U, Eriksson M, Zetterstrom R. (1983) Severe neonatal asphyxia. Incidence and prediction of outcome in the Stockholm area. *Acta Paediatrica Scandinavica.* 72:321-325

Ergenekon E GK, Erbas D, Süheyl Ezgü F, Atalay Y. (1999) Cerebrospinal fluid and serum nitric oxide levels in asphyxiated newborns. *Biology of the Neonate.* 76:200-206

Evans D, Levene M. (1998) Neonatal seizures. *Archives of Disease in Childhood Fetal & Neonatal Edition* 78:70-75

Evans DJ, Levene MI. (2000) Anticonvulsants for preventing mortality and morbidity in full term newborns with perinatal asphyxia. *Cochrane Database of Systematic Reviews.*:CD001240

Evans K RA, Hamilton P, Titchiner N and Hall DMB. (2001) The relationship between neonatal encephalopathy and cerebral palsy : a cohort study. *Journal of Obstetrics and Gynaecology* 21:114-120

Evans MC SJ, Meldrum BS. (1987) An adenosine analogue, 2-chloroadenosine, protects against long term development of ischaemic cell loss in the rat hippocampus. *Neurosci Lett.* 83:287-292

Falciglia HS. (1988) Failure to prevent meconium aspiration syndrome. *Obstetrics & Gynecology.* 71:349-353

Farru O, Rizzardini M, Guzman N. (1986) Transient myocardial ischemia in newborn infants. *Archives des Maladies du Coeur et des Vaisseaux.* 79:633-638

Feldman RC, Tabsh KM, Shields WD. (1985) Correlation of ominous fetal heart rate patterns and brain-specific creatine kinase. *Obstetrics & Gynecology* 65:476-480

Fenichel GM. (1983) Hypoxic-ischemic encephalopathy in the newborn. *Archives of Neurology.* 40:261-266

Ferguson J, Britton F, Simmonds M, Whitelaw A, Thoresen M. (2005) Selective head cooling is safe, cooling by 10 degrees C only mildly prolongs coagulation time in the newborn pig. In: *Early Human Development*, Abstracts Neonatal Society Bristol, p 613

Fineman JR, Soifer SJ, Heymann MA. (1995) Regulation of pulmonary vascular tone in the perinatal period. *Annual Review of Physiology* 57:115-134

Finer N. (1997) Inhaled nitric oxide in neonates. *Arch. Dis. Child. Fetal Neonatal Ed.* 77:81-84

Finer NN, Robertson CM, Peters KL, Coward JH. (1983) Factors affecting outcome in hypoxic-ischemic encephalopathy in term infants. *American Journal of Diseases of Children.* 137:21-25

Finer NN, Robertson CM, Richards RT, Pinnell LE, Peters KL. (1981) Hypoxic-ischemic encephalopathy in term neonates: perinatal factors and outcome. *Journal of Pediatrics.* 98:112-117

Finley JP, Howman-Giles RB, Gilday DL, Bloom KR, Rowe RD. (1979) Transient myocardial ischemia of the newborn infant demonstrated by thallium myocardial imaging. *Journal of Pediatrics.* 94:263-270

Fisher DJ. (1984) Increased regional myocardial blood flows and oxygen deliveries during hypoxemia in lambs. *Pediatric Research.* 18:602-606

Fitzhardinge PM, Flodmark O, Fitz CR, Ashby S. (1981) The prognostic value of computed tomography as an adjunct to assessment of the term infant with postasphyxial encephalopathy. *Journal of Pediatrics* 99:777-781

Fleming SM, O'Gorman T, O'Byrne L, Grimes H, Daly KM, Morrison JJ. (2001) Cardiac troponin I and N-terminal pro-brain natriuretic peptide in umbilical artery blood in relation to fetal heart rate abnormalities during labor. *Pediatric Cardiology.* 22:393-396

Flodmark O, Becker LE, Harwood-Nash DC, Fitzhardinge PM, Fitz CR, Chuang SH. (1980) Correlation between computed tomography and autopsy in premature and full-term neonates that have suffered perinatal asphyxia. *Radiology* 137:93-103

Flores-Nava G, Echevarria-Ybarguengoitia JL, Navarro-Barron JL, Garcia-Alonso A. (1990) Transient myocardial ischemia in newborn babies with perinatal asphyxia (hypoxic cardiomyopathy). *Boletin Medico del Hospital Infantil de Mexico.* 47:809-814

Fortnum H, Davis A. (1997) Epidemiology of permanent childhood hearing impairment in Trent Region, 1985-1993. *British Journal of Audiology.* 31:409-446

Freeman JM, Nelson KB. (1988) Intrapartum asphyxia and cerebral palsy. *Pediatrics.* 82:240-249

Gadian DG, Aicardi J, Watkins KE, Porter DA, Mishkin M, Vargha-Khadem F. (2000) Developmental amnesia associated with early hypoxic-ischaemic injury. *Brain* 3:499-507

Gadkary CS, Alderson P, Signorini DF. (2002) Therapeutic hypothermia for head injury. *Cochrane Database of Systematic Reviews* 4:CD001048

Gaffney G, Flavell V, Johnson A, Squier MV, Sellers S. (1995) Model to identify potentially preventable cerebral palsy of intrapartum origin. *Archives of Disease in Childhood Fetal & Neonatal Edition.* 73:F106-108

Gaffney G, Sellers S, Flavell V, Squier M, Johnson A. (1994) Case-control study of intrapartum care, cerebral palsy, and perinatal death. *BMJ.* 308:743-750

Galambos R, Despland PA. (1980) The auditory brainstem response (ABR) evaluates risk factors for hearing loss in the newborn. *Pediatric Research.* 14:159-163

Garcia-Alix A, Cabanas F, Pellicer A, Hernanz A, Stiris TA, Quero J. (1994) Neuron-specific enolase and myelin basic protein: relationship of cerebrospinal fluid concentrations to the neurologic condition of asphyxiated full-term infants. *Pediatrics* 93:234-240

Gerrits LC BM, Bennet L, Gonzalez H, Gunn AJ. (2005) Epileptiform activity during rewarming from moderate cerebral hypothermia in the near-term fetal sheep. *Pediatr Res.* 57:342-346

Ghidini A, Spong CY. (2001) Severe meconium aspiration syndrome is not caused by aspiration of meconium. *American Journal of Obstetrics & Gynecology.* 185:931-938

Gibson NA, Graham M, Levene MI. (1992) Somatosensory evoked potentials and outcome in perinatal asphyxia. *Archives of Disease in Childhood.* 67:393-398

Gidday JM, Fitzgibbons JC, Shah AR, Kraujalis MJ, Park TS. (1995) Reduction in cerebral ischemic injury in the newborn rat by potentiation of endogenous adenosine. *Pediatric Research* 38:306-311

Ginsberg MD, Busto R. (1989) Rodent models of cerebral ischemia. *Stroke* 20:1627-1642

Giroux JD, Vernotte E, Gagneur A, Metz C, Collet M, de Parscau L. (1997) Transitory hyperinsulinism with hypoglycemia in asphyxia neonatorum. *Archives de Pediatrie.* 4:1213-1216

Gleason CA, Hamm C, Jones MD Jr. (1990) Effect of acute hypoxemia on brain blood flow and oxygen metabolism in immature fetal sheep. *Am J Physiol Heart Circ Physiol* 258:H1064-H1069

Gleason CA, Hamm C, Jones MD, Jr. (1989) Cerebral blood flow, oxygenation, and carbohydrate metabolism in immature fetal sheep in utero. *American Journal of Physiology* 256

Gluckman PD, Guan J, Beilharz EJ, Klempt ND, Klempt M, Miller O, Sirimanne E, Dragunow M, Williams CE. (1993) The role of the insulin-like growth factor system in neuronal rescue. *Annals of the New York Academy of Sciences* 692:138-148

Gluckman PD, Guan J, Williams C, Scheepens A, Zhang R, Bennet L, Gunn A. (1998) Asphyxial brain injury--the role of the IGF system. *Molecular & Cellular Endocrinology* 140:95-99

Gluckman PD, Williams CE. (1992) When and why do brain cells die? *Developmental Medicine & Child Neurology* 34:1010-1014

Gluckman PD, Wyatt JS, Azzopardi D, Ballard R, Edwards AD, Ferriero DM, Polin RA, Robertson CM, Thoresen M, Whitelaw A, Gunn AJ. (2005) Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *Lancet* 365:663-670

Goldaber KG, Gilstrap LC, 3rd, Leveno KJ, Dax JS, McIntire DD. (1991) Pathologic fetal acidemia. *Obstetrics & Gynecology* 78:1103-1107

Goldberg RN, Moscoso P, Bauer CR, Bloom FL, Curless RG, Burke B, Bancalari E. (1986) Use of barbiturate therapy in severe perinatal asphyxia: a randomized controlled trial. *Journal of Pediatrics* 109:851-856

Goodwin TM, Belai I, Hernandez P, Durand M, Paul RH. (1992) Asphyxial complications in the term newborn with severe umbilical acidemia. *American Journal of Obstetrics & Gynecology.* 167:1506-1512

Gordon CJ, Fogelson L. (1991) Comparative effects of hypoxia on behavioral thermoregulation in rats, hamsters, and mice. *American Journal of Physiology* 260

Gorman D, Drewry A, Huang YL, Sames C. (2003) The clinical toxicology of carbon monoxide. *Toxicology* 187:25-38

Gorman D, Huang Y, Williams C. (2005) Blockade of haeme oxygenase and nitric oxide synthetase causes cortical dysfunction in sheep exposed to carbon monoxide. *Toxicology* 209:237-243

Govaert P VD, Achten E, Vanhaesebrouck P, van Rostenberghe H, van Gysel D, Afschrift M. (1992) Noninvasive diagnosis of superior sagittal sinus thrombosis in a neonate. *Am J Perinatol* 9:201-204

Grant A. (1993) Epidemiological principles for the evaluation of monitoring programs--the Dublin experience. *Clinical & Investigative Medicine - Medecine Clinique et Experimentale.* 16:149-158

Grant A, O'Brien N, Joy MT, Hennessy E, MacDonald D. (1989) Cerebral palsy among children born during the Dublin randomised trial of intrapartum monitoring. *Lancet.* 2:1233-1236

Grant DA FC, Wild J, Walker AM. (1995) Continuous measurement of blood flow in the superior sagittal sinus of the lamb. *Am J Physiol* 269:R274-279

Gray PH, Tudehope DI, Masel JP, Burns YR, Mohay HA, O'Callaghan MJ, Williams GM. (1993) Perinatal hypoxic-ischaemic brain injury: prediction of outcome. *Developmental Medicine & Child Neurology* 35:965-973

Greenough A. (1995) Meconium aspiration syndrome--prevention and treatment. *Early Human Development.* 41:183-192

Greenwood C, Lalchandani S, MacQuillan K, Sheil O, Murphy J, Impey L. (2003) Meconium passed in labor: how reassuring is clear amniotic fluid? *Obstetrics & Gynecology* 102:89-93

Greenwood K, Cox P, Mehmet H, Penrice J, Amess PN, Cady EB, Wyatt JS, Edwards AD. (2000) Magnesium sulfate treatment after transient hypoxia-ischemia in the newborn piglet does not protect against cerebral damage. *Pediatric Research.* 48:346-350

Grether JK, Nelson KB. (1997) Maternal infection and cerebral palsy in infants of normal birth weight. *JAMA.* 278:207-211

Grigg-Damberger MM, Coker SB, Halsey CL, Anderson CL. (1989) Neonatal burst suppression: its developmental significance. *Pediatric Neurology* 5:84-92

Groenendaal F, de Vries LS. (2000) Selection of babies for intervention after birth asphyxia. *Seminars in Neonatology.* 5:17-32

Groenendaal F, Lammers H, Smit D, Nikkels P. (2006) Nitrotyrosine in brain tissue of neonates after perinatal asphyxia. *Arch Dis Child Fetal Neonatal Ed.* 91:F429-433

Groenendaal F, Rademaker CM, Toet MC, de Vries LS. (2002) Effects of magnesium sulphate on amplitude-integrated continuous EEG in asphyxiated term neonates. *Acta Paediatrica* 91:1073-1077

Groenendaal F, Vles J, Lammers H, De Vente J, Smit D, Nikkels P. (2008) Nitrotyrosine in human neonatal spinal cord after perinatal asphyxia. *Neonatology* 93:1-6

Guan J, Bennet L, Gluckman PD, Gunn AJ. (2003) Insulin-like growth factor-1 and post-ischemic brain injury. *Progress in Neurobiology* 70:443-462

Guan J, Gunn AJ, Sirimanne ES, Tuffin J, Gunning MI, Clark R, Gluckman PD. (2000) The window of opportunity for neuronal rescue with insulin-like growth factor-1 after hypoxia-ischemia in rats is critically modulated by cerebral temperature during recovery. *Journal of Cerebral Blood Flow & Metabolism* 20:513-519

Guan J, Miller OT, Waugh KM, McCarthy DC, Gluckman PD. (2001) Insulin-like growth factor-1 improves somatosensory function and reduces the extent of cortical infarction and ongoing neuronal loss after hypoxia-ischemia in rats. *Neuroscience* 105:299-306

Guan J, Thomas GB, Lin H, Mathai S, Bachelor DC, George S, Gluckman PD. (2004) Neuroprotective effects of the N-terminal tripeptide of insulin-like growth factor-1, glycine-proline-glutamate (GPE) following intravenous infusion in hypoxic-ischemic adult rats. *Neuropharmacology* 47:892-903

Guardia E, Demestre X, Raspall F, De Juan M, Rovira T, Altirriba O, Ruscalleda J. (1986) Perinatal hypoxic-ischaemic syndrome. Diagnostic and

prognostic value of computed tomography. *Acta Radiologica Supplementum* 369:667-670

Gücüyener K, Ergenekon E, Demiryürek T, Erbas D, Oztürk G, Koç E, Atalay Y. (2002) Cerebrospinal fluid levels of nitric oxide and nitrotyrosine in neonates with mild hypoxic-ischemic encephalopathy. *J Child Neurol.* 17:815-818

Gulbransen G, Hilton J, McKay L, Cox A. (1997) Home birth in New Zealand 1973-93: incidence and mortality. *New Zealand Medical Journal.* 110:87-89

Gunn AJ. (2000) Cerebral hypothermia for prevention of brain injury following perinatal asphyxia. *Current Opinion in Pediatrics.* 12:111-115

Gunn AJ, Parer JT, Mallard EC, Williams CE, Gluckman PD. (1992) Cerebral histologic and electrocorticographic changes after asphyxia in fetal sheep. *Pediatr Res.* 31:486-491

Gunn AJ, Battin M. (2000) Hypothermic centralization: new use for old knowledge? *Pediatrics.* 106:133-134

Gunn AJ, Bennet L. (2001) Is temperature important in delivery room resuscitation? *Seminars in Neonatology.* 6:241-249

Gunn AJ, Bennet L, Gunning MI, Gluckman PD, Gunn TR. (1999a) Cerebral hypothermia is not neuroprotective when started after postischemic seizures in fetal sheep. *Pediatric Research.* 46:274-280

Gunn AJ, Gluckman PD, Gunn TR. (1998a) Selective head cooling in newborn infants after perinatal asphyxia: a safety study. *Pediatrics.* 102:885-892

Gunn AJ, Gunn TR. (1996) Effect of radiant heat on head temperature gradient in term infants. *Archives of Disease in Childhood Fetal & Neonatal Edition.* 74:F200-203

Gunn AJ, Gunn TR. (1997) Changes in risk factors for hypoxic-ischaemic seizures in term infants. *Australian & New Zealand Journal of Obstetrics & Gynaecology.* 37:36-39

Gunn AJ, Gunn TR. (1998) The 'pharmacology' of neuronal rescue with cerebral hypothermia. *Early Human Development.* 53:19-35

Gunn AJ, Gunn TR, de Haan HH, Williams CE, Gluckman PD. (1997) Dramatic neuronal rescue with prolonged selective head cooling after ischemia in fetal lambs. *Journal of Clinical Investigation.* 99:248-256

Gunn AJ, Gunn TR, Gunning MI, Williams CE, Gluckman PD. (1998b) Neuroprotection with prolonged head cooling started before postischemic seizures in fetal sheep. *Pediatrics.* 102:1098-1106

Gunn AJ, Williams CE, Mallard EC, Tan WK, Gluckman PD. (1994) Flunarizine, a calcium channel antagonist, is partially prophylactically neuroprotective in hypoxic-ischemic encephalopathy in the fetal sheep. *Pediatric Research.* 35:657-663

Gunn T, Outerbridge EW. (1978) Effectiveness of neonatal transport. *Canadian Medical Association Journal* 118:646-649

Gunn TR, Wilson NJ, Aftimos S, Gunn AJ. (1999b) Brain hypothermia and QT interval. *Pediatrics.* 103:1079

Haaland K, Loberg EM, Steen PA, Thoresen M. (1997) Posthypoxic hypothermia in newborn piglets. *Pediatric Research.* 41:505-512

Hagberg B, Hagberg G, Beckung E, Uvebrant P. (2001) Changing panorama of cerebral palsy in Sweden. VIII. Prevalence and origin in the birth year period 1991-94. *Acta Paediatrica.* 90:271-277

Hagberg B, Hagberg G, Olow I. (1984) The changing panorama of cerebral palsy in Sweden. IV. Epidemiological trends 1959-78. *Acta Paediatrica Scandinavica.* 73:433-440

Hagberg B, Hagberg G, Olow I. (1993a) The changing panorama of cerebral palsy in Sweden. VI. Prevalence and origin during the birth year period 1983-1986. *Acta Paediatrica.* 82:387-393

Hagberg B, Hagberg G, Olow I, van Wendt L. (1996) The changing panorama of cerebral palsy in Sweden. VII. Prevalence and origin in the birth year period 1987-90. *Acta Paediatrica.* 85:954-960

Hagberg B, Hagberg G, Olow I, von Wendt L. (1989) The changing panorama of cerebral palsy in Sweden. V. The birth year period 1979-82. *Acta Paediatrica Scandinavica.* 78:283-290

Hagberg G, Olow I. (1975) The changing panorama of cerebral palsy in Sweden 1954-1970. II. Analysis of the various syndromes. *Acta Paediatrica Scandinavica.* 64:193-200

Hagberg H. (1999) Glycine and modulation of the NMDA receptor after severe asphyxia. *Acta Paediatrica.* 88:1049-1050

Hagberg H, Andersson P, Kjellmer I, Thiringer K, Thordstein M. (1987) Extracellular overflow of glutamate, aspartate, GABA and taurine in the cortex and basal ganglia of fetal lambs during hypoxia-ischemia. *Neuroscience Letters* 78:311-317

Hagberg H, Thornberg E, Blennow M, Kjellmer I, Lagercrantz H, Thiringer K, Hamberger A, Sandberg M. (1993b) Excitatory amino acids in the cerebrospinal fluid of asphyxiated infants: relationship to hypoxic-ischemic encephalopathy. *Acta Paediatrica.* 82:925-929

Hall RT, Hall FK, Daily DK. (1998) High-dose phenobarbital therapy in term newborn infants with severe perinatal asphyxia: a randomized, prospective study with three-year follow-up. *Journal of Pediatrics* 132:345-348

Hanrahan JD, Cox IJ, Azzopardi D, Cowan FM, Sargentoni J, Bell JD, Bryant DJ, Edwards AD. (1999) Relation between proton magnetic resonance spectroscopy within 18 hours of birth asphyxia and neurodevelopment at 1 year of age. *Developmental Medicine & Child Neurology.* 41:76-82

Hanrahan JD, Sargentoni J, Azzopardi D, Manji K, Cowan FM, Rutherford MA, Cox IJ, Bell JD, Bryant DJ, Edwards AD. (1996) Cerebral metabolism

within 18 hours of birth asphyxia: a proton magnetic resonance spectroscopy study. *Pediatric Research.* 39:584-590

Harbord MG, Weston PF. (1995) Somatosensory evoked potentials predict neurologic outcome in full-term neonates with asphyxia. *Journal of Paediatrics & Child Health* 31:148-151

Hattori H, Wasterlain CG. (1990) Posthypoxic glucose supplement reduces hypoxic-ischemic brain damage in the neonatal rat. *Annals of Neurology.* 28:122-128

Heinonen S, Saarikoski S. (2001) Reproductive risk factors of fetal asphyxia at delivery: a population based analysis. *Journal of Clinical Epidemiology* 54:407-410

Hellstrom-Westas L, Rosen I, Svenningsen NW. (1995) Predictive value of early continuous amplitude integrated EEG recordings on outcome after severe birth asphyxia in full term infants. *Archives of Disease in Childhood Fetal & Neonatal Edition.* 72:F34-38

Hellstrom-Westas L, Rosen I, Swenningsen NW. (1985) Silent seizures in sick infants in early life. Diagnosis by continuous cerebral function monitoring. *Acta Paediatrica Scandinavica* 74:741-748

Herrmann M, Vos P, Wunderlich MT, de Bruijn CH, Lamers KJ. (2000) Release of glial tissue-specific proteins after acute stroke: A comparative analysis of serum concentrations of protein S-100B and glial fibrillary acidic protein. *Stroke* 31:2670-2677

Hicks MJ, Levy ML, Alexander J, Flaitz CM. (1993) Subcutaneous fat necrosis of the newborn and hypercalcemia: case report and review of the literature. *Pediatric Dermatology* 10:271-276

Hill A. (1991) Current concepts of hypoxic-ischemic cerebral injury in the term newborn. *Pediatric Neurology.* 7:317-325

Hill A, Volpe JJ. (1989) Perinatal asphyxia: clinical aspects. *Clinics in Perinatology.* 16:435-457

Hill A, Volpe JJ. (1999) Hypoxic-ischemic cerebral injury in the newborn. In: *Pediatrc Neurology. Principles and Practice* (Swaiman KF AS, ed), St Louis: Mosby Co., pp 191-204

Holmes G, Rowe J, Hafford J, Schmidt R, Testa M, Zimmerman A. (1982) Prognostic value of the electroencephalogram in neonatal asphyxia. *Electroencephalography & Clinical Neurophysiology* 53:60-72

Holmes GL, Gairsa JL, Chevassus-Au-Louis N, Ben-Ari Y. (1998) Consequences of neonatal seizures in the rat: morphological and behavioral effects. *Annals of Neurology* 44:845-857

Holmes GL, Lombroso CT. (1993) Prognostic value of background patterns in the neonatal EEG. *Journal of Clinical Neurophysiology* 10:323-352

Holzer M, Bernard SA, Hachimi-Idrissi S, Roine RO, Sterz F, Mullner M, on behalf of the Collaborative Group on Induced Hypothermia for Neuroprotection After Cardiac A. (2005) Hypothermia for neuroprotection after

cardiac arrest: systematic review and individual patient data meta-analysis. *Critical Care Medicine* 33:414-418

Hope PL, Costello AM, Cady EB, Delpy DT, Tofts PS, Chu A, Hamilton PA, Reynolds EO, Wilkie DR. (1984) Cerebral energy metabolism studied with phosphorus NMR spectroscopy in normal and birth-asphyxiated infants. *Lancet* 2:366-370

Hossmann KA, Kleihues P. (1973) Reversibility of ischemic brain damage. *Archives of Neurology* 29:375-384

Hu B, McDonald JW, Johnston MV, Silverstein FS. (1991) Excitotoxic brain injury suppresses striatal high-affinity glutamate uptake in perinatal rats. *Journal of Neurochemistry* 56:933-937

Huang CC, Wang ST, Chang YC, Lin KP, Wu PL. (1999) Measurement of the urinary lactate:creatinine ratio for the early identification of newborn infants at risk for hypoxic-ischemic encephalopathy. *New England Journal of Medicine* 341:328-335

Hughes WE HM. (1948) Sclerema Neonatorum. *J Pediatr.* 32:676

Hull J, Dodd KL. (1992) Falling incidence of hypoxic-ischaemic encephalopathy in term infants. *British Journal of Obstetrics & Gynaecology.* 99:386-391

Hunt R, Osborn D. (2002) Dopamine for prevention of morbidity and mortality in term newborn infants with suspected perinatal asphyxia. *Cochrane Database of Systematic Reviews* 3:CD003484

Hunt RW, Neil JJ, Coleman LT, Kean MJ, Inder TE. (2004) Apparent diffusion coefficient in the posterior limb of the internal capsule predicts outcome after perinatal asphyxia. *Pediatrics* 114:999-1003

Hunter CJ, Blood AB, White CR, Pearce WJ, Power GG. (2003) Role of nitric oxide in hypoxic cerebral vasodilatation in the ovine fetus. *J Physiol* 549:625-633

Hypothermia after Cardiac Arrest Study G. (2002) Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest. *New England Journal of Medicine* 346:549-556

Iadecola C. (1997) Bright and dark sides of nitric oxide in ischemic brain injury. *Trends in Neurosciences* 20:132-139

Ichiba H, Tamai H, Negishi H, Ueda T, Kim TJ, Sumida Y, Takahashi Y, Fujinaga H, Minami H, Kansai Magnesium Study G. (2002) Randomized controlled trial of magnesium sulfate infusion for severe birth asphyxia. *Pediatrics International* 44:505-509

Ignarro LJ. (1991) Signal transduction mechanisms involving nitric oxide. *Biochemical Pharmacology* 41:485-490

Ignarro LJ, Ross G, Tillisch J. (1991) Pharmacology of endothelium-derived nitric oxide and nitrovasodilators. *Western Journal of Medicine* 154:51-62

Impey L, Greenwood C, MacQuillan K, Reynolds M, Sheil O. (2001a) Fever in labour and neonatal encephalopathy: a prospective cohort study. *BJOG: an International Journal of Obstetrics & Gynaecology* 108:594-597

Impey L, Greenwood C, Sheil O, MacQuillan K, Reynolds M, Redman C. (2001b) The relation between pre-eclampsia at term and neonatal encephalopathy. *Archives of Disease in Childhood Fetal & Neonatal Edition* 85:170-172

Inder TE, Hunt RW, Morley CJ, Coleman L, Stewart M, Doyle LW, Jacobs SE. (2004) Randomized trial of systemic hypothermia selectively protects the cortex on MRI in term hypoxic-ischemic encephalopathy.[see comment]. *Journal of Pediatrics* 145:835-837

Inder TE, Volpe JJ. (2000) Mechanisms of perinatal brain injury. *Seminars in Neonatology.* 5:3-16

Irestedt L, Lagercrantz H, Belfrage P. (1984) Causes and consequences of maternal and fetal syrnpathoadrenal activation during parturition. *Acta Obstetricia et Gynecologica Scandinavica - Supplement.* 118:111-115

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

Jacobs S, Hunt R, Tarnow-Mordi W, Inder T, P. D. (2007) Cooling for newborns with hypoxic ischaemic encephalopathy. *Cochrane Database Syst Rev.* 4:CD003311

Jain L, Ferre C, Vidyasagar D, Nath S, Sheftel D. (1991) Cardiopulmonary resuscitation of apparently stillborn infants: survival and long-term outcome. *Journal of Pediatrics.* 118:778-782

James LS WI, Prince CE, Holaday DA, Apgar V. (1958) The acid-base status of human infants in relation to birth asphyxia and the onset of respiration. *Journal of Pediatrics.* 52:379-394

Janbu T, Nesheim BI. (1987) Uterine artery blood velocities during contractions in pregnancy and labour related to intrauterine pressure. *British Journal of Obstetrics & Gynaecology* 94:1150-1155

Janssen PA, Lee SK, Ryan EM, Etches DJ, Farquharson DF, Peacock D, Klein MC. (2002) Outcomes of planned home births versus planned hospital births after regulation of midwifery in British Columbia.[see comment]. *CMAJ Canadian Medical Association Journal* 166:315-323

Jarvis SN, Holloway JS, Hey EN. (1985) Increase in cerebral palsy in normal birthweight babies. *Archives Disease in Childhood* 60:1113-1121

Jenik AG, Ceriani Cernadas JM, Gorenstein A, Ramirez JA, Vain N, Armadans M, Ferraris JR. (2000) A randomized, double-blind, placebo-controlled trial of the effects of prophylactic theophylline on renal function in term neonates with perinatal asphyxia. *Pediatrics.* 105:E45

Jensen A, Hohmann M, Künzel W. (1987) Dynamic changes in organ blood flow and oxygen consumption during acute asphyxia in fetal sheep. *J Dev Physiol.* 9:543-559

Jensen A GY, Berger R. (1999) Dynamics of fetal circulatory responses to hypoxia and asphyxia. *Eur J Obstet Gynecol Reprod Biol.* 84:155-172

Jiang ZD. (1995) Long-term effect of perinatal and postnatal asphyxia on developing human auditory brainstem responses: peripheral hearing loss. *International Journal of Pediatric Otorhinolaryngology.* 33:225-238

Johnson KC, Daviss BA. (2005) Outcomes of planned home births with certified professional midwives: large prospective study in North America. *BMJ* 330:1416

Johnston MV, Trescher WH, Ishida A, Nakajima W. (2001) Neurobiology of hypoxic-ischemic injury in the developing brain. *Pediatric Research* 49:735-741

Johnston MV, Trescher WH, Taylor GA. (1995) Hypoxic and ischemic central nervous system disorders in infants and children. *Advances in Pediatrics* 42:1-45

Jolly H, Molyneux P, Newell DJ. (1962) A controlled study of the effect of temperature on premature babies. *Journal of Pediatrics* 60:889-894

Jonas RA. (1998) Neurological protection during cardiopulmonary bypass/deep hypothermia. *Pediatric Cardiology* 19:321-330

Jonas RA. (2002) Deep hypothermic circulatory arrest: current status and indications. *Seminars in Thoracic & Cardiovascular Surgery. Pediatric Cardiac Surgery Annual* 5:76-88

Jonas RA BD, Rappaport LA, Wernovsky G, Hickey PR, Farrell DM et al. (1993) Relation of pH Strategy and Developmental Outcome After Hypothermic Circulatory Arrest. *J Thorac Cardiovasc Surg* 106:362-368

Jones CT, Ritchie JW. (1978a) The cardiovascular effects of circulating catecholamines in fetal sheep. *J Physiol.* 285:381-393

Jones CT, Ritchie JW. (1978b) The metabolic and endocrine effects of circulating catecholamines in fetal sheep. *J Physiol.* 285:395-408

Jones CT, Robinson RO. (1975) Plasma catecholamines in foetal and adult sheep. *Journal of Physiology* 248:15-33

Jones M, Jr., Sheldon RE, Peeters LL, Meschia G, Battaglia FC, Makowski EL. (1977) Fetal cerebral oxygen consumption at different levels of oxygenation. *Journal of Applied Physiology: Respiratory* 43:1080-1084

Jongeling BR, Badawi N, Kurinczuk JJ, Thonell S, Watson L, Dixon G, Stanley FJ. (2002) Cranial ultrasound as a predictor of outcome in term newborn encephalopathy. *Pediatric Neurology.* 26:37-42

Karimi A BK, Power GG. (1996) Exogenous infusion of adenosine depresses whole body O2 use in fetal/neonatal sheep. *J Appl Physiol.* 81:541-547.

Karlowicz MG, Adelman RD. (1995) Nonoliguric and oliguric acute renal failure in asphyxiated term neonates. *Pediatric Nephrology.* 9:718-722

Kato H, Liu Y, Kogure K, Kato K. (1994) Induction of 27-kDa heat shock protein following cerebral ischemia in a rat model of ischemic tolerance. *Brain Research* 634:235-244

Kavanagh RJ, Kam PC. (2001) Lazaroids: efficacy and mechanism of action of the 21-aminosteroids in neuroprotection. *British Journal of Anaesthesia* 86:110-119

Khare MD, Merchant RH. (1990) Diagnostic and prognostic value of CT brain scan in term neonates with moderate and severe birth asphyxia. *Indian Pediatrics* 27:267-271

Khare SK. (1977) Neurohypophyseal dysfunction following perinatal asphyxia. *Journal of Pediatrics.* 90:628-629

Kirino T. (2002) Ischemic tolerance. *Journal of Cerebral Blood Flow & Metabolism* 22:1283-1296

Kirpalani H, Barks J, Thorlund K, Guyatt G. (2007) Cooling for neonatal hypoxic ischemic encephalopathy: do we have the answer? *Pediatrics* 120:1126-1130

Kitagawa K, Matsumoto M, Kuwabara K, Tagaya M, Ohtsuki T, Hata R, Ueda H, Handa N, Kimura K, Kamada T. (1991) 'Ischemic tolerance' phenomenon detected in various brain regions. *Brain Research* 561:203-211

Klinger G, Beyene J, Shah P, Perlman M. (2005) Do hyperoxaemia and hypocapnia add to the risk of brain injury after intrapartum asphyxia? *Archives of Disease in Childhood Fetal & Neonatal Edition* 90:F49-52

Koehler RC, Traystman RJ, Jones MD Jr. (1985) Regional blood flow and O2 transport during hypoxic and CO hypoxia in neonatal and adult sheep. *Am J Physiol Heart Circ Physiol* 248:H118-H124

Koehler RC TR, Zeger S, Rogers MC, and Jones MD Jr. (1984) Comparison of cerebrovascular response to hypoxic and carbon monoxide hypoxia in newborn and adult sheep. *J Cereb Blood Flow Metab* 4:115–122

Koht A, Cane R, Cerullo LJ. (1983) Serum potassium levels during prolonged hypothermia. *Intensive Care Medicine* 9:275-277

Koide H. (1985) Neuropathology of brainstem in severe cerebral palsy mainly due to perinatal asphyxia. *No to Hattatsu [Brain & Development].* 17:293-300

Koos B, Doany W, . (1991) Role of plasma adenosine in breathing responses to hypoxia in fetal sheep. *Journal of Developmental Physiology.* 16:81-85

Koos BJ KL, Murray TF. (1997) Source of extracellular brain adenosine during hypoxia in fetal sheep. *Brain Res.* 778:439-442

Kriss VM. (1998) Hyperdense posterior falx in the neonate. *Pediatr Radiol.* 28:817-819

Kumpel B, Wood SM, Anthony PP, Brimblecombe FS. (1983) Umbilical cord serum creatine kinase BB in the diagnosis of brain damage in the newborn: problems in interpretation. *Archives of Disease in Childhood* 58:382-383

Kurth CD, Wagerle LC. (1992) Cerebrovascular reactivity to adenosine analogues in 0.6-07 gestation and near-term fetal sheep. *Am J Physiol Heart Circ Physiol.* 262:H1338-H1342

Lackmann GM, Mader R, Tollner U. (1991) Serum potassium level in healthy neonates and infants with asphyxia in the first 144 hours of life. *Klinische Padiatrie.* 203:399-402

Lackmann GM, Tollner U, Mader R. (1993) Serum enzyme activities in full-term asphyxiated and healthy newborns: enzyme kinetics during the first 144 hours of life. *Enzyme & Protein.* 47:160-172

Lagercrantz H, Slotkin TA. (1986) The "stress" of being born. *Scientific American* 254:100-107

Lagercrantz H, Slotkin TA. (1986) The "stress" of being born. *Scientific American* 254:100-107

Lan J, Hunter CJ, Murata T, Power GG. (2000) Adaptation of laser-Doppler flowmetry to measure cerebral blood flow in the fetal sheep. *J Appl Physiol.* 89:1065-1071

Laptook AR, Corbett RJ. (2002) The effects of temperature on hypoxic-ischemic brain injury. *Clinics in Perinatology* 29:623-649

Laptook AR, Corbett RJ, Burns DK, Sterett R. (1999) A limited interval of delayed modest hypothermia for ischemic brain resuscitation is not beneficial in neonatal swine. *Pediatric Research* 46:383-389

Laptook AR, Corbett RJ, Sterett R, Burns DK, Garcia D, Tollefsbol G. (1997) Modest hypothermia provides partial neuroprotection when used for immediate resuscitation after brain ischemia. *Pediatric Research* 42:17-23

Lee BC VT, Ehrlich ME, Lipper E, Auld PA, Vannucci RC. (1984) Digital intravenous cerebral angiography in neonates. *AJNR Am J Neuroradiol* 5:281-286

Leech RW, Alvord EC, Jr. (1977) Anoxic-ischemic encephalopathy in the human neonatal period. The significance of brain stem involvement. *Archives of Neurology.* 34:109-113

Leech RW, Brumback RA. (1988) Massive brain stem necrosis in the human neonate: presentation of three cases with review of the literature. *Journal of Child Neurology.* 3:258-262

Lees GJ. (1993) The possible contribution of microglia and macrophages to delayed neuronal death after ischemia. *Journal of the Neurological Sciences* 114:119-122

Legido A KC, Mishra OP, Delivoria-Papadopoulous M. (2000) Perinatal hypoxic iscemic encephalopathy:current and future treatments. *International Pediatrics* 15:143-151

Leijon I. (1992) The prognostic significance of antenatal diagnosis of fetal growth retardation. *International Journal of Technology Assessment in Health Care* 1:176-181

Levene M. (1992) Role of excitatory amino acid antagonists in the management of birth asphyxia. *Biology of the Neonate.* 62:248-251

Levene M. (2001) The Asphyxiated Newborn Infant. In: *Fetal and Neonatal Neurology and Neurosurgery* (Levene M, Chervenak F, Whittle M, eds), London: Churchill Livingstone, pp 471-504

Levene M. (2002) The clinical conundrum of neonatal seizures. *Archives of Disease in Childhood Fetal & Neonatal Edition* 86:F75-77

Levene M, Blennow M, Whitelaw A, Hanko E, Fellman V, Hartley R. (1995) Acute effects of two different doses of magnesium sulphate in infants with

birth asphyxia. *Archives of Disease in Childhood Fetal & Neonatal Edition.* 73:F174-177

Levene MI. (1993) Management of the asphyxiated full term infant. *Archives of Disease in Childhood.* 68:612-616

Levene MI, Evans DH. (1983) Continuous measurement of subarachnoid pressure in the severely asphyxiated newborn. *Archives of Disease in Childhood.* 58:1013-1015

Levene MI, Evans DH, Forde A, Archer LN. (1987) Value of intracranial pressure monitoring of asphyxiated newborn infants. *Developmental Medicine & Child Neurology.* 29:311-319

Levene MI, Fenton AC, Evans DH, Archer LN, Shortland DB, Gibson NA. (1989) Severe birth asphyxia and abnormal cerebral blood-flow velocity. *Developmental Medicine & Child Neurology.* 31:427-434

Levene MI, Gibson NA, Fenton AC, Papathoma E, Barnett D. (1990) The use of a calcium-channel blocker, nicardipine, for severely asphyxiated newborn infants. *Developmental Medicine & Child Neurology.* 32:567-574

Levene MI, Sands C, Grindulis H, Moore JR. (1986) Comparison of two methods of predicting outcome in perinatal asphyxia. *Lancet.* 1:67-69

Levene ML, Kornberg J, Williams TH. (1985) The incidence and severity of post-asphyxial encephalopathy in full-term infants. *Early Human Development.* 11:21-26

Leviton A, Dammann O. (2002) Brain damage markers in children. Neurobiological and clinical aspects. *Acta Paediatrica* 91:9-13

Lindenberg JA, Milstein JM, Cox KL. (1987) Sclerema neonatorum: a sign of transient hyperammonemia of the newborn. *Journal of Pediatric Gastroenterology & Nutrition* 6:474-476

Lipper EG, Voorhies TM, Ross G, Vannucci RC, Auld PA. (1986) Early predictors of one-year outcome for infants asphyxiated at birth. *Developmental Medicine & Child Neurology* 28:303-309

Lipp-Zwahlen AE, Deonna T, Micheli JL, Calame A, Chrzanowski R, Cetre E. (1985) Prognostic value of neonatal CT scans in asphyxiated term babies: low density score compared with neonatal neurological signs. *Neuropediatrics* 16:209-217

Lipton SA. (1999) Neuronal protection and destruction by NO. *Cell Death & Differentiation* 6:943-951

Little W. (1862) On the influence of abnormal parturition, difficult labours, premature birth and asphyxia neonatorum on the mental and physical condition of the child, especially in relation to deformities. *Trans Obstet Soc London* 3:293-344

Lorek A, Takei Y, Cady EB, Wyatt JS, Penrice J, Edwards AD, Peebles D, Wylezinska M, Owen-Reece H, Kirkbride V, et al. (1994) Delayed ("secondary") cerebral energy failure after acute hypoxia-ischemia in the newborn piglet: continuous 48-hour studies by phosphorus magnetic resonance spectroscopy. *Pediatric Research.* 36:699-706

Lou HC, Lassen NA, Fris-Hansen B. (1978) Decreased cerebral blood flow after administration of sodium bicarbonate in the distressed newborn infant. *Acta Neurologica Scandinavica* 57:239-247

Lou HC, Tweed WA, Davies JM. (1985) Preferential blood flow increase to the brain stem in moderate neonatal hypoxia: reversal by naloxone. *European Journal of Pediatrics* 144:225-227

Lou HC, Tweed WA, Davis JM. (1989) Endogenous opioids may protect the perinatal brain in hypoxia. *Developmental Pharmacology & Therapeutics.* 13:129-133

Low JA, Galbraith RS, Muir DW, Killen HL, Pater EA, Karchmar EJ. (1984) Factors associated with motor and cognitive deficits in children after intrapartum fetal hypoxia. *Am J Obstet Gynecol.* 148:533-539

Low JA, Pickersgill H, Killen H, Derrick EJ. (2001) The prediction and prevention of intrapartum fetal asphyxia in term pregnancies. *American Journal of Obstetrics & Gynecology.* 184:724-730

Low JA, Simpson LL, Tonni G, Chamberlain S. (1995) Limitations in the clinical prediction of intrapartum fetal asphyxia. *American Journal of Obstetrics & Gynecology.* 172:801-804

Ludbrook GL HS, Gorman DF, Reilly PL, North JB and Grant C,. (1992) The relative effects of hypoxic-hypoxia and carbon monoxide on brain function in rabbits. *Toxicology* 75:71-80

Lundgren J SM, Blennow G, Siesjo BK. (1994) Hyperthermia aggravates and hypothermia ameliorates epileptic brain damage. *Exp Brain Res.* 99:43-55

Lupton BA, Hill A, Roland EH, Whitfield MF, Flodmark O. (1988) Brain swelling in the asphyxiated term newborn: pathogenesis and outcome. *Pediatrics.* 82:139-146

Lutz PL. (1992) Mechanisms for anoxic survival in the vertebrate brain. *Annu Rev Physiol* 54:601-618

MacDonald D, Grant A, Sheridan-Pereira M, Boylan P, Chalmers I. (1985) The Dublin randomized controlled trial of intrapartum fetal heart rate monitoring. *American Journal of Obstetrics & Gynecology* 152:524-539

MacDonald HM, Mulligan JC, Allen AC, Taylor PM. (1980) Neonatal asphyxia. I. Relationship of obstetric and neonatal complications to neonatal mortality in 38,405 consecutive deliveries. *Journal of Pediatrics.* 96:898-902

MacLennan A. (1999) A template for defining a causal relation between acute intrapartum events and cerebral palsy: international consensus statement. *BMJ* 319:1054-1059

Majnemer A, Mazer B. (1998) Neurologic evaluation of the newborn infant: definition and psychometric properties. *Developmental Medicine & Child Neurology* 40:708-715

Malamud N. (1950) Status marmoratus; a form of cerebral palsy following either birth injury or inflammation of the central nervous system. *Journal of Pediatrics* 37:610-619

Marangos PJ, Schmechel DE. (1987) Neuron specific enolase, a clinically useful marker for neurons and neuroendocrine cells. *Annual Review of Neuroscience* 10:269-295

Margileth AM. (1994) Dermatological Conditions. In: *Neonatology Pathophysiology and Management of the Newborn* (Avery GA FM, Mhairi GM,, ed), Philadelphia,: JB Lippincott,, pp 1229-1268

Marks KA, Mallard CE, Roberts I, Williams CE, Gluckman PD, Edwards AD. (1996a) Nitric oxide synthase inhibition attenuates delayed vasodilation and increases injury after cerebral ischemia in fetal sheep. *Pediatric Research* 40:185-191

Marks KA, Mallard EC, Roberts I, Williams CE, Sirimanne ES, Johnston B, Gluckman PD, Edwards AD. (1996b) Delayed vasodilation and altered oxygenation after cerebral ischemia in fetal sheep. *Pediatric Research* 39:48-54

Marks WA, Leech RW, Altshuler GP. (1989) Placental examination in perinatal asphyxia. *Journal of Child Neurology.* 4:124

Marlow N RA, Rands CE, Draper ES. (2005) Neuropsychological and educational problems at school age associated with neonatal encephalopathy. *Arch Dis Child Fetal Neonatal Ed* 90.:F380-387

Martin E, Barkovich AJ. (1995) Magnetic resonance imaging in perinatal asphyxia. *Archives of Disease in Childhood Fetal & Neonatal Edition* 72:F62-70

Martin E, Buchli R, Ritter S, Schmid R, Largo RH, Boltshauser E, Fanconi S, Duc G, Rumpel H. (1996) Diagnostic and prognostic value of cerebral 31P magnetic resonance spectroscopy in neonates with perinatal asphyxia. *Pediatric Research* 40:749-758

Martin-Ancel A, Garcia-Alix A, Gaya F, Cabanas F, Burgueros M, Quero J. (1995) Multiple organ involvement in perinatal asphyxia. *Journal of Pediatrics.* 127:786-793

Martin-Ancel A, Garcia-Alix A, Pascual-Salcedo D, Cabanas F, Valcarce M, Quero J. (1997) Interleukin-6 in the cerebrospinal fluid after perinatal asphyxia is related to early and late neurological manifestations. *Pediatrics* 100:789-794

Matsui TN, T. Kumanishi, T. Asano, T. (1999) Role of nitric oxide in pathogenesis underlying ischemic cerebral damage. *Cellular & Molecular Neurobiology* 19:177-189

McBride MC, Laroia N, Guillet R. (2000) Electrographic seizures in neonates correlate with poor neurodevelopmental outcome. *Neurology* 55:506-513

McDonald JW, Johnston MV. (1990) Physiological and pathophysiological roles of excitatory amino acids during central nervous system development. *Brain Research Brain Research Reviews* 15:41-70

McGuire W, Fowlie PW, Evans DJ. (2004) Naloxone for preventing morbidity and mortality in newborn infants of greater than 34 weeks' gestation with suspected perinatal asphyxia. *Cochrane Database of Systematic Reviews* 1:CD003955.

McLean D. (2004) Computed tomography doses in children. *Lancet* 363:1178

Meara J, Kendall G, Muirhead C, Wall B. (2004) Ionising radiation in infancy and adult cognitive function: protocols for computed tomography must be optimised.[comment]. *Bmj* 328:6

Mehl-Madrona L, Madrona MM. (1997) Physician- and midwife-attended home births. Effects of breech, twin, and post-dates outcome data on mortality rates. *Journal of Nurse-Midwifery.* 42:91-98

Mencher LS, Mencher GT. (1999) Neonatal asphyxia, definitive markers and hearing loss. *Audiology.* 38:291-295

Ment LR, Bada HS, Barnes P, Grant PE, Hirtz D, Papile LA, Pinto-Martin J, Rivkin M, Slovis TL. (2002) Practice parameter: neuroimaging of the neonate: report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. *Neurology* 58:1726-1738

Mercuri E, Guzzetta A, Haataja L, Cowan F, Rutherford M, Counsell S, Papadimitriou M, Cioni G, Dubowitz L. (1999) Neonatal neurological examination in infants with hypoxic ischaemic encephalopathy: correlation with MRI findings. *Neuropediatrics.* 30:83-89

Mercuri E, Ricci D, Cowan FM, Lessing D, Frisone MF, Haataja L, Counsell SJ, Dubowitz LM, Rutherford MA. (2000) Head growth in infants with hypoxic-ischemic encephalopathy: correlation with neonatal magnetic resonance imaging. *Pediatrics.* 106:235-243

Meyer C, Witte J, Hildmann A, Hennecke KH, Schunck KU, Maul K, Franke U, Fahnenstich H, Rabe H, Rossi R, Hartmann S, Gortner L. (1999) Neonatal screening for hearing disorders in infants at risk: incidence, risk factors, and follow-up. *Pediatrics.* 104:900-904

Miller JA, Jr., Miller FS. (1954) Factors in neonatal resistance to anoxia. II. Effects of elevated and reduced temperature upon survival and recovery by neonatal guinea pigs. *Surgery* 36:916-930

Miller JA, Jr., Miller FS, Westin B. (1964) Hypothermia in the treatment of asphyxia neonatorum. *Biologia Neonatorum* 20:148-163

Miller JA, Miller, F.S. (1969) Physiological, biochemical and clinical aspects of hypothermia in neonatal asphyxia. In: *Depressed Metabolism* (Mussachia XJS, J.F., ed), New York: Elsevier, pp 427-452

Miller SP, Latal B, Clark H, Barnwell A, Glidden D, Barkovich AJ, Ferriero DM, Partridge JC. (2004) Clinical signs predict 30-month neurodevelopmental outcome after neonatal encephalopathy. *American Journal of Obstetrics & Gynecology* 190:93-99

Milley JR, Rosenberg AA, Jones MD, Jr. (1984) Retinal and choroidal blood flows in hypoxic and hypercarbic newborn lambs. *Pediatric Research* 18:410-414

Milsom I, Ladfors L, Thiringer K, Niklasson A, Odeback A, Thornberg E. (2002) Influence of maternal, obstetric and fetal risk factors on the prevalence of birth asphyxia at term in a Swedish urban population. *Acta Obstetricia et Gynecologica Scandinavica* 81:909-917

Minamisawa H, Smith ML, Siesjo BK. (1990) The effect of mild hyperthermia and hypothermia on brain damage following 5, 10, and 15 minutes of forebrain ischemia. *Annals of Neurology* 28:26-33

Ministry of Health. (2003) Report on Maternity 2000 & 2001. In: *http://www.moh.govt.nz/moh.nsf/ea6005dc347e7bd44c2566a40079ae6f/dc1cadf8630ae728cc256d0900762153/$FILE/maternityreport00-01.pdf*

Mir NA, Faquih AM, Legnain M. (1989) Perinatal risk factors in birth asphyxia: relationship of obstetric and neonatal complications to neonatal mortality in 16,365 consecutive live births. *Asia-Oceania Journal of Obstetrics & Gynaecology.* 15:351-357

Miyazawa T, Tamura A, Fukui S, Hossmann KA. (2003) Effect of mild hypothermia on focal cerebral ischemia. Review of experimental studies. *Neurological Research* 25:457-464

Mizrahi EM. (1989) Consensus and controversy in the clinical management of neonatal seizures. *Clinics in Perinatology* 16:485-500

Moller JC, Thielsen B, Schaible TF, Reiss I, Kohl M, Welp T, Gortner L. (1998) Value of myocardial hypoxia markers (creatine kinase and its MB-fraction, troponin-T, QT-intervals) and serum creatinine for the retrospective diagnosis of perinatal asphyxia. *Biology of the Neonate.* 73:367-374

Moncada SB, Juan P. (2006) Nitric oxide, cell bioenergetics and neurodegeneration. *Journal of Neurochemistry* 97:1676-1689

Mujsce DJ, Towfighi J, Stern D, Vannucci RC. (1990) Mannitol therapy in perinatal hypoxic-ischemic brain damage in rats. *Stroke* 21:1210-1214

Muttitt SC, Taylor MJ, Kobayashi JS, MacMillan L, Whyte HE. (1991) Serial visual evoked potentials and outcome in term birth asphyxia. *Pediatric Neurology.* 7:86-90

Myers RE. (1975) Four patterns of perinatal brain damage and their conditions of occurrence in primates. *Advances in Neurology.* 10:223-234

Naeye RL, Peters EC, Bartholomew M, Landis JR. (1989) Origins of cerebral palsy. *American Journal of Diseases of Children.* 143:1154-1161

Nagdyman N, Grimmer I, Scholz T, Muller C, Obladen M. (2003) Predictive value of brain-specific proteins in serum for neurodevelopmental outcome after birth asphyxia. *Pediatric Research* 54:270-275

Nagdyman N, Komen W, Ko HK, Muller C, Obladen M. (2001) Early biochemical indicators of hypoxic-ischemic encephalopathy after birth asphyxia. *Pediatric Research* 49:502-506

Nakamura T MO, Sumitani K, Negi T, Itano T, Nagao S. (2003) Do rapid systemic changes of brain temperature have an influence on the brain? *Acta Neurochir (Wien)* 145:301-307

Nakashima K, Todd, MM. (1996) Effects of hypothermia on the rate of excitatory amino acid release after ischemic depolarization. *Stroke* 27:913-918

Nelson KB, Dambrosia JM, Ting TY, Grether JK. (1996) Uncertain value of electronic fetal monitoring in predicting cerebral palsy. *New England Journal of Medicine* 334:613-618

Nelson KB, Ellenberg JH. (1981) Apgar scores as predictors of chronic neurologic disability. *Pediatrics.* 68:36-44

Nelson KB, Ellenberg JH. (1986) Antecedents of cerebral palsy. Multivariate analysis of risk. *New England Journal of Medicine.* 315:81-86

Nelson KB, Leviton A. (1991) How much of neonatal encephalopathy is due to birth asphyxia? *American Journal of Diseases of Children.* 145:1325-1331

Neumar RW, Bircher NG, Sim KM, Xiao F, Zadach KS, Radovsky A, Katz L, Ebmeyer E, Safar P. (1995) Epinephrine and sodium bicarbonate during CPR following asphyxial cardiac arrest in rats. *Resuscitation* 29:249-263

Newton TH, Gooding CA. (1975) Compression of superior sagittal sinus by neonatal calvarial molding. *Radiology* 115:635-640

Niswander K, Henson G, Elbourne D, Chalmers I, Redman C, Macfarlane A, Tizard P. (1984) Adverse outcome of pregnancy and the quality of obstetric care. *Lancet.* 2:827-831

Nolan JP, Morley PT, Hoek TL, Hickey RW, Advancement Life support Task Force of the International Liaison committee on R. (2003) Therapeutic hypothermia after cardiac arrest. An advisory statement by the Advancement Life support Task Force of the International Liaison committee on Resuscitation. *Resuscitation* 57:231-235

Nolan JP MP, Hoek TL, Hickey RW; Advancement Life support Task Force of the International Liaison committee on Resuscitation. (2003) Therapeutic hypothermia after cardiac arrest. An advisory statement by the Advancement Life support Task Force of the International Liaison committee on Resuscitation. *Resuscitation* 57:231-235

Novelli A, Reilly JA, Lysko PG, Henneberry RC. (1988) Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced. *Brain Research* 451:205-212

Oh KS, Bender TM, Bowen A, Godine L, Park SC. (1985) Transient myocardial ischemia of the newborn infant. *Pediatric Radiology.* 15:29-33

Okada D. (1996) Zinc protoporphyrin IX suppresses nitric oxide production through a loss of L-arginine in rat cerebellar slices. *Neurosci Res.* 25:353-358

Ostrea EM, Jr., Odell GB. (1972) The influence of bicarbonate administration on blood pH in a "closed system": clinical implications. *Journal of Pediatrics* 80:671-680

Oswyn G, Vince JD, Friesen H. (2000) Perinatal asphyxia at Port Moresby General Hospital: a study of incidence, risk factors and outcome. *Papua New Guinea Medical Journal.* 43:110-120

Palme-Kilander C. (1992) Methods of resuscitation in low-Apgar-score newborn infants--a national survey. *Acta Paediatrica.* 81:739-744

Palmer C, Towfighi J, Roberts RL, Heitjan DF. (1993) Allopurinol administered after inducing hypoxia-ischemia reduces brain injury in 7-day-old rats. *Pediatric Research* 33:405-411

Palmer C, Vannucci RC, Towfighi J. (1990) Reduction of perinatal hypoxic-ischemic brain damage with allopurinol. *Pediatric Research* 27:332-336

Parer JT. (1983) The influence of beta-adrenergic activity on fetal heart rate and the umbilical circulation during hypoxia in fetal sheep. *American Journal of Obstetrics & Gynecology* 147:592-597

Parer JT. (1984) The effect of atropine on heart rate and oxygen consumption of the hypoxic fetus. *Am J Obstet Gynecol.* 148:1118-1122

Park TS, Van Wylen DG, Rubio R, Berne RM. (1988) Brain interstitial adenosine and sagittal sinus blood flow during systemic hypotension in piglet. *Journal of Cerebral Blood Flow & Metabolism.* 8:822-828

Pasternak JF, Predey TA, Mikhael MA. (1991) Neonatal asphyxia: vulnerability of basal ganglia, thalamus, and brainstem. *Pediatric Neurology.* 7:147-149

Patel J, Edwards AD. (1997) Prediction of outcome after perinatal asphyxia. *Current Opinion in Pediatrics.* 9:128-132

Pattison N, Teele R. (2001) A plea for a comprehensive perinatal database. *New Zealand Medical Journal.* 114:439-440

Pearce W. (2006) Hypoxic regulation of the fetal cerebral circulation. *J Appl Physiol.* 100:731-738

Pearce WJ. (1995) Mechanisms of hypoxic cerebral vasodilatation. *Pharmacol Ther* 65:75-91

Peeters LL, Sheldon RE, Jones MD, Jr., Makowski EL, Meschia G. (1979) Blood flow to fetal organs as a function of arterial oxygen content. *American Journal of Obstetrics & Gynecology.* 135:637-646

Peeters-Scholte C, Koster J, Veldhuis W, van den Tweel E, Zhu C, Kops N, Blomgren K, Bar D, van Buul-Offers S, Hagberg H, Nicolay K, van Bel F, Groenendaal F. (2002) Neuroprotection by selective nitric oxide synthase inhibition at 24 hours after perinatal hypoxia-ischemia. *Stroke* 33:2304-2310

Peliowski A, Finer N. (1992) Birth asphyxia in the term infant. In: *Effective Care Of The Newborn* (Sinclair JC BM, ed), Oxford: Oxford University Press, pp 249 - 279

Penning DH, Patrick J, Jimmo S, Brien JF. (1991) Release of glutamate and gamma-aminobutyric acid in the ovine fetal hippocampus: ontogeny and effect of hypoxia. *Journal of Developmental Physiology.* 16:301-307

Penrice J, Amess PN, Punwani S, Wylezinska M, Tyszczuk L, D'Souza P, Edwards AD, Cady EB, Wyatt JS, Reynolds EO. (1997a) Magnesium sulfate after transient hypoxia-ischemia fails to prevent delayed cerebral energy failure in the newborn piglet. *Pediatric Research.* 41:443-447

Penrice J, Lorek A, Cady EB, Amess PN, Wylezinska M, Cooper CE, D'Souza P, Brown GC, Kirkbride V, Edwards AD, Wyatt JS, Reynolds EO. (1997b) Proton magnetic resonance spectroscopy of the brain during acute hypoxia-

ischemia and delayed cerebral energy failure in the newborn piglet. *Pediatric Research.* 41:795-802

Perlman JM. (1989) Systemic abnormalities in term infants following perinatal asphyxia: relevance to long-term neurologic outcome. *Clinics in Perinatology.* 16:475-484

Perlman JM, Risser R. (1996) Can asphyxiated infants at risk for neonatal seizures be rapidly identified by current high-risk markers? *Pediatrics.* 97:456-462

Perlman JM, Tack ED. (1988) Renal injury in the asphyxiated newborn infant: relationship to neurologic outcome. *Journal of Pediatrics.* 113:875-879

Perlman JM, Tack ED, Martin T, Shackelford G, Amon E. (1989) Acute systemic organ injury in term infants after asphyxia. *American Journal of Diseases of Children.* 143:617-620

Pezzani C, Radvanyi-Bouvet MF, Relier JP, Monod N. (1986) Neonatal electroencephalography during the first twenty-four hours of life in full-term newborn infants. *Neuropediatrics* 17:11-18

Pharoah PO, Cooke T, Johnson MA, King R, Mutch L. (1998) Epidemiology of cerebral palsy in England and Scotland, 1984-9. *Archives of Disease in Childhood Fetal & Neonatal Edition.* 79:F21-25

Phelan JP, Ahn MO, Korst L, Martin GI, Wang YM. (1998) Intrapartum fetal asphyxial brain injury with absent multiorgan system dysfunction. *Journal of Maternal-Fetal Medicine.* 7:19-22

Phillippe M. (1983) Fetal catecholamines. *American Journal of Obstetrics & Gynecology* 146:840-855

Pierrat V, Haouari N, Liska A, Thomas D, Subtil D, Truffert P, Groupe d'Etudes en Epidémiologie Périnatale. (2005) Prevalence, causes, and outcome at 2 years of age of newborn encephalopathy: population based study. *Arch Dis Child Fetal Neonatal Ed.* 90:F257-261

Prabhakar NR. (1998) Endogenous carbon monoxide in control of respiration. *Respir Physiol.* 114:57-64

Prechtl HF, Ferrari F, Cioni G. (1993) Predictive value of general movements in asphyxiated fullterm infants. *Early Human Development* 35:91-120

Procianoy RS, Giacomini CB, Oliveira ML. (1988) Fetal and neonatal cortical adrenal function in birth asphyxia. *Acta Paediatrica Scandinavica.* 77:671-674

Pryds O, Edwards AD. (1996) Cerebral blood flow in the newborn infant. *Archives of Disease in Childhood Fetal & Neonatal Edition* 74:F63-69

Ramirez J, Folkow L, Blix A. (2007) Hypoxia tolerance in mammals and birds: from the wilderness to the clinic. *Annu Rev Physiol.* 69:113-143

Ramji S, Ahuja S, Thirupuram S, Rootwelt T, Rooth G, Saugstad OD. (1993) Resuscitation of asphyxic newborn infants with room air or 100% oxygen. *Pediatric Research* 34:809-812

Ranjit MS. (2000) Cardiac abnormalities in birth asphyxia. *Indian Journal of Pediatrics.* 67:S26-29

Reed RL, 2nd, Bracey AW, Jr., Hudson JD, Miller TA, Fischer RP. (1990) Hypothermia and blood coagulation: dissociation between enzyme activity and clotting factor levels. *Circulatory Shock* 32:141-152

Rees K, Beranek-Stanley M, Burke M, Ebrahim S. (2001) Hypothermia to reduce neurological damage following coronary artery bypass surgery. *Cochrane Database of Systematic Reviews* 1:CD002138

Reid DL, Parer JT, Williams K, Darr D, Phernetton TM, Rankin JH. (1991) Effects of severe reduction in maternal placental blood flow on blood flow distribution in the sheep fetus. *Journal of Developmental Physiology.* 15:183-188

Reith J, Jorgensen HS, Pedersen PM, Nakayama H, Raaschou HO, Jeppesen LL, Olsen TS. (1996) Body temperature in acute stroke: relation to stroke severity, infarct size, mortality, and outcome.[see comment]. *Lancet* 347:422-425

Rennie JM. (1997) Neonatal seizures. *European Journal of Pediatrics* 156:83-87

Rennie JM, Boylan GB. (2003) Neonatal seizures and their treatment. *Current Opinion in Neurology* 16:177-181

Rennie JM, Chorley G, Boylan GB, Pressler R, Nguyen Y, Hooper R. (2004) Non-expert use of the cerebral function monitor for neonatal seizure detection. *Archives of Disease in Childhood Fetal & Neonatal Edition* 89:F37-40

Richardson BS. (1989) Fetal adaptive responses to asphyxia. *Clinics in Perinatology* 16:595-611

Richardson BS, Carmichael L, Homan J, Patrick JE. (1992) Electrocortical activity, electroocular activity, and breathing movements in fetal sheep with prolonged and graded hypoxemia. *American Journal of Obstetrics & Gynecology* 167:553-558

Richmond S. (2007) ILCOR and neonatal resuscitation 2005. *Arch Dis Child Fetal Neonatal Ed.* 92 .F163-165

Riikonen RS, Kero PO, Simell OG. (1992) Excitatory amino acids in cerebrospinal fluid in neonatal asphyxia. *Pediatric Neurology* 8:37-40

Riikonen RS, Korhonen LT, Lindholm DB. (1999) Cerebrospinal nerve growth factor--a marker of asphyxia? *Pediatric Neurology* 20:137-141

Rivkin MJ, Volpe JJ. (1993) Hypoxic-ischemic brain injury in the newborn. *Semin Neurol* 13:30-39

Robertson C, Finer N. (1985) Term infants with hypoxic-ischemic encephalopathy: outcome at 3.5 years. *Developmental Medicine & Child Neurology.* 27:473-484

Robertson CM, Finer NN. (1988) Educational readiness of survivors of neonatal encephalopathy associated with birth asphyxia at term. *Journal of Developmental & Behavioral Pediatrics.* 9:298-306

Robertson CM, Finer NN. (1993) Long-term follow-up of term neonates with perinatal asphyxia. *Clinics in Perinatology.* 20:483-500

Robertson CM, Finer NN, Grace MG. (1989) School performance of survivors of neonatal encephalopathy associated with birth asphyxia at term. *Journal of Pediatrics.* 114:753-760

Robertson RL, Ben-Sira L, Barnes PD, Mulkern RV, Robson CD, Maier SE, Rivkin MJ, du Plessis A. (1999) MR line-scan diffusion-weighted imaging of term neonates with perinatal brain ischemia. *AJNR: American Journal of Neuroradiology* 20:1658-1670

Rohrer MJ, Natale AM. (1992) Effect of hypothermia on the coagulation cascade. *Critical Care Medicine* 20:1402-1405

Roland EH, Flodmark O, Hill A. (1990) Thalamic hemorrhage with intraventricular hemorrhage in the full-term newborn. *Pediatrics.* 85:737-742

Roland EH, Hill A, Norman MG, Flodmark O, MacNab AJ. (1988) Selective brainstem injury in an asphyxiated newborn. *Annals of Neurology.* 23:89-92

Roland EH, Jan JE, Hill A, Wong PK. (1986) Cortical visual impairment following birth asphyxia. *Pediatric Neurology.* 2:133-137

Rordorf G, Uemura Y, Bonventre JV. (1991) Characterization of phospholipase A2 (PLA2) activity in gerbil brain: enhanced activities of cytosolic, mitochondrial, and microsomal forms after ischemia and reperfusion. *Journal of Neuroscience* 11:1829-1836

Rose AL, Lombroso CT. (1970) A study of clinical, pathological, and electroencephalographic features in 137 full-term babies with a long-term follow-up. *Pediatrics* 45:404-425

Rosenberg AA. (1992) Response of the cerebral circulation to hypocarbia in postasphyxia newborn lambs. *Pediatric Research* 32:537-541

Rosenberg AA, Jones MD, Jr., Traystman RJ, Simmons MA, Molteni RA. (1982) Response of cerebral blood flow to changes in PCO2 in fetal, newborn, and adult sheep. *American Journal of Physiology* 242

Rosenbloom L. (1994) Dyskinetic cerebral palsy and birth asphyxia. *Developmental Medicine & Child Neurology* 36:285-289

Rosenbloom L. (1995) Diagnosis and management of cerebral palsy. *Archives of Disease in Childhood.* 72:350-354

Rosenbloom L, Rennie JM. (2000) Establishing probable cause in cerebral palsy. There are problems with the consensus statement. *BMJ.* 320:1076

Rosenthal TB. (1948) The effect of temperature on the pH of blood and plasma in vitro. *J. biol. Chem.* 173:25-30

Rossi EM, Philipson EH, Williams TG, Kalhan SC. (1989) Meconium aspiration syndrome: intrapartum and neonatal attributes. *American Journal of Obstetrics & Gynecology.* 161:1106-1110

Roth SC, Baudin J, Cady E, Johal K, Townsend JP, Wyatt JS, Reynolds EO, Stewart AL. (1997) Relation of deranged neonatal cerebral oxidative metabolism with neurodevelopmental outcome and head circumference at 4 years. *Developmental Medicine & Child Neurology* 39:718-725

Roth SC, Edwards AD, Cady EB, Delpy DT, Wyatt JS, Azzopardi D, Baudin J, Townsend J, Stewart AL, Reynolds EO. (1992) Relation between cerebral

oxidative metabolism following birth asphyxia, and neurodevelopmental outcome and brain growth at one year. *Developmental Medicine & Child Neurology.* 34:285-295

Rowe RD, Hoffman T. (1972) Transient myocardial ischemia of the newborn infant: a form of severe cardiorespiratory distress in full-term infants. *Journal of Pediatrics.* 81:243-250

Rurak DW. (1978) Plasma vasopressin levels during hypoxaemia and the cardiovascular effects of exogenous vasopressin in foetal and adult sheep. *J Physiol.* 277:341-357

Russell GA, Cooke RW. (1995) Randomised controlled trial of allopurinol prophylaxis in very preterm infants. *Archives of Disease in Childhood Fetal & Neonatal Edition* 73:F27-31

Ruth V, Korkman M, Liikanen A, Paetau P. (1991) High-dose phenobarbitol treatment to prevent postasphyxial brain damage: a 6 year follow-up. *Pediatric Research* 30:638A

Ruth VJ. (1989) Prognostic value of creatine kinase BB-isoenzyme in high risk newborn infants. *Archives of Disease in Childhood* 64:563-568

Ruth VJ, Raivio KO. (1988) Perinatal brain damage: predictive value of metabolic acidosis and the Apgar score. *BMJ.* 297:24-27

Rutherford M, Counsell S, Allsop J, Boardman J, Kapellou O, Larkman D, Hajnal J, Edwards D, Cowan F. (2004) Diffusion-weighted magnetic

resonance imaging in term perinatal brain injury: a comparison with site of lesion and time from birth. *Pediatrics* 114:1004-1014

Rutherford M, Pennock J, Schwieso J, Cowan F, Dubowitz L. (1996) Hypoxic-ischaemic encephalopathy: early and late magnetic resonance imaging findings in relation to outcome. *Archives of Disease in Childhood Fetal & Neonatal Edition* 75:F145-151

Rutherford MA AD, Whitelaw A, Cowan F, Renowden S, Edwards AD, Thoresen M. (2005) Mild hypothermia and the distribution of cerebral lesions in neonates with hypoxic-ischemic encephalopathy. *Pediatrics.* 116:1001-1006

Rutherford MA, Pennock JM, Counsell SJ, Mercuri E, Cowan FM, Dubowitz LM, Edwards AD. (1998) Abnormal magnetic resonance signal in the internal capsule predicts poor neurodevelopmental outcome in infants with hypoxic-ischemic encephalopathy. *Pediatrics.* 102:323-328

Rutherford MA, Pennock JM, Dubowitz LM. (1994) Cranial ultrasound and magnetic resonance imaging in hypoxic-ischaemic encephalopathy: a comparison with outcome. *Developmental Medicine & Child Neurology* 36:813-825

Rutherford MA, Pennock JM, Schwieso JE, Cowan FM, Dubowitz LM. (1995) Hypoxic ischaemic encephalopathy: early magnetic resonance imaging findings and their evolution. *Neuropediatrics* 26:183-191

Saigal S. (1991) Follow-up of high risk infants:methodological issues, current status, and future trends. In: *Reproductive and Perinatal Epidemiology* (M K, ed), Boca Raton: CRC Press, pp 337-355

Saili A, Sarna MS, Gathwala G, Kumari S, Dutta AK. (1990) Liver dysfunction in severe birth asphyxia. *Indian Pediatrics.* 27:1291-1294

Saint Hilaire MH, Burke RE, Bressman SB, Brin MF, Fahn S. (1991) Delayed-onset dystonia due to perinatal or early childhood asphyxia. *Neurology* 41:216-222

Salhab WA, Wyckoff MH, Laptook AR, Perlman JM. (2004) Initial hypoglycemia and neonatal brain injury in term infants with severe fetal acidemia. *Pediatrics* 114:361-366

Sann L. (1999) Transitory hyperinsulinism with hypoglycemia in asphyxia neonatorum. *Archives de Pediatrie.* 6:228

Sarnat HB, Sarnat MS. (1976) Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Archives of Neurology.* 33:696-705

Sato T, Takahashi K, Kojima M. (1977) Sclerema neonatorum associated with systemic fibrosis and endocardial fibroelastosis. *Acta Pathologica Japonica* 27:917-925

Saugstad OD, Rootwelt T, Aalen O. (1998) Resuscitation of asphyxiated newborn infants with room air or oxygen: an international controlled trial: the Resair 2 study. *Pediatrics.* 102:e1

Scher MS, Redline RW, Bangert BA. (2002) Delayed onset of status epilepticus after transient asphyxia in an asymptomatic full-term neonate. *Journal of Child Neurology.* 17:780-783

Schneider H, Ballowitz L, Schachinger H, Hanefeld F, Droszus JU. (1975) Anoxic encephalopathy with predominant involvement of basal ganglia, brain stem and spinal cord in the perinatal period. Report on seven newborns. *Acta Neuropathologica.* 32:287-298

Schubert A. (1995) Side effects of mild hypothermia. *Journal of Neurosurgical Anesthesiology* 7:139-147

Schultz K, Soltesz G. (1991) Transient hyperinsulinism in asphyxiated newborn infants. *Acta Paediatrica Hungarica.* 31:47-52

Schulzke S, Buchner S, Fahnenstich H. (2005) Subcutaneous fat necrosis of the newborn. *Swiss Medical Weekly* 135:122-123

Schulzke S, Rao S, Patole S. (2007) A systematic review of cooling for neuroprotection in neonates with hypoxic ischemic encephalopathy - are we there yet?. *BMC Pediatrrics* 7:30

Scott H. (1976) Outcome of very severe birth asphyxia. *Archives of Disease in Childhood.* 51:712-716

Sensenbrenner M, Lucas M, Deloulme JC. (1997) Expression of two neuronal markers, growth-associated protein 43 and neuron-specific enolase, in rat glial cells. *Journal of Molecular Medicine* 75:653-663

Shah P, Ohlsson A, Perlman M. (2007) Hypothermia to treat neonatal hypoxic ischemic encephalopathy: systematic review. *Arch Pediatr Adolesc Med.* 161:951-958

Shah S, Tracy M, Smyth J. (2004) Postnatal lactate as an early predictor of short-term outcome after intrapartum asphyxia. *Journal of Perinatology* 24:16-20

Shalak L, Perlman JM. (2004) Hypoxic-ischemic brain injury in the term infant-current concepts. *Early Human Development* 80:125-141

Shalak LF, Laptook AR, Velaphi SC, Perlman JM. (2003) Amplitude-integrated electroencephalography coupled with an early neurologic examination enhances prediction of term infants at risk for persistent encephalopathy. *Pediatrics* 111:351-357

Shankaran S. (2002) The postnatal management of the asphyxiated term infant. *Clinics in Perinatology.* 29:675-692

Shankaran S LA, Ehrenkranz R, Tyson J, McDonald S, Donavan, Fanaroff A, Poole K, Wright L, Higgins R, Goldberg R. NICHD Neonatal Research Network, Bethesda, MD. (2005) Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. N Engl J Med. 353(15):1574-84

Shankaran S, Laptook A, Wright LL, Ehrenkranz RA, Donovan EF, Fanaroff AA, Stark AR, Tyson JE, Poole K, Carlo WA, Lemons JA, Oh W, Stoll BJ, Papile LA, Bauer CR, Stevenson DK, Korones SB, McDonald S. (2002) Whole-body hypothermia for neonatal encephalopathy: animal observations

as a basis for a randomized, controlled pilot study in term infants. *Pediatrics.* 110:377-385

Shankaran S, Woldt E, Koepke T, Bedard MP, Nandyal R. (1991) Acute neonatal morbidity and long-term central nervous system sequelae of perinatal asphyxia in term infants. *Early Human Development.* 25:135-148

Sheldon RA, Partridge JC, Ferriero DM. (1992) Postischemic hyperglycemia is not protective to the neonatal rat brain. *Pediatric Research.* 32:489-493

Sheldon RE, Peeters LL, Jones MD, Jr., Makowski EL, Meschia G. (1979) Redistribution of cardiac output and oxygen delivery in the hypoxemic fetal lamb. *American Journal of Obstetrics & Gynecology.* 135:1071-1078

Shevell MI SK, O'Gorman AM, Watters GV, Montes JL. (1989) Neonatal dural sinus thrombosis. *Pediatr Neurol.* 5:161-165

Shi Y LH, Pan J, Qin S, Yao Z, Jiang D, Shen J. (1997) Evidence of increased endogenous carbon monoxide production in newborn rat endotoxicosis. *Chin Med Sci J.* 12:212-215

Shi Y PF, Li H, Pan J, Qin S, Jiang D, Shen C. (2000a) Plasma carbon monoxide levels in term newborn infants with sepsis. *Biol Neonate.* 78:230-232

Shi Y PF, Li H, Pan J, Qin S, Shen C. (2000b) Role of carbon monoxide and nitric oxide in newborn infants with postasphyxial hypoxic-ischemic encephalopathy. *Pediatrics.* 106:1447-1451

Shinomura T NS, Mori K. (1994) Reduction of depolarization-induced glutamate release by heme oxygenase inhibitor: possible role of carbon monoxide in synaptic transmission. *Neurosci Lett.* 166:131-134

Siegel MJ, Shackelford GD, Perlman JM, Fulling KH. (1984) Hypoxic-ischemic encephalopathy in term infants: diagnosis and prognosis evaluated by ultrasound. *Radiology* 152:395-399

Silverman WA, Fertig JW, Berger AP. (1958) The influence of the thermal environment upon the survival of newly born premature infants. *Pediatrics* 22:876-886

Silverstein FS, Buchanan K, Johnston MV. (1986) Perinatal hypoxia-ischemia disrupts striatal high-affinity [3H]glutamate uptake into synaptosomes. *Journal of Neurochemistry* 47:1614-1619

Simbruner G, Haberl C, Harrison V, Linley L, Willeitner AE. (1999) Induced brain hypothermia in asphyxiated human newborn infants: a retrospective chart analysis of physiological and adverse effects. *Intensive Care Medicine* 25:1111-1117

Simon NP. (1999) Long-term neurodevelopmental outcome of asphyxiated newborns. *Clinics in Perinatology* 26:767-778

Singh D KP, Majumdar S, Narang A,. (2004) Effect of phenobarbital on free radicals in neonates with hypoxic ischemic encephalopathy - a randomized controlled trial. *Journal of Perinatal Medicine* 32:278-281

Sizonenko S, Bednarek N, Gressens P. (2007) Growth factors and plasticity. *Semin Fetal Neonatal Med.* 12:241-249.

Slovis TL. (2002a) The ALARA concept in pediatric CT: myth or reality? *Radiology* 223:5-6

Slovis TL. (2002b) CT and computed radiography: the pictures are great, but is the radiation dose greater than required? *AJR. American Journal of Roentgenology* 179:39-41

Slovis TL. (2003) Children, computed tomography radiation dose, and the As Low As Reasonably Achievable (ALARA) concept.[comment]. *Pediatrics* 112:971-972

Smith J, Wells L, Dodd K. (2000) The continuing fall in incidence of hypoxic-ischaemic encephalopathy in term infants. *BJOG: an International Journal of Obstetrics & Gynaecology.* 107:461-466

Snyder SH JS, Zakhary R. (1998) Nitric oxide and carbon monoxide: parallel roles as neural messengers. *Brain Res Brain Res Rev.* 26:167-175

Soll RF. (2000a) Prophylactic natural surfactant extract for preventing morbidity and mortality in preterm infants. *Cochrane Database Syst Rev.* 2:CD000511

Soll RF MC. (2000b) Synthetic surfactant for respiratory distress syndrome in preterm infants. *Cochrane Database Syst Rev.* 2:CD001149

Sprung J CE, Gamulin S, Kampine JP, Bosnjak ZJ. (1991) Effects of acute hypothermia and beta-adrenergic receptor blockade on serum potassium concentration in rats. *Crit Care Med* 19:1545-1551

Sprung J, Cheng EY, Gamulin S, Kampine JP, Bosnjak ZJ. (1992) The effect of acute hypothermia and serum potassium concentration on potassium cardiotoxicity in anesthetized rats. *Acta Anaesthesiologica Scandinavica* 36:825-830

Sprung J, Gamulin S, Bosnjak ZJ, Kampine JP. (1990) Potassium correction of hypothermic hypokalemia induces hyperkalemia after rewarming. *Canadian Journal of Anaesthesia* 37

Stanley FJ, Blair E. (1991) Why have we failed to reduce the frequency of cerebral palsy? *Medical Journal of Australia.* 154:623-626

Stecker MM CA, Pochettino A, Kent GP, Patterson T, Weiss SJ, Bavaria JE. (2001) Deep hypothermic circulatory arrest: II. Changes in electroencephalogram and evoked potentials during rewarming. *Ann Thorac Surg.* 71:22-28

Steinberg GK, Bell TE, Yenari MA. (1996) Dose escalation safety and tolerance study of the N-methyl-D-aspartate antagonist dextromethorphan in neurosurgery patients. *Journal of Neurosurgery* 84:860-866

Steinberg GK, Kunis D, DeLaPaz R, Poljak A. (1993) Neuroprotection following focal cerebral ischaemia with the NMDA antagonist dextromethorphan, has a favourable dose response profile. *Neurological Research* 15:174-180

Stern S, Braun K. (1970) Pulmonary arterial and venous response to cooling: role of alpha-adrenergic receptors. *American Journal of Physiology* 219:982-985

Strauss DJ, Shavelle RM, Anderson TW. (1998) Life expectancy of children with cerebral palsy. *Pediatric Neurology.* 18:143-149

Strijbos PJ, Leach MJ, Garthwaite J. (1996) Vicious cycle involving Na+ channels, glutamate release, and NMDA receptors mediates delayed neurodegeneration through nitric oxide formation. *Journal of Neuroscience* 16:5004-5013

Suehiro E PJ. (2001) Exacerbation of traumatically induced axonal injury by rapid posthypothermic rewarming and attenuation of axonal change by cyclosporin A. *J Neurosurg. .* 94:493-498

Sutton L, Bajuk B, Berry G, Sayer GP, Richardson V, Henderson-Smart DJ. (2002) Score of neonatal acute physiology as a measure of illness severity in mechanically ventilated term babies. *Acta Paediatrica.* 91:415-423

Svenningsen NW, Blennow G, Lindroth M, Gaddlin PO, Ahlstrom H. (1982) Brain-orientated intensive care treatment in severe neonatal asphyxia. Effects of phenobarbitone protection. *Archives of Disease in Childhood.* 57:176-183

Swan H. (1973) Clinical hypothermia: a lady with a past and some promise for the future. *Surgery* 73:736-758

Szabo C. (1996) Physiological and pathophysiological roles of nitric oxide in the central nervous system. *Brain Research Bulletin* 41:131-141

Szabó C. (2007) Hydrogen sulphide and its therapeutic potential. *Nat Rev Drug Discov* 6:917-935

Takagi K, Ginsberg MD, Globus MY, Martinez E, Busto R. (1994) Effect of hyperthermia on glutamate release in ischemic penumbra after middle cerebral artery occlusion in rats. *American Journal of Physiology* 267

Takahashi K HE, Suzuki H, Sasano H, Shibahara S. (1996) Expression of heme oxygenase isozyme mRNAs in the human brain and induction of heme oxygenase-1 by nitric oxide donors. *J Neurochem.* 67:482-489

Tan WK, Williams CE, During MJ, Mallard CE, Gunning MI, Gunn AJ, Gluckman PD. (1996) Accumulation of cytotoxins during the development of seizures and edema after hypoxic-ischemic injury in late gestation fetal sheep. *Pediatric Research* 39:791-797

Taylor G. (1992) Intracranial venous system in the newborn: evaluation of normal anatomy and flow characteristics with color Doppler US. *Radiology* 183:449-452

Taylor MJ, Murphy WJ, Whyte HE. (1992) Prognostic reliability of somatosensory and visual evoked potentials of asphyxiated term infants. *Developmental Medicine & Child Neurology* 34:507-515

Thibeault DW, Hall FK, Sheehan MB, Hall RT. (1984) Postasphyxial lung disease in newborn infants with severe perinatal acidosis. *American Journal of Obstetrics & Gynecology.* 150:393-399

Thompson CM, Puterman AS, Linley LL, Hann FM, van der Elst CW, Molteno CD, Malan AF. (1997) The value of a scoring system for hypoxic ischaemic encephalopathy in predicting neurodevelopmental outcome. *Acta Paediatrica* 86:757-761

Thomson AJ, Searle M, Russell G. (1977) Quality of survival after severe birth asphyxia. *Archives of Disease in Childhood.* 52:620-626

Thordstein M, Bagenholm R, Thiringer K, Kjellmer I. (1993) Scavengers of free oxygen radicals in combination with magnesium ameliorate perinatal hypoxic-ischemic brain damage in the rat. *Pediatric Research* 34:23-26

Thoresen M. (2000) Cooling the newborn after asphyxia - physiological and experimental background and its clinical use. *Seminars in Neonatology.* 5:61-73

Thoresen M, Bagenholm R, Loberg EM, Apricena F, Kjellmer I. (1996a) Posthypoxic cooling of neonatal rats provides protection against brain injury. *Archives of Disease in Childhood Fetal & Neonatal Edition.* 74:F3-9

Thoresen M, Haaland K, Loberg EM, Whitelaw A, Apricena F, Hanko E, Steen PA. (1996b) A piglet survival model of posthypoxic encephalopathy. *Pediatric Research.* 40:738-748

Thoresen M, Penrice J, Lorek A, Cady EB, Wylezinska M, Kirkbride V, Cooper CE, Brown GC, Edwards AD, Wyatt JS, et al. (1995) Mild hypothermia after severe transient hypoxia-ischemia ameliorates delayed cerebral energy failure in the newborn piglet. *Pediatric Research.* 37:667-670

Thoresen M, Satas S, Loberg EM, Whitelaw A, Acolet D, Lindgren C, Penrice J, Robertson N, Haug E, Steen PA. (2001) Twenty-four hours of mild hypothermia in unsedated newborn pigs starting after a severe global hypoxic-ischemic insult is not neuroprotective. *Pediatric Research.* 50:405-411

Thoresen M, Satas S, Puka-Sundvall M, Whitelaw A, Hallstrom A, Loberg EM, Ungerstedt U, Steen PA, Hagberg H. (1997) Post-hypoxic hypothermia reduces cerebrocortical release of NO and excitotoxins. *Neuroreport.* 8:3359-3362

Thoresen M TJ, Satas S. (2003) Cooling amerliorates seizures in encephalopathic newborn infants. In: *Pediatric Research Society*, Baltimore: Pediatric Research, p 23A

Thoresen M, Whitelaw A. (2000) Cardiovascular changes during mild therapeutic hypothermia and rewarming in infants with hypoxic-ischemic encephalopathy. *Pediatrics.* 106:92-99

Thoresen M, Wyatt J. (1997) Keeping a cool head, post-hypoxic hypothermia--an old idea revisited. *Acta Paediatrica* 86:1029-1033

Thornberg E, Thiringer K, Hagberg H, Kjellmer I. (1995a) Neuron specific enolase in asphyxiated newborns: association with encephalopathy and cerebral function monitor trace. *Archives of Disease in Childhood Fetal & Neonatal Edition.* 72:F39-42

Thornberg E, Thiringer K, Odeback A, Milsom I. (1995b) Birth asphyxia: incidence, clinical course and outcome in a Swedish population. *Acta Paediatrica.* 84:927-932

Thorngren-Jerneck K, Ohlsson T, Sandell A, Erlandsson K, Strand SE, Ryding E, Svenningsen NW. (2001) Cerebral glucose metabolism measured by positron emission tomography in term newborn infants with hypoxic ischemic encephalopathy. *Pediatric Research* 49:495-501

Thorp PS, Levin SD, Garnett ES, Nahmias C, Firnau G, Toi A, Upton AR, Nobbs PT, Sinclair JC. (1988) Patterns of cerebral glucose metabolism using 18FDG and positron tomography in the neurologic investigation of the full term newborn infant. *Neuropediatrics* 19:146-153

Ting P, Pan Y. (1994) The effects of naloxone on the post-asphyxic cerebral pathophysiology of newborn lambs. *Neurological Research* 16:359-364

Toet MC, Hellstrom-Westas L, Groenendaal F, Eken P, de Vries LS. (1999) Amplitude integrated EEG 3 and 6 hours after birth in full term neonates with hypoxic-ischaemic encephalopathy. *Archives of Disease in Childhood Fetal & Neonatal Edition* 81:F19-23

Toet MC, van der Meij W, de Vries LS, Uiterwaal CS, van Huffelen KC. (2002) Comparison between simultaneously recorded amplitude integrated electroencephalogram (cerebral function monitor) and standard electroencephalogram in neonates. *Pediatrics* 109:772-779

Toh VC. (2000) Early predictors of adverse outcome in term infants with post-asphyxial hypoxic ischaemic encephalopathy. *Acta Paediatrica* 89:343-347

Tolcos M, Harding R, Loeliger M, Breen S, Cock M, Duncan J, Rees S. (2003) The fetal brainstem is relatively spared from injury following intrauterine hypoxemia. *Brain Res Dev Brain Res* 143:73-81

Tooley J, Satas S, Eagle R, Silver IA, Thoresen M. (2002) Significant selective head cooling can be maintained long-term after global hypoxia ischemia in newborn piglets. *Pediatrics.* 109:643-649

Tooley JR ER, Satas S, Thoresen M. (2005) Significant head cooling can be achieved while maintaining normothermia in the newborn piglet. *Arch Dis Child Fetal Neonatal Ed.* 90:F262-266

Tooley JR, Satas S, Porter H, Silver IA, Thoresen M. (2003) Head cooling with mild systemic hypothermia in anesthetized piglets is neuroprotective. *Annals of Neurology* 53:65-72

Torfs CP, van den Berg B, Oechsli FW, Cummins S. (1990) Prenatal and perinatal factors in the etiology of cerebral palsy. *Journal of Pediatrics.* 116:615-619

Towell ME. (1966) The influence of labor on the fetus and the newborn. *Pediatric Clinics of North America.* 13:575-598

Trescher WH, Ishiwa S, Johnston MV. (1997) Brief post-hypoxic-ischemic hypothermia markedly delays neonatal brain injury. *Brain & Development* 19:326-338

Trevisanuto D, Lachin M, Zaninotto M, Pellegrino PA, Plebani M, Cantarutti F, Zanardo V. (1998) Cardiac troponin T in newborn infants with transient myocardial ischemia. *Biology of the Neonate.* 73:161-165

Tseng EE, Brock MV, Lange MS, Blue ME, Troncoso JC, Kwon CC, Lowenstein CJ, Johnston MV, Baumgartner WA. (1997) Neuronal nitric oxide

synthase inhibition reduces neuronal apoptosis after hypothermic circulatory arrest. *Annals of Thoracic Surgery* 64:1639-1647

Vajro P, Amelio A, Stagni A, Paludetto R, Genovese E, Giuffre M, DeCurtis M. (1997) Cholestasis in newborn infants with perinatal asphyxia. *Acta Paediatrica.* 86:895-898

Vajro P, Paludetto R, DeCurtis M. (1999) Transient neonatal cholestasis and perinatal asphyxia. *Journal of Pediatrics.* 134:795

Valeri CR, Feingold H, Cassidy G, Ragno G, Khuri S, Altschule MD. (1987) Hypothermia-induced reversible platelet dysfunction. *Annals of Surgery* 205:175-181

Van Bel F, Shadid M, Moison RM, Dorrepaal CA, Fontijn J, Monteiro L, Van De Bor M, Berger HM. (1998) Effect of allopurinol on postasphyxial free radical formation, cerebral hemodynamics, and electrical brain activity. *Pediatrics* 101:185-193

Van Bel F, Walther FJ. (1990) Myocardial dysfunction and cerebral blood flow velocity following birth asphyxia. *Acta Paediatrica Scandinavica.* 79:756-762

van den Berg PP, Nelen WL, Jongsma HW, Nijland R, Kollée LA, Nijhuis JG, Eskes TK. (1996) Neonatal complications in newborns with an umbilical artery pH < 7.00. *Am J Obstet Gynecol.* 175:1152-1157

van Lieshout HB, Jacobs JW, Rotteveel JJ, Geven W, v't Hof M. (1995) The prognostic value of the EEG in asphyxiated newborns. *Acta Neurologica Scandinavica* 91:203-207

Vannucci RC. (1990) Experimental biology of cerebral hypoxia-ischemia: relation to perinatal brain damage. *Pediatric Research* 27:317-326

Vannucci RC, Duffy TE. (1977) Cerebral metabolism in newborn dogs during reversible asphyxia. *Annals of Neurology* 1:528-534

Vannucci RC, Perlman JM. (1997) Interventions for perinatal hypoxic-ischemic encephalopathy. *Pediatrics.* 100:1004-1014

Vannucci RC, Vannucci SJ. (1997) A model of perinatal hypoxic-ischemic brain damage. *Annals of the New York Academy of Sciences* 835:234-249

Vannucci RC, Yager JY. (1992) Glucose, lactic acid, and perinatal hypoxic-ischemic brain damage. *Pediatric Neurology.* 8:3-12

Vargas-Origel A, Espinosa-García JO, Muñiz-Quezada E, Vargas-Nieto MA, Aguilar-García G. (2004) Prevention of hypoxic-ischemic encephalopathy with high-dose, early phenobarbital therapy.
[Article in Spanish]. *Gac Med Mex.* 140:147-153

Veen S, de Haan J, Martens S, Riijken M, Van Bel F, Berger HM. (1999) Allopurinol treatment following severe asphyxia: Follow-up at two years of age. *Pediatric Research* 45:230A

Vento M, Asensi M, Sastre J, Garcia-Sala F, Vina J. (2001) Six years of experience with the use of room air for the resuscitation of asphyxiated newly born term infants. *Biology of the Neonate.* 79:261-267

Vento M, Asensi M, Sastre J, Lloret A, Garcia-Sala F, Vina J. (2003) Oxidative stress in asphyxiated term infants resuscitated with 100% oxygen. *Journal of Pediatrics.* 142:240-246

Viggedal G, Lundalv E, Carlsson G, Kjellmer I. (2002) Follow-up into young adulthood after cardiopulmonary resuscitation in term and near-term newborn infants. II. Neuropsychological consequences. *Acta Paediatrica.* 91:1218-1226

Volpe J. (2001) Hypoxic ischemic encephalopathy. In: *Neurology of the Newborn* (Volpe JJ, ed), Philedelphia: WB Saunders, pp 217-396

Volpe JJ, Herscovitch P, Perlman JM, Kreusser KL, Raichle ME. (1985) Positron emission tomography in the asphyxiated term newborn: parasagittal impairment of cerebral blood flow. *Annals of Neurology.* 17:287-296

Von Lubitz D. (1999) Adenosine and cerebral ischemia: therapeutic future or death of a brave concept? *Eur J Pharmacol.* 365:9-25

Voorhies TM LE, Lee BC, Vannucci RC, Auld PA. (1984) Occlusive vascular disease in asphyxiated newborn infants. *J Pediatr.* 105:92-96

Walker NI, Harmon BV, Gobe GC, Kerr JF. (1988) Patterns of cell death. *Methods & Achievements in Experimental Pathology* 13:18-54

Walther FJ, Siassi B, Ramadan NA, Wu PY. (1985) Cardiac output in newborn infants with transient myocardial dysfunction. *Journal of Pediatrics.* 107:781-785

Wass CT, Waggoner JR, 3rd, Cable DG, Schaff HV, Schroeder DR, Lanier WL. (1998a) Selective convective brain cooling during hypothermic cardiopulmonary bypass in dogs. *Annals of Thoracic Surgery* 66:2008-2014

Wass CT, Waggoner JR, 3rd, Cable DG, Schaff HV, Schroeder DR, Lanier WL. (1998b) Selective convective brain cooling during normothermic cardiopulmonary bypass in dogs. *Journal of Thoracic & Cardiovascular Surgery* 115:1350-1357

Wayenberg JL, Vermeylen D, Bormans J, Magrez P, Muller MF, Pardou A. (1994) Diagnosis of severe birth asphyxia and early prediction of neonatal neurological outcome in term asphyxiated newborns. *Journal of Perinatal Medicine* 22:129-136

Weinrauch V, Safar P, Tisherman S, Kuboyama K, Radovsky A. (1992) Beneficial effect of mild hypothermia and detrimental effect of deep hypothermia after cardiac arrest in dogs. *Stroke* 23:1454-1462

Wertheim D, Mercuri E, Faundez JC, Rutherford M, Acolet D, Dubowitz L. (1994) Prognostic value of continuous electroencephalographic recording in full term infants with hypoxic ischaemic encephalopathy. *Archives of Disease in Childhood.* 71:F97-102

West C CL, Battin M, Harding J, McCowan L, Belgrave S, Knight D, Westgate J. (2005a) Antenatal antecedents of moderate or severe neonatal encephalopathy in term infants - a regional review. *ANZJOG* 45:207-210.

West C HJ, Knight D, Battin M. (2005b) Demographic Characteristics And Clinical Course In Infants Admitted To Neonatal Intensive Care With Moderate Or Severe Neonatal Encephalopathy. *ANZJOG* 45:151-154.

Westgate J, Wibbens B, Bennet L, Wassink G, Parer J, Gunn A. (2007) The intrapartum deceleration in center stage: a physiologic approach to the interpretation of fetal heart rate changes in labor. *Am J Obstet Gynecol.* 197:e1-11

Westgate JA, Gunn AJ, Gunn TR. (1999) Antecedents of neonatal encephalopathy with fetal acidaemia at term. *British Journal of Obstetrics & Gynaecology.* 106:774-782

Westin B, Miller JA, Jr., Nyberg R, Wedenberg E. (1959) Neonatal asphyxia pallida treated with hypothermia alone or with hypothermia and transfusion of oxygenated blood. *Surgery* 45:868-879

Westin B, Nyberg R, Miller JA, Jr., Wedenberg E. (1962) Hypothermia and transfusion with oxygenated blood in the treatment of asphyxia neonatorum. *Acta Paediatrica* 51:1-80

White CR, Hamade MW, Siami K, Chang MM, Mangalwadi A, Frangos JA, Pearce WJ. (2005) Maturation enhances fluid shear-induced activation of eNOS in perfused ovine carotid arteries. *Am J Physiol Heart Circ Physiol* 289:H2220-H2227

Whitelaw A. (2000) Systematic review of therapy after hypoxic-ischaemic brain injury in the perinatal period. *Seminars in Neonatology* 5:33-40

Whitelaw A, Thoresen M. (2002) Clinical trials of treatments after perinatal asphyxia. *Current Opinion in Pediatrics.* 14:664-668

Whyte HE. (1993) Visual-evoked potentials in neonates following asphyxia. *Clinics in Perinatology* 20:451-461

Wiadrowski TP MG. (2001) Subcutaneous fat necrosis of the newborn following hypothermia and complicated by pain and hypercalcaemia. *Australas. J. Dermatol.* 42:207-210

Williams CE, Gunn A, Gluckman PD. (1991) Time course of intracellular edema and epileptiform activity following prenatal cerebral ischemia in sheep. *Stroke* 22:516-521

Williams CE, Gunn AJ, Mallard C, Gluckman PD. (1992) Outcome after ischemia in the developing sheep brain: an electroencephalographic and histological study. *Annals of Neurology* 31:14-21

Williams CE, Gunn AJ, Synek B, Gluckman PD. (1990) Delayed seizures occurring with hypoxic-ischemic encephalopathy in the fetal sheep. *Pediatric Research* 27:561-565

Williams GW, Luders HO, Brickner A, Goormastic M, Klass DW. (1985) Interobserver variability in EEG interpretation. *Neurology* 35:1714-1719

Williams LJ, Lucci AP. (1990) Placental examination can help determine cause of brain damage in neonates. *Texas Medicine.* 86:33-38

Willis F, Summers J, Minutillo C, Hewitt I. (1997) Indices of renal tubular function in perinatal asphyxia. *Archives of Disease in Childhood Fetal & Neonatal Edition.* 77:F57-60

Winn HR RG, Berne RM. (1981) The role of adenosine in the regulation of cerebral blood flow. *J Cereb Blood Flow Metab.* 1:239-244

Wirrell EC, Armstrong EA, Osman LD, Yager JY. (2001) Prolonged seizures exacerbate perinatal hypoxic-ischemic brain damage. *Pediatric Research* 50:445-454

Wiswell TE. (2001a) Advances in the treatment of the meconium aspiration syndrome. *Acta Paediatrica. Supplement.* 90:28-30

Wiswell TE. (2001b) Handling the meconium-stained infant. *Seminars in Neonatology.* 6:225-231

Wiswell TE, Gannon CM, Jacob J, Goldsmith L, Szyld E, Weiss K, Schutzman D, Cleary GM, Filipov P, Kurlat I, Caballero CL, Abassi S, Sprague D, Oltorf C, Padula M. (2000) Delivery room management of the apparently vigorous meconium-stained neonate: results of the multicenter, international collaborative trial. *Pediatrics.* 105:1-7

Wong VK, LeMesurier J, Franceschini R, Heikali M, Hanson R. (1987) Cerebral venous thrombosis as a cause of neonatal seizures. *Pediatric Neurology* 3:235-237

Wood SC. (1991) Interactions between hypoxia and hypothermia. *Annual Review of Physiology* 53:71-85

Wood SC, Gonzales R. (1996) Hypothermia in hypoxic animals: mechanisms, mediators, and functional significance. *Comparative Biochemistry & Physiology. Part B* Biochemistry & Molecular Biology. 113:37-43

Wu YW, Backstrand KH, Zhao S, Fullerton HJ, Johnston SC. (2004) Declining diagnosis of birth asphyxia in California: 1991-2000. *Pediatrics* 114:1584-1590

Yager JY. (2002) Hypoglycemic injury to the immature brain. *Clinics in Perinatology* 29:651-674

Yager JY, Armstrong EA, Jaharus C, Saucier DM, Wirrell EC. (2004) Preventing hyperthermia decreases brain damage following neonatal hypoxic-ischemic seizures. *Brain Research* 1:48-57

Yager JY, Armstrong EA, Miyashita H, Wirrell EC. (2002) Prolonged neonatal seizures exacerbate hypoxic-ischemic brain damage: correlation with cerebral energy metabolism and excitatory amino acid release. *Developmental Neuroscience.* 24:367-381

Zeinstra E, Fock JM, Begeer JH, van Weerden TW, Maurits NM, Zweens MJ. (2001) The prognostic value of serial EEG recordings following acute neonatal asphyxia in full-term infants. *European Journal of Paediatric Neurology* 5:155-160

Zhu N WH. (1994) Effect of hypoxic and carbon monoxide-induced hypoxia on regional myocardial segment work and oxygen consumption. *Res. Exp. Med.* 194:97-107

Ziino AJ, Davies MW, Davis PG. (2003) Epinephrine for the resuscitation of apparently stillborn or extremely bradycardic newborn infants. *Cochrane Database of Systematic Reviews.*:CD003849

Zurcher SD, Ong-Veloso GL, Akopov SE, Pearce WJ. (1998) Maturational modification of hypoxic relaxation in ovine carotid and cerebral arteries: role of endothelium. . *Biology of Neonate* 74:222-232

Zydlewski AW, Hasbargen JA. (1998) Hypothermia-induced hypokalemia. *Military Medicine* 163:719-721