

# Hypothermia: A Neuroprotective Therapy for Neonatal Hypoxic Ischemic Encephalopathy

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The incidence of hypoxic ischemic encephalopathy in the United States is 2.8/1000 live births of which 10–15% of the infants succumb and 25–30% suffer permanent neurologic damage: mental retardation, cerebral palsy and epilepsy. The incidence is tenfold higher in the developing world [1–3]. Experimental evidence has confirmed that HIE is an evolving process lasting hours to weeks. Understanding the mechanisms that culminate in neuronal death has enabled the development of therapeutic strategies that limit the extent of the injury. Mild hypothermia is the most rigorously tested of these strategies and has been the subject of several recently published international multicenter randomized controlled trials. The results provide extensive information regarding the effectiveness and safety of this treatment in the management of the infants most at risk of significant brain injury. In this review, we present mechanisms of neuronal injury, the effect of hypothermia on these processes, and the results of the clinical trials. In addition, we present our experience with mild hypothermia in the treatment of moderate-severe HIE at Soroka Medical Center.

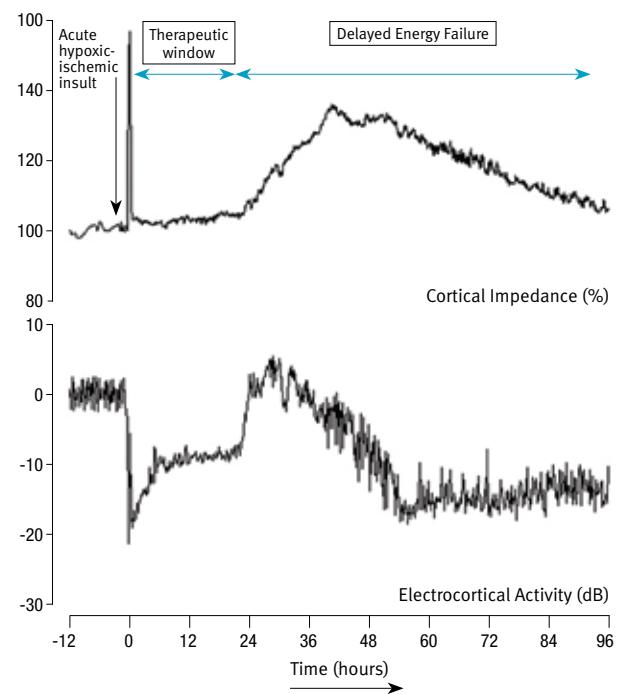
## SECONDARY OR DELAYED ENERGY FAILURE

Experimental evidence on the mechanism of delayed neuronal injury has determined that hypoxia-ischemia triggers a cascade of biochemical events during and after the insult that leads to cell death many hours, and even days, later. In newborn infants with moderate to severe HIE, normal cerebral oxidative metabolism has been observed by magnetic resonance spectroscopy shortly after birth [4]. However, a fall in concentration of high energy phosphates represents a delayed energy failure 8–12 hours later. The extent of this delayed energy failure is closely related to neurodevelopment-

tal outcome at 18 months and 4 years [5,6]. Delayed energy failure has also been demonstrated in fetal sheep, rat and piglet models of HIE, and the extent has been shown to be related to the severity of neuronal loss on neuropathologic examination [7] [Figure 1]. Similarly, persisting abnormalities in mitochondrial function, demonstrated as a persistent elevation in cerebral lactate and intracellular pH, are also associated with abnormal neurodevelopmental outcome and reduced head growth [8].

**Figure 1.** Fetal sheep model of severe transient cerebral ischemia demonstrating delayed energy failure.

Continuous measurement of electrocortical activity and cortical impedance demonstrates the presence of a delayed increase in cortical impedance and intense electrocortical activity. These changes reflect a delayed fall in cerebral energetics together with intense seizure activity which leads to cytotoxic edema as a consequence of ionic pump failure. Neuroprotective manipulations instituted during the therapeutic window are most effective.



HIE = hypoxic ischemic encephalopathy

The important events shown to have a role in these secondary events include accumulation of excitatory neurotransmitters, generation of reactive free radicals, intracellular calcium accumulation, and mitochondrial dysfunction. In addition, increased apoptosis plays a critical role in the culmination of neuronal death in the developing brain [9,10]. Apoptosis, an active biochemical process requiring energy, might be more amenable to neuronal rescue therapies [11-13].

### **EXPERIMENTAL EVIDENCE OF NEUROPROTECTION WITH HYPOTHERMIA**

Experimental evidence has confirmed that neuroprotective strategies, implemented after resuscitation but prior to the onset of the delayed cerebral energy failure, are most effective in reducing brain injury [14]. The concept of a *therapeutic window* has allowed for the investigation of novel pharmaceutical products with neuroprotective properties. These include calcium channel blockers, free radical scavengers, glutamate receptor blockers, anti-inflammatory and anti-apoptotic agents, and growth factors to promote repair [15]. These agents have not yet been the subject of randomized controlled trials. Mild hypothermia critically affects biological processes and has been widely investigated both in experimental models and newborn infants. In experimental models, mild hypothermia to 32.5°C initiated immediately after the hypoxic ischemic injury, and maintained for 3 hours, improves histologic outcome [16]. Mild hypothermia is even more effective in limiting delayed cerebral injury and neuronal loss when provided for 12 hours following the hypoxic ischemic event [17,18]. Furthermore, delayed prolonged hypothermia, initiated 2–6 hours post-resuscitation and maintained for 28–72 hours, provides neuroprotection in numerous animal models [19-23].

### **POTENTIAL MECHANISM OF NEUROPROTECTION BY HYPOTHERMIA**

Hypothermia reduces brain injury by its impact on several biological processes [24]. It reduces vasogenic edema, hemorrhage and neutrophil infiltration. It limits the release of excitatory neurotransmitters and the accumulation of intracellular calcium. The production of free radicals is restricted by hypothermia, and thus cells and organelles are protected from oxidative damage during reperfusion. Hypothermia reduces the activation of cytokine and coagulation cascades by increasing the concentration of interleukin-10, an anti-inflammatory cytokine, and reducing tumor necrosis factor-alpha. Mild hypothermia helps to maintain cerebral metabolism during and after cerebral insults by decreasing the metabolic rate of glucose and oxygen. Most importantly,

by reducing caspase-3 activity and increasing the expression of the anti-apoptotic protein BCL-2, hypothermia limits neuronal apoptotic death [25].

### **CLINICAL STUDY OF HYPOTHERMIA IN NEONATES**

#### **SAFETY AND FEASIBILITY STUDIES**

The first pilot studies aimed to: a) determine the safety of mild hypothermia in the asphyxiated infant, b) determine criteria to select the asphyxiated infants most at risk of neurologic sequelae, and c) determine the feasibility of initiating selective head cooling or total body hypothermia within the 6 hour time limit. The safety issues addressed were based on the complications observed in greater degrees of hypothermia, and included cardiac arrhythmias, hypotension, hyperviscosity syndromes, thrombocytopenia, coagulopathies, sepsis/pneumonia, hypoglycemia, persistent pulmonary hypertension, and persistent metabolic acidosis. The studies assessed the role of clinical assessment and electroencephalography in selecting infants most likely to benefit from treatment. Two methods of cooling were used: a)

selective head cooling, with a cooling cap on the infants' head combined with mild systemic hypothermia, aimed to achieve adequate cerebral cooling with minimal systemic cooling; and

b) whole-body surface cooling that relies on the assumption that brain temperature is close to core body temperature.

A pilot study of whole-body hypothermia recruited 16 infants with severe birth asphyxia and recorded amplitude integrated EEG during the first 6 hours [26]. The 10 infants with the worst prognostic findings on aEEG received whole-body hypothermia to a rectal temperature of  $33.2 \pm 0.6^\circ\text{C}$  for 48 hours. All cooled infants developed HIE grades 2-3, while the infants with normal aEEG were neurologically intact without seizures. Cooling was associated with a reduction in heart rate and elevation of blood pressure, which reversed on rewarming and was not associated with complications. Blood viscosity and coagulation studies were not affected by the cooling.

The safety of selective head cooling, begun within 6 hours and continued for 72 hours, with rectal temperature maintained at 34.5–35°C, was assessed in 13 asphyxiated term infants [27]. The infants selected had Apgar scores of  $\leq 6$  at 5 minutes and clinical evidence of encephalopathy. Selective head cooling was achieved by circulating water at 10°C through a cap placed on the infant's head. Cooling was associated with bradycardia and prolonged Q-T interval but no increased incidence of cardiac arrhythmias. The incidence of thrombocytopenia, hypoglycemia and positive blood cultures was similar in the cooled and normothermic groups. Further preliminary studies confirmed that cooling to 33–35°C for 48–72 hours following resuscitation

aEEG = amplitude integrated EEG

EEG = electroencephalography

in asphyxiated term infants was not associated with increased blood viscosity, arrhythmias, pulmonary hypertension, or sepsis [28,29]. Preliminary studies also described the distribution of cerebral abnormalities on magnetic resonance imaging. Cooling appeared to reduce lesions in cortical, thalamic and basal ganglia with no increase in the incidence of cerebral thrombosis or hemorrhage [30].

#### PHASE 2 TRIALS

These trials aimed to determine the efficacy of mild hypothermia on reducing mortality and improving long-term neurodevelopmental outcome. Eicher and co-workers [31,32] conducted a small randomized trial in 65 infants of ≥ 35 weeks gestation with abnormal neurologic signs together with two of the following: fetal bradycardia < 80 beats/min for 15 minutes, pH < 7.1, base deficit > 13, Apgar < 6 at 10 minutes, and postnatal desaturation < 70% for 20 minutes. Within 6 hours of birth, the infants were randomly assigned to either normothermia (37°C) or whole-body cooling to a rectal temperature of 33°C for 48 hours. Neurodevelopmental outcome was assessed at 12 months. The neonatal mortality was 10 (31%) in the hypothermia group (n=32) and 14 (42%) in the normothermic group (n=33). Among the normothermic infants, 84% died or had a severe motor disability, while in the hypothermia group 52% died or had a severe motor disability ( $P = 0.019$ ) [31]. A single-center trial from China studied the neuroprotective efficacy of mild hypothermia via selective head cooling (n=30) compared to normothermia (n=28) in full-term

infants with perinatal asphyxia and encephalopathy [40]. Computed tomographic brain scans at postnatal age 5–7 days and neurologic assessments at 7–10 days were significantly improved in the hypothermic group.

#### PHASE 3 TRIALS

Randomized controlled trials were designed to determine the effectiveness of mild hypothermia in reducing mortality and improving long-term neurodevelopmental outcome in infants with moderate-severe HIE. Table 1 is a summary of the Phase 2 and 3 trials published to date. The first large multicenter trial, the Cool Cap trial, was published in 2005 [33]. Infants of ≥ 36

weeks of gestation were selected if they had evidence of perinatal asphyxia ( $pH < 7.0$ , base deficit 16, Apgar < 6 at 5 minutes, or need for resuscitation at 10 minutes), abnormal neurologic signs, and abnormal aEEG

#### Mild hypothermia to 33–34°C for 72 hours in newborns with moderate-severe hypoxic ischemic encephalopathy reduces mortality and improves neurodevelopmental outcome at 2 years

(moderately or severely depressed amplitude or seizures). Randomization was completed by 5.5 hours and infants were allocated to either normothermia (rectal temperature 37°C) or selective head cooling for 72 hours via a cap through which water was circulated at a controlled temperature with mild systemic hypothermia (rectal temperature 34.5°C). A total of 234 infants were recruited and follow-up data at 18 months were available for 218 (93%). Of the normothermic infants, 66% died or were disabled versus 55% of the hypothermic infants ( $P = 0.1$ ). However, the investigators hypothesized a priori that hypothermia would not be effective in the infants with the most severe aEEG changes (severe depression and seizures before randomization). When these infants (21%) were

**Table 1.** Summary of completed randomized controlled trials of cooling for newborns with hypoxic ischemic encephalopathy

Completed studies	No. in trial	Entry criteria	Primary outcome	Cooling method and duration	Rectal temperature	Results favoring therapeutic hypothermia vs. normothermia
Eicher 2005 Multicenter [32]	65	• > 35 weeks, > 2 kg • Perinatal asphyxia • Neonatal encephalopathy	Death or severe impairment 12 mos	Systemic, 48 hrs	32.5–33.5°C	$P = 0.019$
Gluckman 2005 Multicenter [33]	234	• ≥ 36 wks • Perinatal asphyxia • Moderate/severe encephalopathy • Abnormal aEEG	Death or severe impairment 18 mos Outcome in subgroup with less severe aEEG	Selective, 72 hrs	34–35°C	RR 0.61, 95%CI 0.34–1.09, $P = 0.1$ RR 0.42, 95%CI 0.42, $P = 0.009$
Shankaran 2005 Multicenter [34]	208	• ≥ 36 wks • Perinatal asphyxia • Encephalopathy/seizures	Death or moderate/severe impairment 18–22 mos	Systemic, 72 hrs	33–34°C	RR 0.72, 95%CI 0.54–0.95, $P = 0.01$
Lin 2006 Single center [40]	58	• Full-term • Perinatal asphyxia • Clinical encephalopathy	CT brain scan 5–7 days Neonatal behavioral neurologic assessment score	Selective, 72 hrs	34–35°C	$P < 0.01$ $P < 0.01$
Azzopardi 2009 Multicenter [37]	325	• ≥ 36 wks • Perinatal asphyxia • Encephalopathy • Abnormal aEEG	Death or severe impairment 18 mos Survival in cooled group without neurologic abnormality	Systemic, 72 hrs	33–34°C	RR 0.86, 95%CI 0.68–1.07, $P = 0.17$ RR 1.57, 95%CI 1.16–2.12, $P = 0.003$

aEEG = amplitude integrated electroencephalogram, CT = computed tomography, RR = relative risk, CI = confidence interval

excluded only 48% died or were disabled in the hypothermic group versus 66% in the normothermic group ( $P = 0.02$ ), and severe neuromotor disability was reduced from 28% to 12% ( $P = 0.03$ ). No increase in serious adverse events was observed in the hypothermic group. Sinus bradycardia, transient elevation in plasma glucose and temporary scalp edema were not clinically significant. Platelet counts, prothrombin times and hypotension were similar in the two treatment groups.

The second large multicenter trial from the National Institute for Child Health and Human Development (NICHD), published in 2005, enrolled 208 infants from 16 Neonatal Research Network centers and tested the effect of whole-body cooling in moderate to severe HIE [34]. Eligibility criteria included gestational age  $\geq 36$  weeks, severe acidosis or perinatal complications, resuscitation at birth, moderate or severe HIE according to clinical signs based on Sarnat and Sarnat criteria, and age less than 6 hours [35]. Infants were randomly assigned to normothermia or whole-body cooling to an esophageal temperature of 33.5°C for 72 hours, followed by slow rewarming (hypothermia group, n=102). At 18 months, the primary outcome status was known for 205/208 infants (98%); death or moderate/severe disability occurred in 44% (45/102) of the hypothermia and 62% (64/103) of the control group (risk ratio 0.72, 95% confidence interval 0.54–0.95;  $P = 0.01$ ) indicating that 6 infants needed to be treated to result in a better outcome in 1 infant. Twenty-four infants (24%) in the hypothermia group and 38 (37%) in the control group died (RR 0.68, 95% CI 0.44–1.05;  $P = 0.08$ ). There was no increase in major disability among survivors; the rate of cerebral palsy was 15/77 (19%) in the hypothermia group as compared with 19/64 (30%) in the control group (RR 0.68, 95% CI 0.38–1.22;  $P = 0.2$ ). The frequency of adverse events was similar: 19% in the hypothermia group and 15% in the control group.

The last randomized control trial, the TOBY trial, in which Soroka Medical Center participated, was published in 2009 [36]. Of 325 infants with perinatal asphyxial encephalopathy, 163 were randomized at less than 6 hours of age to intensive care with total body cooling to 33.5°C for 72 hours, and 162 received intensive care alone. The primary outcome of death or severe disability at 18 months was similar between the groups (RR for either outcome: 0.86, 95% CI 0.68–1.07;  $P = 0.17$ ). However, the cooled group had an increased survival without neurologic abnormality (RR 1.57, 95% CI 1.16–2.12;  $P = 0.003$ ).

The preliminary results of two additional randomized controlled trials – the Infant Cooling Evaluation trial [38] and a trial by Simbruner and the neo.nEuro. network [39] – were presented

at the "Hot Topics in Neonatology" conference in Washington, December 2008. The trials shared similarities to the published reports in entry criteria, methodology, and outcomes.

The Australian ICE trial [38] enrolled subjects from a wide geographic region using simplified protocols. Hypothermia was achieved by turning off ambient heating systems and applying "Hot-Cold" gel packs (at 10°C) around the infant's head and chest to achieve a rectal temperature of 33–34°C for 72 hours. Although the results of the primary outcome of survival free of major disability at 2 years (n=221) are not yet available, again the safety of hypothermia as used was confirmed. The results of the European neo.nEURO.network Trial [39] showed a marked improvement in neurodevelopmental outcome in the cooled group at 2 years. The trial used the same degree of systemic hypothermia (33–34°C by a cooling blanket for 72 hours), but employed routine analgesia and sedation with morphine in both groups.

Published studies of acceptable quality have been the subject of meta-analyses determining both the effectiveness of hypothermia as measured by survival without moderate to severe neurodevelopmental disability in infancy and childhood, and the safety of hypothermia vs. normothermia [35,37]. The data analyzed represent only half of the infants who were recruited to trials and await analysis. The reviews included eight studies comprising 638 term infants with moderate/severe encephalopathy and evidence of intrapartum asphyxia. Therapeutic hypothermia resulted in a statistically significant and clinically important reduction in the combined outcome of mortality or major disability to 18 months of age (RR 0.76, 95% CI 0.65–0.89). Cooling also resulted in statistically significant reductions in mortality (RR 0.74, CI 0.58–0.84) and in neurodevelopmental disability in the survivors (RR 0.68, 95% CI 0.51–0.92). Some clinically insignificant adverse effects were demonstrated in the cooled infants: an increase in hypotension treated with inotropes (RR 1.17, 95% CI 1.00–1.38) and an increase in thrombocytopenia (platelets  $< 150 \times 10^9/L$ , RR 0.83, 95% CI 0.03–0.15). The results of the meta-analysis confirm the benefits of therapeutic hypothermia for term infants with moderate to severe HIE in reducing the composite outcome of mortality or long-term neurodevelopmental disability at 18 months, with a relative risk reduction of 23%, absolute risk reduction of 15% and number needed to treat of 7. This reduction in death or major disability remains significant in the subgroup analysis for severe encephalopathy (NNT 6), although the numbers are small. The analysis of all infants recruited in completed trials will be important to further clarify the effec-

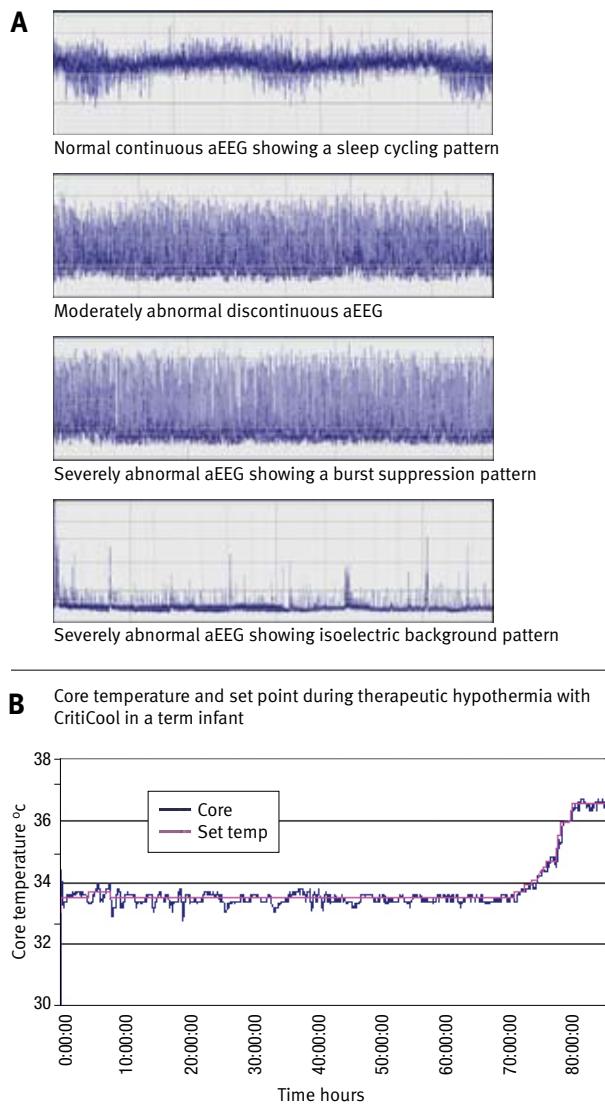
**Infants with moderate to severe hypoxic ischemic encephalopathy should be treated with mild hypothermia according to protocols of published randomized controlled trials**

tiveness of cooling and to provide more information on the safety of therapeutic hypothermia.

### THERAPEUTIC HYPOTHERMIA AT SOROKA MEDICAL CENTER, BEER SHEVA

Of the 13,000 infants delivered annually at Soroka Medical Center, 10–12 have moderate to severe HIE. Since 2008, all term infants with perinatal asphyxia admitted to the neonatal intensive care unit are managed with therapeutic whole-body hypothermia according to the TOBY study protocol. Having collaborated in the TOBY trial during the period 2005–2007, the staff of the NICU is experienced in managing infants treated with mild systemic hypothermia. Furthermore, our experience with interpreting aEEG recordings in term infants has allowed us to identify, shortly after birth, the infants at risk of moderate-severe HIE. Asphyxiated infants are eligible for therapeutic hypothermia if they fulfill the following three criteria: a) evidence of perinatal asphyxia (Apgar  $\leq$  5 at 10 minutes or need for 10 minute resuscitation at birth, pH  $<$  7.00 or base excess  $>$  16 mmol/L within 1 hour of birth); b) altered state of consciousness (lethargy, stupor, coma), with abnormal neurologic examination (hypotonia, abnormal primitive reflexes, absent or weak suck or clinical seizures); and c) at least 30 minutes of abnormal aEEG recordings (seizures, moderately abnormal or suppressed activity, continuous seizure activity). Infants with criterion (a) are passively cooled (no overhead heater) to 35°C. Whole-body cooling to 33–34°C using a servo-controlled cooling garment (CertiCool®, Mennen Medical, Israel) is begun within 6 hours of birth as soon as entry criteria (b) and (c) are fulfilled. Hypothermia is continued for 72 hours with continuous aEEG recording. Gradually rewarming (no faster than 0.4°C per hour) is begun after 72 hours treatment. Over a period of 1 year, eight infants fulfilled the entry criteria. The Apgar score (mean  $\pm$  SD) at 10 minutes was  $4.8 \pm 1.6$ , and cord pH  $6.86 \pm 0.23$ . All infants had severely abnormal aEEG recordings within 6 hours of birth and all progressed to HIE grades 2–3 within the first 48 hours [Figure 2A]. Two infants succumbed during the first 48 hours to severe HIE following a decision to re-warm and withhold further intensive care. In all infants, signs of multi-organ involvement developed: troponin T  $>$  0.1 ng/ml, raised liver enzymes, creatinine  $>$  1.0 mg/dl, hematuria), although none of the infants developed thrombocytopenia. Cardiac arrhythmias other than sinus bradycardia, pulmonary hypertension, or systemic hypotension requiring inotropic support did not occur in any infant. Whole-body hypothermia to 33–34°C was maintained throughout the study period with minimal fluctuations [Figure 2B]. On discharge, one infant required

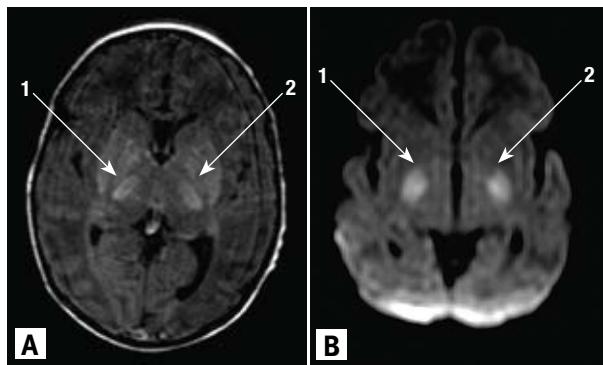
**Figure 2. [A]** Patterns of abnormal continuous aEEG tracing following birth asphyxia. **[B]** The set point temperature and core temperature measured rectally throughout the cooling period using the CertiCool® system. 0:00:00 represents the starting time of cooling at age 4 hours; gradual rewarming was begun after 72 hours treatment.



gastrostomy while five were discharged with fully established feeding. MRI, performed in four surviving infants, was normal in one and abnormal in three, demonstrating a varying spectrum of damage to the cortical parasagittal region, the thalamus and the posterior limb of the internal capsule. In the infant who required a gastrostomy, diffusion weighted imaging also revealed damage to the whole lentiform nucleus [Figure 3]. All infants had a neurodevelopmental examination at discharge by a trained child physiotherapist and are being closely followed in the child development center.

NICU = neonatal intensive care unit

**Figure 3.** [A] Axial T1 weighted MRI, showing increased striate and lateral thalamic signal (1). The posterior limb of the internal capsule (2) is less bright than the adjacent posterior putamina and thalamai. [B] Axial diffusion weighted MRI, showing reduced diffusivity in deep gray matter structure (arrows)



### HYPOTHERMIA AS STANDARD CARE

Hypothermic "rescue" of neonates with HIE is a therapy in evolution. In many of the experienced centers involved in the multicenter trials, hypothermia was a novel treatment and has become "standard care." The NICU departments that participated in the Network trial in the States [39] and the TOBY trial in the United Kingdom [37] are now cooling babies who meet eligibility criteria of the original trials, with or without informed consent and without randomization. The UK TOBY cooling register during its first 17 months registered 174 infants from 29 different neonatal units. Several centers that participated in the "Cool Cap" trial have offered brain cooling for over 2 years, under a Food and Drug Administration-authorized continued access protocol, and 150 babies have been enrolled.

### FUTURE DIRECTIONS

The benefit of hypothermia in reducing death and major disability in the survivors has been confirmed, but a number of important questions remain. The optimal depth of cooling, found to be 32–34°C in animal studies, has yet to be determined in asphyxiated infants. Similarly, optimal duration remains unclear. The optimal mode of delivery – selective head vs. whole-body cooling – has not been studied. Furthermore, the impact of time of initiation of hypothermia is unclear: will earlier initiation of hypothermia further improve long-term outcome; and is therapeutic hypothermia initiated after 6 hours also beneficial under certain conditions? Further analysis of aEEG recordings following resuscitation may allow for more selective inclusion of infants most likely to benefit. Though many questions still remain unanswered,

there is no longer equipoise to randomize to normothermia in future studies that will address these issues.

The question remains as to how the neonatal community should proceed with the issue of offering hypothermia as a treatment option while preserving the critical need for further research? The dilemma is further magnified since neonatal encephalopathy secondary to intrapartum hypoxia-ischemia is a relatively infrequent event in industrialized countries and most small neonatal units will treat only four to six cases annually. The numbers are clearly insufficient to achieve competency with the technique and/or enhance treatment outcomes even if data are submitted to a national register. Suggested solutions are: a) the establishment of regional cooling centers with a dedicated team of neonatologists, neurologists and neonatal nurses; and b) the establishment of national registries that collect data on infants with moderate-severe HIE that include perinatal complications, neonatal morbidity and mortality, and long-term developmental outcome. These pooled data will fulfill the phase IV data required to identify rarer adverse effects and allow hypothermia to be the standard of care for all infants with moderate to severe HIE while preserving the critical need for additional research.

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