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Hypothermia for hypoxic–ischemic encephalopathy

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Abstract

Moderate to severe hypoxic–ischemic injury in newborn infants, manifested as encephalopathy immediately or within hours after birth, is associated with a high risk of either death or a lifetime with disability. In recent multicenter clinical trials, hypothermia initiated within the first 6 postnatal hours has emerged as a therapy that reduces the risk of death or impairment among infants with hypoxic–ischemic encephalopathy. Prior to hypothermia, no therapies directly targeting neonatal encephalopathy secondary to hypoxic–ischemic injury had convincing evidence of efficacy. Hypothermia therapy is now becoming increasingly available at tertiary centers. Despite the deserved enthusiasm for hypothermia, obstetric and neonatology caregivers, as well as society at large, must be reminded that in the clinical trials more than 40% of cooled infants died or survived with impairment. Although hypothermia is an evidence-based therapy, additional discoveries are needed to further improve outcome after HIE. In this article, we briefly present the epidemiology of neonatal encephalopathy due to hypoxic–ischemic injury, describe the rationale for the use of hypothermia therapy for hypoxic–ischemic encephalopathy, and present results of the clinical trials that have demonstrated the efficacy of hypothermia. We also present findings noted during and after these trials that will guide care and direct research for this devastating problem.

Keywords

HIE; hyperthermia; hypothermia; hypoxic–ischemic encephalopathy; neonate; perinatal asphyxia

What is hypoxic–ischemic encephalopathy & what is its impact on health outcomes?

Neonatal encephalopathy, or a persistently abnormal neurologic examination that includes one or more of the following: altered consciousness, abnormal muscle tone or reflexes, altered respirations, and sometimes seizures in the first postnatal hours to days – can be caused by a number of etiologies, including acute perinatal asphyxia, intracranial bleeding and stroke, injuries secondary to birth trauma, infections, metabolic disorders including hypoglycemia, and congenital brain abnormalities. Encephalopathy due to hypoxic–ischemic injury – hypoxic–ischemic encephalopathy (HIE) – is defined as brain injury caused by the combination of inadequate blood flow and oxygen delivery to the brain. Attributing neonatal encephalopathy

to perinatal hypoxic–ischemic injury requires combinations of parameters indicative of metabolic acidosis in the first postnatal hours with low cord pH (<7.0), base deficit of over 12, and evidence of a need for respiratory support also starting in the first minutes, with low Apgar scores at and beyond 5 min. The American College of Obstetrics and Gynecology maintains that only specific types of long-term neurologic injury, including specific types of cerebral palsy, can be connected to perinatal hypoxic–ischemic injury [1].

In a recent review, Graham *et al.* found that HIE occurs in an estimated 2.5 (1.2–7.7) of every 1000 term births in high-income countries [2]. In less affluent countries, the incidence is reported to be higher; in one example, the incidence was 26 per 1000 [3]. Worldwide, approximately a quarter of all neonatal (first postnatal month) deaths are attributed to perinatal asphyxia, a leading cause of HIE [4,5]. Cerebral palsy is a significant morbidity associated with HIE, but the percentage of cerebral palsy cases associated with prior HIE is estimated to be 15% [2]. Children born at term who develop cerebral palsy following newborn encephalopathy have a poorer prognosis (twice as likely to have a severe composite disability score and three-times more likely to die in the first 5 postnatal years) than those with cerebral palsy who were not encephalopathic in the first week of life [6].

Clinicians caring for neonates with HIE generally describe their clinical appearance in the first postnatal days in terms of Sarnat scores, or slightly modified variants of these scores. The scores are based on serial neurobehavioral evaluations (TABLE 1) [7]. Neonates with mild encephalopathy (Sarnat I) do not have an increased risk of motor or cognitive deficits. Neonates with persistent severe encephalopathy (Sarnat III) have a risk of death, and an increased risk of cerebral palsy and mental retardation among survivors that approaches 100%. Neonates with moderate encephalopathy (Sarnat II) are less likely to have severe impairments, but may still have significant deficits, memory impairment, visual motor or visual perceptive dysfunction, increased hyperactivity, and delayed school readiness [8,9]. Adding serial MRI neuroimaging, inclusive of diffusion-weighted imaging, which measures the diffusion of water in tissues (less diffusion is proportional to more injury) to demonstrate evolving pathology in the first postnatal months can add to the predictive value of the early neonatal period exams [10]. Neuromonitoring with conventional EEG and amplitude-integrated EEG (aEEG; discussed later) can also provide additional information regarding current status and can be helpful in predicting long-term outcome [11–13]. Combinations of all three, serial physical exam assessment, MRI, and EEG and aEEG, are likely to be most useful for prognosis, but the availability of EEG and MRI with diffusion-weighted imaging capability for neonates may be limited in many clinical settings.

What is the pathophysiology of HIE?

The pathophysiology of brain injury secondary to hypoxic–ischemic injury can be simplified into two phases of pathologic events that culminate, after weeks, in brain injury, and after months to years, in measureable neurodevelopmental dysfunction. These phases are primary and secondary energy failure, with a latency period of varying length inbetween (FIGURE 1). This pathophysiologic model for hypoxic–ischemic brain injury is based on characteristics of the temporal sequence of cerebral energy state in timed injury newborn animal models [14]. Primary energy failure is characterized by reductions in cerebral blood flow and, consequently, delivery of oxygen and substrates to brain tissue [14,15]. High-energy phosphorylated compounds are reduced, and brain tissue acidosis is prominent. This phase is a prerequisite for all subsequent deleterious events. Primary energy failure is associated with acute intracellular derangements, including loss of membrane ionic homeostasis, release and blocked reuptake of excitatory neurotransmitters, defective osmoregulation and inhibition of protein synthesis [16]. Excessive stimulation of excitatory neurotransmitter receptors and loss of ionic homeostasis mediate an increase in intracellular calcium and osmotic dysregulation. Elevation

in intracellular calcium triggers a number of destructive pathways by activating lipases, proteases and endonucleases, ultimately resulting in acute cell death and necrosis [17].

Resolution of hypoxia–ischemia within a specific time interval reverses the primary energy failure fall in high-energy phosphorylated metabolites and intracellular pH, and promotes recycling of neurotransmitters. The duration of time for hypoxia–ischemia to be successfully reversed and promote recovery is likely influenced by maturation, preconditioning events, substrate availability, body temperature and simultaneous disease processes, and possibly individual genetic variations. Following primary energy failure, cerebral metabolism may recover following reperfusion–reoxygenation, only to deteriorate in a secondary energy failure. This secondary energy failure differs from primary energy failure in that declines in phosphocreatine and ATP are not accompanied by brain acidosis [14]. The presence and severity of secondary energy failure depends on the extent of primary energy failure. The pathogenesis of secondary energy failure is not as well understood as primary energy failure, but likely involves multiple pathophysiologic processes, including the accumulation of excitatory neurotransmitters such as glutamate, oxidative injury, anerobic metabolism and free radical production from the synthesis of xanthenes and uric acid, inflammation and, ultimately, an untimely initiation of apoptosis in many cells. In addition to classic injury concepts, in the newborn, secondary energy failure also leads to altered growth factor and protein synthesis, which ultimately adds the ongoing insult of altered brain development to the established hypoxic–ischemic injury [11,18–22]. More extensive secondary energy failure and disruption of oxidative metabolism worsens the outcome [23].

Much of the later injury after secondary energy failure occurs secondary to the process of apoptosis, or programmed cell death. In contrast to the cell membrane disruptions that lead to necrosis in primary energy failure, apoptosis is a more nuclear phenomenon that ultimately results in DNA fragmentation and condensation [24,25]. Apoptosis occurs as an important component of normal brain development. Cell connections and pathways are refined via apoptosis [26], but when the process is accelerated and apoptosis occurs in regions of the brain where it is unintended, as it does in the context of hypoxic–ischemic injury, there are significant problems. The cellular signals after hypoxic–ischemic injury accelerate apoptosis in normal brain tissue, contributing significantly to the later evolving injury noted in infants with HIE [27–29]. Although acute cellular necrosis and apoptosis both are noted post-hypoxic–ischemic injury in animal models and human infants, apoptotic cell death appears to be a more significant contributor in the developing brain of the term infant, and is the biggest problem in secondary energy failure [30,31].

The interval between primary and secondary energy failure represents a latent phase that corresponds to an optimal available therapeutic window, as we clinicians do not always know the precise time of onset and resolution of primary energy failure in newborns with HIE. Some diagnoses, such as cord prolapse or uterine rupture may suggest a time of onset of primary energy failure. In the National Institute of Child Health and Human Development (NICHD) Hypothermia trial, cord prolapse, maternal hemorrhage or uterine rupture accounted for slightly less than half of the deliveries resulting in enrolled infants [32]. Initiation of therapies during the latent phase between resolved primary energy failure and the initial phase of secondary energy failure in perinatal animals with timed initial injury has been successful in reducing brain damage, possibly through alteration or avoidance of the secondary energy failure. These results substantiate the presence of a therapeutic window in human newborns and offered hope for postnatal intervention to improve outcome for neonatal HIE [22,26,27]. Hypothermia is one intervention that has been tested during the latency period after timed initial injury and primary energy failure [30].

Why might hypothermia work?

In most cases, a neonatologist cannot address primary energy failure. Even in deliveries in which an acute event such as cord prolapse or acute abruption is noted, minutes pass before delivery occurs and neonatal resuscitation can begin, and sometimes there is uncertainty as to whether or not there was subacute compromise prior to the noted acute event, and whether or not the initial resuscitation was adequate. Owing to the uncertainty around identification of the exact timing of primary energy failure, identifying a postnatal therapy to ameliorate secondary energy failure and its subsequent significant detrimental impact on brain development is a logical target. In an anecdotal report, hypothermia had been used after cardiac arrest in four adult patients, and three had unexpected normal neurologic function afterwards [33].

Previously, hypothermia had been used to decrease 'brain swelling' after injury or the removal of brain tumors [34]. In the first report of use of hypothermia for term infants with apparent HIE, Westin reported using immersion in cold water to achieve core body temperatures of 23–25°C for no more than 20 min in six patients. Five of the infants survived to apparently normal outcomes at 6 and 30 months [35]. This was followed by similar reports by others using similar cooling protocols [36–38]. Despite these promising results, concurrent randomized clinical trials (vs the case series' reported for the cooling strategies for HIE) demonstrating harm from using hypothermia in preterm infants [39], and animal studies that failed to show benefit from hypothermia after hypoxic injury [40,41], led to questioning of the use of hypothermia for HIE, with recommendations to maintain skin-measured euthermia at 36.5°C to reduce heat loss and oxygen consumption in the immediate postnatal period for term infants with HIE [42,43].

Fortunately, investigators continued testing hypothermia in asphyxiated animal models. Interest in hypothermia as a therapeutic measure resumed in 1987, with the publication of work by Busto *et al.* who reported that small differences in brain temperature during ischemia had a critical effect on the extent of histologic brain injury in adult rats [44]. This work led investigators interested in hypoxic–ischemic injury in newborns to test hypothermia in animal models of hypoxic–ischemic injury to explore mechanisms that could explain hypothermia's anecdotal successes in newborns [14,30,45,46].

In these early 'second era' animal studies, investigators measured how physiologic and anatomic responses to hypoxia and ischemia were modified by moderate hypothermia (no less than 6°C below normal). Thoresen *et al.* used phosphorus magnetic resonance spectroscopy to demonstrate that hypoxic injury to piglets lowered the cerebral phosphocreatine:inorganic phosphate ratio to almost zero, and the nucleotide triphosphate:exchangeable phosphate pool ratio to below 30% of baseline. High-energy phosphate pools in cooled animals recovered and maintained recovery, but the injured animals maintained at normothermia experienced short-lived recovery and then a prolonged depletion [45]. Edwards *et al.* demonstrated that the brains of cooled animals versus those maintained at normothermia had significant reductions in apoptosis, an established key element of secondary energy failure and ultimate cell death [30]. As a potential explanation for the decreased apoptosis, Fukuda *et al.* examined the effects of hypothermia on caspase-3 activation in 7-day-old rat pups. Caspase-3 is a cysteine protease present in various kinds of cells, including neuronal cells [47]. When caspase-3 is activated, it cleaves endogenous substrate proteins, such as inhibitory protein of caspase-activated DNase, finally causing the degradation of chromosomal DNA characteristic of apoptosis [48]. This caspase-3 activation has been detected in hypoxic–ischemic-induced neuronal cell death [49]. In Fukuda's experiment, 24 h of hypothermia initiated 1 h after hypoxic–ischemic (permanent left carotid ligation) injury suppressed caspase-3 activation following injury, leading to excellent brain protection assessed at multiple time points through 168 h [47]. Laptook *et al.* demonstrated that a decrease in brain temperature from normothermic values reduced brain energy utilization by approximately 5% for every 1°C cooled. This lowering of energy utilization could contribute to neuroprotection by enhancing the maintenance of high-energy ATP stores during hypoxia–ischemia [50,51]. Other neuroprotective effects of cerebral

hypothermia during the latency and secondary energy failure periods include normalization (rather than decrease) of protein synthesis, reduction in free radicals and modulation of activation of microglia and cytokine production. Another key mechanistic finding for hypothermia's potential as a therapeutic agent for hypoxic-ischemic brain injury was the reduction in the excitatory neurotransmitter glutamate when hypothermia was used compared with controls. In piglets, a 4°C temperature reduction attenuated the increase in excitatory amino acids and nitric oxide concentrations in brain extracellular fluid following hypoxia-ischemia [46].

With such promising results for a potential treatment initiated in the latency phase that could ameliorate multiple mechanisms of secondary energy failure that caused brain pathology in animals, investigators continued work to identify optimal dose and timing of cooling in anticipation of having to choose a degree and duration of cooling for clinical trials in human infants. The duration and degree of cooling have been shown to interact. In infant rats, a 2–3°C decrease in brain temperature for 72 h from the end of hypoxia prevented brain injury, while 6 h of cooling had an intermediate effect [52]. In the same model, a greater reduction in body temperature, of 5°C for only 6 h, starting immediately after the insult, gave significant neuroprotection and neurobehavioral improvement 1 and 6 weeks later [53]. However, in the current clinical setting, the immediate initiation of active cooling after perinatal hypoxic-ischemic injury is not feasible while assessing the degree of HIE, but strategies to avoid overwarming while assessing the encephalopathic newborn can be implemented without adding significant risk. For this reason, investigators explored how time of initiation of cooling after injury affected outcomes in animals, targeting the latent phase between primary and secondary energy failure. In the most cited series of experiments, Gunn *et al.* induced hypothermia in fetal sheep at various times after injury, and before and after the onset of predictable electrographic seizures in the animals, which occur between 5.5 and 6 h after injury [54]. These studies revealed that moderate hypothermia induced by 90 min after reperfusion, and continued until 72 h after ischemia, prevented brain injury and improved electroencephalographic recovery [55]. When the start of hypothermia was delayed until just before the expected onset of post-injury seizures in this animal model (5.5 h after reperfusion), partial neuroprotection was seen [56]. With further delay until after seizures were established (8.5 h after reperfusion), there was no electrophysiological or overall histological protection with cooling [57]. With the mounting evidence supporting a physiologic mechanism for hypothermia, and identification of optimal time of initiation, degree and duration of cooling in large animal studies, the initial infant trials of hypothermia for HIE were initiated.

Hypothermia clinical trials

The pilot trials of moderate hypothermia for HIE in term infants demonstrated acceptable safety using one of two modalities: selective head cooling [58] or whole-body hypothermia [59]. The pilot studies led to the first two successful multicenter randomized trials of moderate hypothermia for HIE [32,60]. These two, plus two additional completed large multicenter trials (one recently presented and one published in the *New England Journal of Medicine*) are reviewed here [61] [ICE TRIAL, UNPUBLISHED DATA].

The industry-sponsored (Olympic Medical) multicenter randomized study of selective head cooling enrolled 243 infants with moderate or severe encephalopathy and an abnormal aEEG, who were either cared for using the combination of a cap with circulating water and an overhead radiant warmer to maintain a rectal temperature of 34–35°C for 72 h after study entry in the first 6 postnatal hours, or had temperatures managed by servo-control by an overhead radiant warmer with a goal to maintain the rectal temperature at 36.8–37.2°C. The primary outcome of the study was death or severe disability at 18 months. A total of 16 infants (7%) were lost to follow-up, but among those followed, death or severe disability occurred in 66% of infants

randomized to conventional care and 55% randomized to the cooled group (odds ratio: 0.61; 95% CI: 0.34–1.09; $p = 0.10$). In accordance with the hypothesis of the lead investigators in this study, head cooling for infants with the most severe aEEG changes was not protective; however, head cooling to achieve a rectal temperature of 34–35°C for infants with less severe aEEG changes ($n = 172$) was protective, with an odds ratio of 0.42, and a 95% CI of 0.22–0.80 ($p = 0.009$) [60].

The NICHD Neonatal Research Network trial evaluated 102 infants randomized to hypothermia with whole-body cooling using a temperature-control blanket with circulating water to an esophageal temperature of 33.5°C for 72 h compared with 106 control infants randomized to conventional care. Enrolled infants met blood gas as well as neurologic exam criteria in the first 6 postnatal hours (TABLE 2). Skin temperature for the control group was maintained between 36.5 and 37.0°C with servo-controlled overhead warmers. Esophageal temperatures were monitored, but were not used to guide management in the control group. The primary outcome of the Network trial was death, or moderate or severe disability at 18 months of age (in the CoolCap trial, the primary outcome was death or severe disability). A total of 44% of infants in the hypothermia group died or survived with moderate to severe impairment at 18–22 month follow-up, compared with 62% of infants in the control group, with a risk ratio of 0.72 (95% CI: 0.54–0.95), for a number needed to treat to avoid the primary outcome of death or moderate to severe impairment of six. For the hypothermia group versus the control group, respectively, the disabling cerebral palsy occurred in 19.2 and 30.0%, for a risk ratio of 0.68 and a 95% CI of 0.38–1.22. Unlike the CoolCap study, where the benefit appeared to be limited to infants with moderate encephalopathy as defined by aEEG criteria, there was a trend for cooling to benefit infants in both moderate and severe encephalopathy groups noted in subgroup analyses. It is important to note that a prior aEEG was not included in the enrolment criteria for the Network Whole Body Hypothermia study. Eligibility was determined by biochemical and medical history parameters, and a baseline neurological examination. The eligibility and exclusion criteria are included in Box 1 [59]. Two additional multicenter trials were recently completed. In the Total Body Hypothermia for Neonatal Encephalopathy (TOBY) trial, 325 infants were enrolled and randomized if they were at least 36 weeks gestational age, plus, at 10 min after birth, had either an Apgar score of 5 or less or a continued need for resuscitation, or, within 60 min after birth, metabolic acidosis (defined as any occurrence of umbilical cord, arterial or capillary pH of <7 or base deficit of ≥ 16 mmol/l). They also had to demonstrate encephalopathy and have at least 30 min of abnormal background activity or seizures on amplitude integrated electroencephalography. Cooled infants received 72 h of hypothermia to 33.5°C for 72 h with slow rewarming, much like the NICHD trial. The control group was maintained on servo-controlled radiant warmers with skin temperature probes, to maintain the rectal temperature at $37.0 \pm 0.2^\circ\text{C}$. A total of 42 infants died and 32 survived with severe neurodevelopmental disability in the cooled group, and 44 infants died and 42 had severe disability in the noncooled group ($p = 0.17$). The rate of survival without a neurologic abnormality was significantly increased in the cooled group (71 out of 163 infants [44%] vs 45 out of 162 [28%] in the noncooled group; $p = 0.003$). Among survivors, cooling resulted in reduced risks of cerebral palsy ($p = 0.03$). The authors' conclusions were that the induction of moderate hypothermia for 72 h in infants who had perinatal asphyxia did not significantly reduce the combined rate of death or severe disability, but resulted in improved neurologic outcomes in survivors. The authors noted the consistency of effect in improving survival free of disabilities among the three published trials [61].

The in-hospital outcomes and mortality results from a fourth pivotal trial, the Infant Cooling Evaluation (ICE) trial were reported at the Hot Topics in Neonatology annual conference in Washington DC (USA), in December 2008. In the ICE trial, infants were recruited from wide geographic areas, and were cooled on transport using HotCold gel packs cooled to 10°C. The cooled group core rectal temperature goal was 33–34°C for 72 h, and the control group rectal

temperature goal was 36.7–37.3°C. Infants were excluded if they required supplemental oxygen with a fractional inspired oxygen (FiO₂) greater than 0.80. Additional inclusion and exclusion criteria for the ICE trial are listed in Box 1. This study enrolled 221 infants between February 2001 and July 2007. Enrolment ended in July 2007 because investigators at study sites had lost equipoise following publication of the studies by Shankaran and Gluckman [32,60] and meta-analysis of these studies plus the pilot studies by Gunn *et al.* [58] and the study of whole-body hypothermia by Eicher *et al.* [62] demonstrating a consistent benefit [63]. A 2-year follow-up of infants enrolled in the ICE trial was completed in July 2009. Pre-peer review results were promising, but we await further word on mortality plus morbidities from this fourth pivotal multicenter randomized study.

Box 1. TOBY and ICE trial enrolment criteria

TOBY trial enrolment criteria [61]

For TOBY, three sets of criteria were required (A, B and C).

- A.** Infants >36 weeks gestation admitted to the NICU with at least one of the following:
- Apgar score of <5 at 10 min after birth
  - Continued need for resuscitation, including endotracheal or mask ventilation, at 10 min after birth
  - Acidosis within 60 min of birth (defined as any occurrence of umbilical cord, arterial or capillary pH <7)
  - Base deficit >16 mmol/l in any blood sample (arterial, venous or capillary) within 60 min of birth

Infants that meet criteria A will be assessed for whether they meet criteria B:

- B.** Moderate-to-severe encephalopathy, consisting of altered state of consciousness (lethargy, stupor or coma) AND at least one of the following:
- Hypotonia
  - Abnormal reflexes including oculomotor or papillary abnormalities
  - An absent or weak suck
  - Clinical seizures, as recorded by study personnel

Infants that meet criteria A and B will be assessed by aEEG (read by trained personnel), as criteria C:

- C.** At least 30-min duration of aEEG recording that shows abnormal background aEEG activity or seizures, there must be one of the following:
- Normal background with some seizure activity
  - Moderately abnormal activity
  - Suppressed activity
  - Continuous seizure activity

ICE trial enrolment criteria [100]

- 35 weeks of age
- Evidence of moderate or severe encephalopathy

- Evidence of intrapartum hypoxia-ischemia with at least two of the following:
  - Apgar score of 5 or less at 10 min
  - Ongoing resuscitation in the form of mechanical ventilation at 10 min
  - Metabolic acidosis (cord pH <7 or an arterial pH <7, or base deficit of 12 or more) within 60 min of birth

aEEG: Amplitude-integrated EEG; ICE: Infant Cooling Evaluation; NICU: Neonatal intensive care unit; TOBY: Total Body Hypothermia for Neonatal Encephalopathy.

In all of the clinical trials, safety outcomes were tracked, including disorders of glucose homeostasis. The risks of hypothermia include hypothermia-induced insulin-resistant hyperglycemia, which has been noted in adults treated with hypothermia. Hyperglycemia alters brain tissue metabolism, increases neurotoxic glutamate and damages neuronal cell membranes [64]. In addition to the threat of hyperglycemia, hypoglycemia may occur along with severe hypoxic–ischemic injury, as glucose is rapidly and anerobically metabolized [65,66]. The degree of hypoglycemia contributes to the severity of asphyxia injury in an animal model [67], and is associated with more severe encephalopathy [68] and accumulation of biomarkers consistent with cellular injury [66]. Hypothermia may exacerbate hypoglycemia [69], but to date, no excess hypoglycemia or hyperglycemia attributable to use of moderate hypothermia in neonates with HIE has been reported from the clinical trials [60,62,70].

Meta-analyses of the hypothermia clinical trials: consistent efficacy

Azzopardi and Edwards conducted a speculative meta-analysis of the Eicher, CoolCap and NICHD studies [32,60,62–63]. They acknowledged the differences in the primary outcome among the three studies (Eicher reported 12-month outcome, and had high loss to follow-up; CoolCap reported death or severe disability; the NICHD study reported death or moderate and severe disability), and included them as ‘any’ disability. The cumulative results for 237 cooled infants and 241 controls were a relative risk (RR) of death or disability of 0.76, with a 95% CI of 0.65–0.89 [71].

Schulzke *et al.* reviewed five cooling trials involving 552 neonates. They identified the differences among studies – specifically, cooling techniques, the definition and severity of neurodevelopmental disability differences between studies, and the varying loss to follow-up. They included three studies for analysis of the primary outcome of mortality and disability at 18–22 months. These three studies included 223 cooled infants and 226 controls [57,60]. Hypothermia had a significant impact on the primary composite outcome of death or disability, with a RR of 0.78 and a 95% CI of 0.66–0.92. The estimated number needed to treat to have one infant avoid death or disability was eight [72].

Shah *et al.* reported a meta-analysis of four of the cooling trials, combining the results of Gunn, Gluckman, Shankaran and Eicher, with the primary outcome of death or impairment in childhood [32,58,60,62,63]. A total of 249 cooled infants and 248 control infants were included. There was a significant reduction in the risk of death or moderate to severe neurodevelopmental disability in infants who received hypothermia compared with control infants, with a RR of 0.76, and a 95% CI of 0.65–0.88. The number of encephalopathic infants to treat to avoid one infant to die or survive with moderate-to-severe disability was estimated to be six [63].

The fourth meta-analysis of hypothermia is included in the *Cochrane* registry, and was led by Susan Jacobs, who is also the principle investigator of the ICE trial. In the primary analysis, the Network and CoolCap trials, as well as the Eicher pilot study and the Gunn pilot study,

were included, with 228 cooled and 251 control infants among the four studies. Their results were quite similar to the other reviews: typical RR of 0.76 with a 95% CI of 0.65–0.89, and a number needed to treat of seven [73].

All four meta-analyses included an assessment of whether or not treatment and safety effects were heterogeneous, and all found that the results of the included studies were homogenous – that is, findings of efficacy and safety of use of hypothermia in the clinical trials were similar and consistent. Assessments of secondary outcomes, including mortality separately, and disability separately, also demonstrated benefit. This is important because there were concerns that if mortality was averted by cooling, more survivors would be devastated. As there has been reduction in both mortality and disability alone, this does not seem to be the case. In the Network trial, mortality was reduced in the hypothermia group, 24% compared with 37% in the control group (RR: 0.68; 95% CI: 0.43–1.01), and the risk of disabling cerebral palsy was 19.2% for the hypothermia group and 30.0% among the control group for a RR of 0.68 and a 95% CI of 0.38–1.22 [73]. In the more recently published TOBY trial, mortality was the same for cooled and regular care, but among survivors, disability was significantly decreased [61]. Referring back to FIGURE 1, the results of the multicenter clinical trials indicate that the critical period for initiation of cooling appears to occur in the first 6 postnatal hours.

Predicting outcome after HIE: hypothermia & aEEG impact on predictions

Prior to the studies and use of hypothermia for HIE, longitudinal evaluation of neurologic status over the first postnatal days to weeks was predictive of outcome [7,8]. Secondary analyses of data collected in the two pivotal hypothermia trials are likely to enable clinicians to have a better prognostic ability in the context of cooling. In the CoolCap study, information on severity of encephalopathy at randomization, and at day 4 was available for a subset of 177 infants. Infants in the cooled group and infants in the control group with grade 3 or the most severe HIE who improved their grade of encephalopathy by day 4 showed higher rates of favorable outcome than those that did not improve. All infants except three in the cooled group who had severe HIE at day 4 had an unfavorable outcome, and most infants (75%) with mild or normal status at day 4 had a favorable outcome in both groups. In both groups, infants with moderate HIE prerandomization who had an improved HIE grade on day 4 had a favorable prognosis, whereas infants who deteriorated did consistently poorly. Among infants whose moderate encephalopathy did not change over the 4 days, the cooled infants were more likely to end up with a positive outcome than controls (24 out of 31 cooled with good outcome vs 10 out of 29 controls; $p = 0.002$). This suggests that even if moderate encephalopathy persists after use of the CoolCap protocol, there is still a chance of improved outcome compared with prior epochs without hypothermia [74]. This is consistent with the potential hypothermia effect of modifying secondary energy failure and the repair and remodeling that is induced by hypoxic–ischemic injury to the still-developing brain.

The TOBY and CoolCap trials, as mentioned previously, used aEEG criteria for enrolment, while the NICHD and ICE trials did not. aEEG is a bedside tool that can be used for cerebral function monitoring in term and near-term infants. The aEEG records a single channel EEG from biparietal electrodes, and the signal is then filtered, rectified, smoothed and amplitude integrated. The aEEG interpretation is based primarily on pattern recognition, and the aEEG correlates well with conventional EEG. Owing to its simplicity of operations relative to conventional EEG, it can be more feasibly used in the intensive care unit immediately after birth and throughout a cooling interval. One group has found 100% agreement between aEEG and conventional EEG findings in infants with HIE [75].

Spitzmiller *et al.* reviewed the evidence for EEG and aEEG predicting long-term outcome after hypoxic–ischemic injury and found several studies confirming the strong predictive value of

conventional EEG. Patients with EEGs that improved in the first 12 postnatal hours were very likely to have normal outcomes [76]. Several of the reviewed studies found that improvement in aEEG during the first postnatal days was also associated with good prognosis [77,78]. In the NICHD Network trial there was a trend toward benefit in neonates with both moderate and severe encephalopathy by physical examination criteria [32]. There is no information currently available on whether whole-body hypothermia in the range and for the duration used in the NICHD Network trial has any potential benefit for infants demonstrating specifically moderate or severe encephalopathy aEEG criteria, but from the data collected in the NICHD study Shankaran *et al.* found that improvement in clinical examination severity of HIE and hypothermia treatment was associated with lower risk, and persistence of severe HIE was associated with a greater risk of death or disability following neonatal HIE [79]. The CoolCap study had similar findings [74]. The Network collected aEEG data on cooled infants, and we await results of their analyses assessing how cooling affects the aEEG trends, and how cooling influences the associations between early aEEG findings and neurologic outcome. Eventually, findings from combinations of alterations in MRI, aEEG, EEG and the evolving clinical examination may be useful in directed care or improving prognostic ability. The future could also include biomarkers that classify injury and help predict outcome, as they are showing promise in the rapid diagnosis of ischemic stroke in adults [80,81].

Hyperthermia–pyrexia

Both the Network and the CoolCap trials identified worse outcomes among infants with HIE who experienced high core body temperatures. In the CoolCap study, control patients had core temperatures monitored, and clinicians were directed to maintain rectal temperatures within the study range of 36.8–37.2°C. A total of 34 control patients had rectal temperatures of 38°C and over at any time during the 76-h monitoring period, 28 of whom had unfavorable outcomes; 76 control patients did not have pyrexia and 45 of these had unfavorable outcomes (OR: 3.2; 95% CI: 1.2–8.4; $p = 0.028$). Only 11 cooled patients (including one patient who was not cooled) had a rectal temperature of 38°C and over at randomization or at rewarming, and nine of these had unfavorable outcomes; while 50 of the 97 patients in the cooled group who did not experience pyrexia had unfavorable outcomes (OR: 4.2; 95% CI: 0.97–18; $p = 0.11$) [77]. In the NICHD trial, esophageal temperature in the control group was monitored, but the goal temperatures for control infants in the study were those used for routine care, which has been servo-controlled skin temperatures maintained on radiant warmers, rather than direct measures of core temperatures. Of the 102 infants randomized to the usual care group, 50 infants had a maximum esophageal temperature over 38°C. Higher core temperatures were associated with significant increases in risk of death or impairment, with odds of death or disability increased 3.6- to fourfold for each 1°C increase in the highest quartile of skin or esophageal temperatures [82].

Additional strategies & therapies to prevent or treat HIE

Cooling is a postnatal therapy for infants presenting with HIE. Initiation of cooling soon after a hypoxic–ischemic insult improves the chances of a good outcome in animal models of HIE [54,55]; initiation of cooling closer to the time of perinatal injury could improve outcomes. Although clinicians were optimistic that electronic fetal cardiotocography and fetal pulse oximetry might improve outcomes, neither one nor the combination has reliably provided obstetricians with definitive information that would lead to reducing the risk of hypoxic–ischemic injury via identification of infants that would benefit from operative delivery in the clinical trials of these monitoring techniques [83,84]. Although summary evidence in combined clinical trials has not suggested that these interventions (fetal cardiotocography and fetal saturation monitoring) will lead to changed outcomes, this may be in part because the prevalence of hypoxic–ischemic injury in the clinical trials of these monitoring techniques was

low. The combined assessment of the standard fetal heart rate tracing with an automated analysis of the fetal electrocardiogram (ST-waveform analysis; STAN, Neoventa Medical, Moelndal, Sweden) has shown promise in reducing metabolic acidosis at delivery; however, this promising modality requires a combination of educational and technological resources for proper implementation before it can be accepted [85,86].

As the clinical trials of fetal oximetry and cardiotocography have been completed and results reported, and the use of novel techniques such as STAN monitoring and conventional fetal heart rate monitoring are discussed, obstetric practice changes. As obstetric practice changes, the incidence of moderate to severe HIE may change as well. In some regional epidemiologic reports, increasing cesarean section rates and less frequent post-term deliveries have been associated with a lower incidence of HIE-classified infants [87].

Even if changes in obstetric practice and improvements in monitoring techniques reduce metabolic acidosis, it is unlikely that all occurrences of hypoxic–ischemic injury will be prevented. We know from the cooling clinical trials that more than 40% of the cooled infants either died or survived with impairments; therefore, the search for effective interventions for neonates must continue beyond hypothermia. There are promising agents in the pipeline, but none at the stage of Phase III clinical trials.

Postnatal infusions of magnesium sulfate have been tested in a study, which enrolled 40 infants (20 treated with magnesium), with promising results. Magnesium may work by increasing extracellular magnesium concentrations to block neuronal influx of calcium enough to overcome the axonal depolarization associated with hypoxic–ischemia, but magnesium infusions may also produce unwanted hypotension [88,89]. Its use specifically for HIE in neonates requires further study.

Xenon, a noble gas that also acts as a *N*-methyl-D-aspartic acid antagonist, has been shown to have additive benefits to hypothermia when tested in a rodent model of brain injury, with benefit of the combination of hypothermia plus xenon resulting in greater short- and long-term neuroprotection than from either treatment alone [90]. More recently, in a piglet model of HIE, xenon alone or xenon combined with hypothermia was associated with lower use of inotropes than in control animals or those treated with hypothermia alone [91]. A pilot clinical trial of xenon and hypothermia is listed on ClinicalTrials.gov [101] but has not started enrolment as of this writing. Tested inhalation doses have been 50% inhaled xenon. If higher than 50% FiO₂ is clinically required, lower concentrations of inhaled xenon will have to be used.

Recombinant human erythropoietin (rEpo) is neuroprotective in neonatal models of brain injury. Proposed mechanisms of neuroprotection include the activation of gene pathways that decrease oxidative injury, inflammation and apoptosis, while increasing vasculogenesis and neurogenesis [92]. A Phase II study of rEpo for HIE testing doses in the range of those used to prevent anemia of prematurity has been completed by investigators in China. Death or moderate/severe disability occurred for 35 (43.8%) out of 80 infants in the control group and 18 (24.6%) out of 73 infants in the erythropoietin group ($p = 0.017$) at 18 months [93]. The current US studies of rEpo for neuroprotection, a Phase II study for preterm infants [102] and a Phase I study for term infants with HIE [103], are currently underway.

At Duke University (NC, USA), we have started enrolment in a Phase I trial of autologous cord blood cells for HIE with infusions to begin before 14 postnatal days [104]. Human cord blood cells contain multiple cell types, including hematopoietic and endothelial progenitor cells, and when administered in adult animals with hypoxia plus ischemic injury, they ameliorate injury and improve behavioral outcomes [94–96]. In one study of rat pups, human umbilical cord blood cells infused 24 h after hypoxic–ischemic injury migrated selectively to the injured area of the brain, and engraftment was associated with substantial alleviation of spastic paresis

[97]. Not every study in newborn rodents has found similar positive results [98] ; however, we believe there is a need to initiate Phase I trials of autologous cord blood for HIE in human infants with available autologous cells and high risk of poor outcome even with hypothermia, before non-evidence-based clinical use becomes prevalent.

Expert commentary

The efficacy of hypothermia for HIE in term and near-term neonates with HIE has been consistently demonstrated in the multiple clinical trials to date. The effectiveness in clinical practice, and for longer term school-age outcomes, remains to be seen. Hypothermia should be used in concordance with the protocols used in the published randomized controlled trials, with initiation of cooling in the first 6 postnatal hours. Cooled infants must be closely followed for their neurodevelopmental outcomes. Results of the practice must be reported and institutions providing cooling must participate in registries or databases that will allow for future analyses of implementation and results of hypothermia in clinical practice [99]. While early initiation of hypothermia (in the first 6 postnatal hours) becomes part of usual care, questions remain. Should we cool infants who are born in a referring hospital and appear to meet entry criteria in the first hour, but on arrival at a tertiary center appear vigorous and have a normalized neurological exam? What should we do with infants who cannot be transferred to a tertiary cooling center by 6 postnatal hours? What should be done with infants who suffer a hypoxic–ischemic event in the first days after birth? These questions are being addressed, as in the Neonatal Research Network's Late Hypothermia Clinical Trial [105], or will be addressed in ongoing and upcoming clinical trials. As we learn more about hypothermia, the evidence supports obstetricians and neonatologists partnering to advocate for the development of appropriate cooling protocols in regional centers that will develop expertise and maintain close neurodevelopmental follow-up of cooled infants and report results to large databases where effectiveness of hypothermia can be analyzed.

Key issues

- Hypoxic–ischemic encephalopathy (HIE) and perinatal asphyxia contribute significantly to poor outcomes among neonates in all countries.
- The pathophysiology of HIE suggests that an intervention that reduces cerebral oxygen consumption during the latency period between primary and secondary energy failure could be effective, as numerous pathways to late cell death could be avoided.
- Hypothermia, as used in major clinical trials, is efficacious, but slight variations in the trials' sample populations and interventions are important to consider.
- Institution of hypothermia protocols used in the clinical trials at tertiary centers will increase, so surveillance of the impact of this new therapy must be closely monitored.
- The hypothermia clinical trials have informed practitioners about the hazards of undiagnosed hyperthermia, so even if hypothermia is not immediately adopted, taking steps to avoid hyperthermia among infants diagnosed with HIE is appropriate.
- Current clinical diagnostic methodologies to predict outcomes before and during the 3-day hypothermia treatment course are improving, but further study is needed.

- With 40% or more of cooled infants still dying or suffering moderate or severe long-term impairment, more work to discover additional neuroprotective strategies is required.

Five-year view

Given the consistency of results of the clinical trials, hypothermia protocols are increasingly implemented in tertiary care centers. The results of the ICE trial will influence whether or not standardized regimens for cooling on transport are initiated and whether or not relatively simple cooling techniques that could be operable in the developing world reduce mortality and morbidity from HIE. Further exploration of aEEG results will inform clinicians as to whether or not this tool adds to prognostic accuracy and should guide therapy before, during and after hypothermia. Additional trials of duration and degree of cooling, and whether or not cooling initiated after the first 6 postnatal hours will influence outcome, will be completed and further inform clinicians of the best use of this therapy. Trials of additional interventions targeting neonatal HIE are needed and must continue. We are optimistic that the safety and efficacy of additional treatments will be established. Given the experience of the hypothermia clinical trials requiring 2–3 years to complete enrolment and an additional 2 years to complete neurodevelopmental follow-up, it will be 5 years at the earliest that the new adjunct therapies will be ready for widespread clinical use. Alternative methodologies to assess neurologic outcomes earlier, or trials that include larger numbers of enrolling centers, will reduce the wait. Hypothermia is likely also to be tested for its ability to provide neuroprotection for infants in the developing world, for more preterm neonates (33–35-weeks gestational age) with evidence of hypoxic–ischemic injury, neonates with heart disease requiring cardiac bypass surgery and neonates on extracorporeal membrane oxygenation.

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References

Papers of special note have been highlighted as:

- of interest
- of considerable interest

1. American College of Obstetricians and Gynecologists and American Academy of Pediatrics. Neonatal Encephalopathy and cerebral palsy. Defining the pathogenesis. 2003
2. Graham EM, Ruis KA, Hartman AL, Northington FJ, Fox HE. A systematic review of the role of intrapartum hypoxia–ischemia in the causation of neonatal encephalopathy. *Am. J. Obstet. Gynecol* 2008;199(6):587–595. [PubMed: 19084096] **Current comprehensive review of the epidemiology of hypoxic–ischemic encephalopathy (HIE), from its incidence to the associated mortality and morbidity rates.**
3. Airede AI. Birth asphyxia and hypoxic–ischaemic encephalopathy: incidence and severity. *Ann. Trop. Paediatr* 1991;11(4):331–335. [PubMed: 1721789]

4. Lawn J, Shibuya K, Stein C. No cry at birth: global estimates of intrapartum stillbirths and intrapartum-related neonatal deaths. *Bull. World Health Organ* 2005;83(6):409–417. [PubMed: 15976891]
5. Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: when? Where? Why? *Lancet* 2005;365(9462): 891–900. [PubMed: 15752534] **Provides information on the progress made and suggestions for what further can be done to reduce the world's 4 million neonatal deaths per year, with a particular discussion of approaches to reduce the 23% of these deaths attributable to asphyxia.**
6. Badawi N, Felix JF, Kurinczuk JJ, et al. Cerebral palsy following term newborn encephalopathy: a population-based study. *Dev. Med. Child. Neurol* 2005;47(5):293–298. [PubMed: 15892370]
7. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Arch. Neurol* 1976;33(10):696–705. [PubMed: 987769]
8. Robertson CM, Finer NN, Grace MG. School performance of survivors of neonatal encephalopathy associated with birth asphyxia at term. *J. Pediatr* 1989;114(5):753–760. [PubMed: 2469789]
9. Shankaran S, Woldt E, Koepke T, Bedard MP, Nandyal R. Acute neonatal morbidity and long-term central nervous system sequelae of perinatal asphyxia in term infants. *Early Hum. Dev* 1991;25(2): 135–148. [PubMed: 1713544]
10. McKinsty RC, Miller JH, Snyder AZ, et al. A prospective, longitudinal diffusion tensor imaging study of brain injury in newborns. *Neurology* 2002;59(6):824–833. [PubMed: 12297561]
11. Freeman JP. Brain injury in the term neonate. *Semin. Perinatol* 2004;(28):415–424. [PubMed: 15693398]
12. Toet MC, Hellstrom-Westas L, Groenendaal F, Eken P, de Vries LS. Amplitude integrated EEG 3 and 6 hours after birth in full term neonates with hypoxic–ischaemic encephalopathy. *Arch. Dis. Child. Fetal Neonatal Ed* 1999;81(1):F19–F23. [PubMed: 10375357]
13. Toet MC, Lemmers PM. Brain monitoring in neonates. *Early Hum. Dev* 2009;85(2):77–84. [PubMed: 19150756] **Describes the current state of use of brain-monitoring technologies for HIE by the leaders in the field of amplitude-integrated EEG in neonates.**
14. Lorek A, Takei Y, Cady EB, et al. Delayed ('secondary') cerebral energy failure after acute hypoxia–ischemia in the newborn piglet: continuous 48-hour studies by phosphorus magnetic resonance spectroscopy. *Pediatr. Res* 1994;36(6):699–706. [PubMed: 7898977] **Provides an early, but subsequently validated, picture of the sequence of events occurring after hypoxic–ischemic injury, and is widely cited by subsequent articles investigating particular aspects of HIE pathophysiology.**
15. Laptook AR, Corbett RJ, Ruley J, Olivares E. Blood flow and metabolism during and after repeated partial brain ischemia in neonatal piglets. *Stroke* 1992;23(3):380–387. [PubMed: 1542900]
16. Johnston MV, Trescher WH, Ishida A, Nakajima W. Neurobiology of hypoxic–ischemic injury in the developing brain. *Pediatr. Res* 2001;49(6):735–741. [PubMed: 11385130]
17. Siesjo BK, Bengtsson F. Calcium fluxes, calcium antagonists, and calcium-related pathology in brain ischemia, hypoglycemia, and spreading depression: a unifying hypothesis. *J. Cereb. Blood Flow Metab* 1989;9(2):127–140. [PubMed: 2537841]
18. Mehmet H, Yue X, Squier MV, et al. Increased apoptosis in the cingulate sulcus of newborn piglets following transient hypoxia–ischaemia is related to the degree of high energy phosphate depletion during the insult. *Neurosci. Lett* 1994;181(1–2):121–125. [PubMed: 7898750]
19. Fellman V, Raivio KO. Reperfusion injury as the mechanism of brain damage after perinatal asphyxia. *Pediatr. Res* 1997;41(5):599–606. [PubMed: 9128279]
20. Liu XH, Kwon D, Schielke GP, Yang GY, Silverstein FS, Barks JD. Mice deficient in interleukin-1 converting enzyme are resistant to neonatal hypoxic–ischemic brain damage. *J. Cereb. Blood Flow Metab* 1999;19(10):1099–1108. [PubMed: 10532634]
21. Tan WK, Williams CE, During MJ, et al. Accumulation of cytotoxins during the development of seizures and edema after hypoxic–ischemic injury in late gestation fetal sheep. *Pediatr. Res* 1996;39(5):791–797. [PubMed: 8726230]
22. Gluckman PD, Guan J, Williams C, et al. Asphyxial brain injury – the role of the IGF system. *Mol. Cell. Endocrinol* 1998;140(1–2):95–99. [PubMed: 9722175]
23. Roth SC, Edwards AD, Cady EB, et al. Relation between cerebral oxidative metabolism following birth asphyxia, and neurodevelopmental outcome and brain growth at one year. *Dev. Med. Child. Neurol* 1992;34(4):285–295. [PubMed: 1572514]

24. Samejima K, Tone S, Kottke TJ, et al. Transition from caspase-dependent to caspase-independent mechanisms at the onset of apoptotic execution. *J. Cell Biol* 1998;143(1):225–239. [PubMed: 9763434]
25. Jacobson MD, Weil M, Raff MC. Programmed cell death in animal development. *Cell* 1997;88(3):347–354. [PubMed: 9039261]
26. Blaschke AJ, Staley K, Chun J. Widespread programmed cell death in proliferative and postmitotic regions of the fetal cerebral cortex. *Development* 1996;122(4):1165–1174. [PubMed: 8620843]
27. Martin LJ, Al-Abdulla NA, Brambrink AM, Kirsch JR, Sieber FE, Portera-Cailliau C. Neurodegeneration in excitotoxicity, global cerebral ischemia, and target deprivation: a perspective on the contributions of apoptosis and necrosis. *Brain Res. Bull* 1998;46(4):281–309. [PubMed: 9671259]
28. Ferriero DM. Neonatal brain injury. *N. Engl. J. Med* 2004;351(19):1985–1995. [PubMed: 15525724]
29. Hu BR, Liu CL, Ouyang Y, Blomgren K, Siesjo BK. Involvement of caspase-3 in cell death after hypoxia–ischemia declines during brain maturation. *J. Cereb. Blood Flow Metab* 2000;20(9):1294–1300. [PubMed: 10994850]
30. Edwards AD, Yue X, Squier MV, et al. Specific inhibition of apoptosis after cerebral hypoxia–ischemia by moderate post-insult hypothermia. *Biochem. Biophys. Res. Commun* 1995;217(3):1193–1199. [PubMed: 8554576]
31. Rothstein RP, Levison SW. Gray matter oligodendrocyte progenitors and neurons die caspase-3 mediated deaths subsequent to mild perinatal hypoxic/ischemic insults. *Dev. Neurosci* 2005;27(2–4):149–159. [PubMed: 16046849]
32. Shankaran S, Laptook AR, Ehrenkranz RA, et al. Whole-body hypothermia for neonates with hypoxic–ischemic encephalopathy. *N. Engl. J. Med* 2005;353(15):1574–1584. [PubMed: 16221780]
Second multicenter Phase III trial demonstrated efficacy for whole-body hypothermia after HIE, with a number needed to treat of six to avoid one death or an infant with moderate or severe disability.
33. Williams GR, Spencer FC. The clinical use of hypothermia following cardiac arrest. *Ann. Surg* 1958;148(3):462–468. [PubMed: 13571922]
34. Loughheed WM, Kahn DS. Circumvention of anoxia during arrest of cerebral circulation for intracranial surgery. *J. Neurosurg* 1955;12(3):226–239. [PubMed: 14381930]
35. Westin B, Miller JA Jr, Nyberg R, Wedenberg E. Neonatal asphyxia pallida treated with hypothermia alone or with hypothermia and transfusion of oxygenated blood. *Surgery* 1959;45(5):868–879. [PubMed: 13659328]
36. Cordey R. Hypothermia in resuscitating newborns in white asphyxia; a report of 14 cases. *Obstet. Gynecol* 1964;24:760–767. [PubMed: 14227613]
37. Drage JS, Berendes H. Apgar scores and outcome of the newborn. *Pediatr. Clin. North. Am* 1966;13(3):637–643. [PubMed: 5949897]
38. Dunn JM, Miller JA Jr. Hypothermia combined with positive pressure ventilation in resuscitation of the asphyxiated neonate. Clinical observations in 28 infants. *Am. J. Obstet. Gynecol* 1969;104(1):58–67. [PubMed: 4888017]
39. Silverman WA, Fertig JW, Berger AP. The influence of the thermal environment upon the survival of newly born premature infants. *Pediatrics* 1958;22(5):876–886. [PubMed: 13600915]
40. Daniel SS, Dawes GS, James LS, Ross BB, Windle WF. Hypothermia and the resuscitation of asphyxiated fetal rhesus monkeys. *J. Pediatr* 1966;68(1):45–53. [PubMed: 4954933]
41. Oates RK, Harvey D. Failure of hypothermia as treatment for asphyxiated newborn rabbits. *Arch. Dis. Child* 1976;51(7):512–516. [PubMed: 989263]
42. Behrman RE, James LS, Klaus M, Nelson N, Oliver T. Treatment of the asphyxiated newborn infant. Current opinions and practices as expressed by a panel. *J. Pediatr* 1969;74(6):981–988. [PubMed: 5781807]
43. Westin B. Hypothermia in the resuscitation of the neonate: a glance in my rear-view mirror. *Acta Paediatr* 2006;95(10):1172–1174. [PubMed: 16982485]
44. Busto R, Dietrich WD, Globus MY, Valdes I, Scheinberg P, Ginsberg MD. Small differences in intraischemic brain temperature critically determine the extent of ischemic neuronal injury. *J. Cereb. Blood Flow Metab* 1987;7(6):729–738. [PubMed: 3693428] **This study ‘broke the ice’,**

demonstrating benefit from hypothermia in an (adult) animal model of hypoxic-ischemic injury to the brain, which re-invigorated interest in hypothermia for neonatal HIE.

45. Thoresen M, Bagenholm R, Loberg EM, Apricena F, Kjellmer I. Posthypoxic cooling of neonatal rats provides protection against brain injury. *Arch. Dis. Child. Fetal Neonatal Ed* 1996;74(1):F3–F9. [PubMed: 8653432]
46. Thoresen M, Satas S, Puka-Sundvall M, et al. Post-hypoxic hypothermia reduces cerebrocortical release of NO and excitotoxins. *Neuroreport* 1997;8(15):3359–3362. [PubMed: 9351672]
47. Fukuda H, Tomimatsu T, Watanabe N, et al. Post-ischemic hypothermia blocks caspase-3 activation in the newborn rat brain after hypoxia-ischemia. *Brain Res* 2001;910(1–2):187–191. [PubMed: 11489270]
48. Enari M, Sakahira H, Yokoyama H, Okawa K, Iwamatsu A, Nagata S. A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature* 1998;391(6662):43–50. [PubMed: 9422506]
49. Namura S, Zhu J, Fink K, et al. Activation and cleavage of caspase-3 in apoptosis induced by experimental cerebral ischemia. *J. Neurosci* 1998;18(10):3659–3668. [PubMed: 9570797]
50. Laptook AR, Corbett RJ, Sterett R, Garcia D, Tollefsbol G. Quantitative relationship between brain temperature and energy utilization rate measured *in vivo* using ³¹P and ¹H magnetic resonance spectroscopy. *Pediatr. Res* 1995;38(6):919–925. [PubMed: 8618794]
51. Laptook AR, Shalak L, Corbett RJ. Differences in brain temperature and cerebral blood flow during selective head versus whole-body cooling. *Pediatrics* 2001;108(5):1103–1110. [PubMed: 11694688]
52. Sirimanne ES, Blumberg RM, Bossano D, et al. The effect of prolonged modification of cerebral temperature on outcome after hypoxic-ischemic brain injury in the infant rat. *Pediatr. Res* 1996;39(4 Pt 1):591–597. [PubMed: 8848330]
53. Bona E, Hagberg H, Loberg EM, Bagenholm R, Thoresen M. Protective effects of moderate hypothermia after neonatal hypoxia-ischemia: short- and long-term outcome. *Pediatr. Res* 1998;43(6):738–745. [PubMed: 9621982]
54. Gunn AJ, Gunn TR. The ‘pharmacology’ of neuronal rescue with cerebral hypothermia. *Early Hum. Dev* 1998;53(1):19–35. [PubMed: 10193924] **Provided clinical trialists with evidence for a ‘therapeutic window’ for optimal benefit from hypothermia for HIE.**
55. Gunn AJ, Gunn TR, de Haan HH, Williams CE, Gluckman PD. Dramatic neuronal rescue with prolonged selective head cooling after ischemia in fetal lambs. *J. Clin. Invest* 1997;99(2):248–256. [PubMed: 9005993]
56. Gunn AJ, Gunn TR, Gunning MI, Williams CE, Gluckman PD. Neuroprotection with prolonged head cooling started before postischemic seizures in fetal sheep. *Pediatrics* 1998;102(5):1098–1106. [PubMed: 9794940]
57. Gunn AJ, Bennet L, Gunning MI, Gluckman PD, Gunn TR. Cerebral hypothermia is not neuroprotective when started after postischemic seizures in fetal sheep. *Pediatr. Res* 1999;46(3):274–280. [PubMed: 10473041]
58. Gunn AJ, Gluckman PD, Gunn TR. Selective head cooling in newborn infants after perinatal asphyxia: a safety study. *Pediatrics* 1998;102(4 Pt 1):885–892. [PubMed: 9755260]
59. Shankaran S, Laptook A, Wright LL, et al. Whole-body hypothermia for neonatal encephalopathy: animal observations as a basis for a randomized, controlled pilot study in term infants. *Pediatrics* 2002;110(2 Pt 1):377–385. [PubMed: 12165594]
60. Gluckman PD, Wyatt JS, Azzopardi D, et al. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *Lancet* 2005;365(9460):663–670. [PubMed: 15721471] **First multicenter Phase III trial of cooling for HIE reported positive results.**
61. Azzopardi DV, Strohm B, Edwards AD, et al. Moderate hypothermia to treat perinatal asphyxial encephalopathy. *N. Engl. J. Med* 2009;361(14):1349–1358. [PubMed: 19797281] **Third (and largest) Phase III trial of hypothermia for HIE also demonstrated efficacy and safety.**
62. Eicher DJ, Wagner CL, Katikaneni LP, et al. Moderate hypothermia in neonatal encephalopathy: efficacy outcomes. *Pediatr. Neurol* 2005;32(1):11–17. [PubMed: 15607598]
63. Shah PS, Ohlsson A, Perlman M. Hypothermia to treat neonatal hypoxic ischemic encephalopathy: systematic review. *Arch. Pediatr. Adolesc. Med* 2007;161(10):951–958. [PubMed: 17909138]

64. Hayashi N. Management of pitfalls for the successful clinical use of hypothermia treatment. *J. Neurotrauma* 2009;26(3):445–453. [PubMed: 19292696]
65. Jones MD Jr, Burd LI, Makowski EL, Meschia G, Battaglia FC. Cerebral metabolism in sheep: a comparative study of the adult, the lamb, and the fetus. *Am. J. Physiol* 1975;229(1):235–239. [PubMed: 1147049]
66. Basu P, Som S, Choudhuri N, Das H. Contribution of the blood glucose level in perinatal asphyxia. *Eur. J. Pediatr* 2009;168(7):833–838. [PubMed: 18843505]
67. Vannucci RC. Cerebral carbohydrate and energy metabolism in perinatal hypoxic–ischemic brain damage. *Brain Pathol* 1992;2(3):229–234. [PubMed: 1343838]
68. Salhab WA, Wyckoff MH, Laptook AR, Perlman JM. Initial hypoglycemia and neonatal brain injury in term infants with severe fetal acidemia. *Pediatrics* 2004;114(2):361–366. [PubMed: 15286217]
69. Gandy GM, Adamson K, Cunningham N, et al. Thermal environment and acid–base homeostasis in human infants during the first few hours of life. *J. Clin. Invest* 1964;41:751–758. [PubMed: 14149926]
70. Shankaran S, Pappas A, Laptook AR, et al. Outcomes of safety and effectiveness in a multicenter randomized, controlled trial of whole-body hypothermia for neonatal hypoxic–ischemic encephalopathy. *Pediatrics* 2008;122(4):e791–e798. [PubMed: 18829776]
71. Edwards AD, Azzopardi DV. Therapeutic hypothermia following perinatal asphyxia. *Arch. Dis. Child. Fetal Neonatal Ed* 2006;91(2):F127–F131. [PubMed: 16492950]
72. Schulzke SM, Rao S, Patole SK. A systematic review of cooling for neuroprotection in neonates with hypoxic ischemic encephalopathy – are we there yet? *BMC Pediatr* 2007;7:30. [PubMed: 17784966]
73. Jacobs S, Hunt R, Tarnow-Mordi W, Inder T, Davis P. Cooling for newborns with hypoxic ischaemic encephalopathy. *Cochrane Database Syst. Rev* 2007;4:CD003311. [PubMed: 17943788]
74. Gunn AJ, Wyatt JS, Whitelaw A, et al. Therapeutic hypothermia changes the prognostic value of clinical evaluation of neonatal encephalopathy. *J. Pediatr* 2008;152(1):55–58. 58.e1. [PubMed: 18154900]
75. Toet MC, van der Meij W, de Vries LS, Uiterwaal CS, van Huffelen KC. Comparison between simultaneously recorded amplitude integrated electroencephalogram (cerebral function monitor) and standard electroencephalogram in neonates. *Pediatrics* 2002;109(5):772–779. [PubMed: 11986435]
76. Spitzmuller RE, Phillips T, Meinzen-Derr J, Hoath SB. Amplitude-integrated EEG is useful in predicting neurodevelopmental outcome in full-term infants with hypoxic–ischemic encephalopathy: a meta-analysis. *J. Child Neurol* 2007;22(9):1069–1078. [PubMed: 17890403]
77. ter Horst HJ, Sommer C, Bergman KA, Fock JM, van Weerden TW, Bos AF. Prognostic significance of amplitude-integrated EEG during the first 72 hours after birth in severely asphyxiated neonates. *Pediatr. Res* 2004;55(6):1026–1033. [PubMed: 15155870]
78. van Rooij LG, Toet MC, Osredkar D, van Huffelen AC, Groenendaal F, de Vries LS. Recovery of amplitude integrated electroencephalographic background patterns within 24 hours of perinatal asphyxia. *Arch. Dis. Child. Fetal Neonatal Ed* 2005;90(3):F245–F251. [PubMed: 15846017]
79. Shankaran S, Laptook A, Tyson J, et al. Evolution of encephalopathy with whole body hypothermia for neonatal hypoxic–ischemic encephalopathy (HIE) 2009 Pediatric Academic Society. E-Pas 2009:2160.9.
80. Laskowitz DT, Kasner SE, Saver J, Remmel KS, Jauch EC. Clinical usefulness of a biomarker-based diagnostic test for acute stroke: the Biomarker Rapid Assessment in Ischemic Injury (BRAIN) study. *Stroke* 2009;40(1):77–85. [PubMed: 18948614]
81. Saenger AK, Christenson RH. Stroke biomarkers: progress and challenges for diagnosis, prognosis, differentiation, and treatment. *Clin. Chem* 2010;56(1):21–33. [PubMed: 19926776]
82. Laptook A, Tyson J, Shankaran S, et al. Elevated temperature after hypoxic–ischemic encephalopathy: risk factor for adverse outcomes. *Pediatrics* 2008;122(3):491–499. [PubMed: 18762517] **For clinicians, this report demonstrating harm from elevated core temperature in infants with HIE is crucial to clinicians caring for infants with HIE.**
83. Alfrevic Z, Devane D, Gyte GM. Continuous cardiotocography (CTG) as a form of electronic fetal monitoring (EFM) for fetal assessment during labour. *Cochrane Database Syst. Rev* 2006;3:CD006066. [PubMed: 16856111]

84. East CE, Chan FY, Colditz PB, Begg LM. Fetal pulse oximetry for fetal assessment in labour. *Cochrane Database Syst. Rev* 2007;2:CD004075. [PubMed: 17443538]
85. Noren H, Blad S, Carlsson A, et al. STAN in clinical practice – the outcome of 2 years of regular use in the city of Gothenburg. *Am. J. Obstet. Gynecol* 2006;195(1):7–15. [PubMed: 16643829]
86. Devoe LD, Ross M, Wilde C, et al. United States multicenter clinical usage study of the STAN 21 electronic fetal monitoring system. *Am. J. Obstet. Gynecol* 2006;195(3):729–734. [PubMed: 16949404]
87. Smith J, Wells L, Dodd K. The continuing fall in incidence of hypoxic–ischaemic encephalopathy in term infants. *BJOG* 2000;107(4):461–466. [PubMed: 10759262]
88. Bhat MA, Charoo BA, Bhat JI, Ahmad SM, Ali SW, Mufti MU. Magnesium sulfate in severe perinatal asphyxia: a randomized, placebo-controlled trial. *Pediatrics* 2009;123(5):e764–e769. [PubMed: 19349375]
89. Levene M, Blennow M, Whitelaw A, Hanko E, Fellman V, Hartley R. Acute effects of two different doses of magnesium sulphate in infants with birth asphyxia. *Arch. Dis. Child. Fetal Neonatal Ed* 1995;73(3):F174–F177. [PubMed: 8535876]
90. Hobbs C, Thoresen M, Tucker A, Aquilina K, Chakkarapani E, Dingley J. Xenon and hypothermia combine additively, offering long-term functional and histopathologic neuroprotection after neonatal hypoxia/ischemia. *Stroke* 2008;39(4):1307–1313. [PubMed: 18309163]
91. Chakkarapani E, Dingley J, Hoque N, Liu X, Thoresen M. Xenon is cardiosupportive in newborn piglets after global hypoxia–ischaemia. *E-Pas* 2009;2335.8.
92. Juul SE, McPherson RJ, Bammler TK, Wilkerson J, Beyer RP, Farin FM. Recombinant erythropoietin is neuroprotective in a novel mouse oxidative injury model. *Dev. Neurosci* 2008;30(4):231–242. [PubMed: 17960054]
93. Zhu C, Kang W, Xu F, et al. Erythropoietin improved neurologic outcomes in newborns with hypoxic–ischemic encephalopathy. *Pediatrics* 2009;124(2):e218–e226. [PubMed: 19651565]
94. Chen J, Sanberg PR, Li Y, et al. Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats. *Stroke* 2001;32(11):2682–2688. [PubMed: 11692034]
95. Vendrame M, Cassady J, Newcomb J, et al. Infusion of human umbilical cord blood cells in a rat model of stroke dose-dependently rescues behavioral deficits and reduces infarct volume. *Stroke* 2004;35(10):2390–2395. [PubMed: 15322304]
96. Taguchi A, Soma T, Tanaka H, et al. Administration of CD34+ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. *J. Clin. Invest* 2004;114(3):330–338. [PubMed: 15286799]
97. Meier C, Middelani J, Wasielewski B, et al. Spastic paresis after perinatal brain damage in rats is reduced by human cord blood mononuclear cells. *Pediatr. Res* 2006;59(2):244–249. [PubMed: 16439586]
98. de Paula S, Vitola AS, Greggio S, et al. Hemispheric brain injury and behavioral deficits induced by severe neonatal hypoxia–ischemia in rats are not attenuated by intravenous administration of human umbilical cord blood cells. *Pediatr. Res* 2009;65(6):631–635. [PubMed: 19430381]
99. Higgins RD, Raju TN, Perlman J, et al. Hypothermia and perinatal asphyxia: executive summary of the National Institute of Child Health and Human Development workshop. *J. Pediatr* 2006;148(2):170–175. [PubMed: 16492424]
100. Inder TE, Hunt RW, Morley CJ, et al. Randomized trial of systemic hypothermia selectively protects the cortex on MRI in term hypoxic–ischemic encephalopathy. *J. Pediatr* 2004;145(6):835–837. [PubMed: 15580212]

Websites

101. ClinicalTrials.gov. Neuroprotective Effects of Hypothermia Combined With Inhaled Xenon Following Perinatal Asphyxia (NCT00934700)
<http://clinicaltrials.gov/ct2/results?term=NCT00934700>
102. ClinicalTrials.gov. High-Dose Erythropoietin in Extremely Premature Infants to Prevent/Attenuate Brain Injury: A Phase II Study (NCT00589953)
<http://clinicaltrials.gov/ct2/results?term=NCT00589953>

103. ClinicalTrials.gov. Neonatal Erythropoietin in Asphyxiated Term Newborns (NCT00719407)
<http://clinicaltrials.gov/ct2/results?term=NCT00719407>
104. ClinicalTrials.gov. Cord Blood for Neonatal Hypoxic–ischemic Encephalopathy (NCT00593242)
<http://clinicaltrials.gov/ct2/results?term=NCT00593242>
105. ClinicalTrials.gov. Late Hypothermia for Hypoxic–Ischemic Encephalopathy (NCT00614744)
<http://clinicaltrials.gov/ct2/results?term=NCT00614744>

Figure 1. Mechanisms of brain injury in the term neonate

Oxidative stress and excitotoxicity, through downstream intracellular signaling, produce both inflammation and repair. Cell death begins immediately and continues during a period of days to weeks. The cell death phenotype changes from an early necrotic morphology to a pathology resembling apoptosis. This evolution is called the necrosis–apoptosis continuum. Reproduced with permission from [28].

Table 1

Sarnat stages of neonatal encephalopathy.

Assessment	Stage 1	Stage 2	Stage 3
Mental status	Hyperalert	Lethargic	Stuporous
Suck	Weak or absent	Weak or absent	Absent
Moro	Strong	Weak	Absent
Muscle tone	Normal	Hypotonia	Flaccid
Autonomic function	Generalized sympathetic	Generalized parasympathetic	Absent
Pupils	Mydriasis	Miosis	
Seizures	None	Common	Variable
EEG	Normal (awake)	Early: low-voltage δ and θ	Early: periodic pattern with isopotential phases Late: isopotential
Duration	<24 h	2–14 days	Hours to weeks

Adapted from [7].

Table 2

Enrolment Criteria for the National Institute of Child Health and Human Development Neonatal Research Network Hypothermia Study.

Infants were evaluated in two steps; first by clinical and biochemical criteria (Step A), followed by a neurological examination (Step B)			
Step A			
If blood gas is available		If blood gas is not available or pH between 7.0 and 7.15, base deficit 10–15.9 mEq/l	
A1		A2	
Infant should have: Cord pH or first postnatal blood gas within 1 h with pH ≤ 7.0 OR Base deficit on cord gas or first postnatal blood gas within 1 h at ≥ 16 mEq/l		Infant should have: Acute perinatal event and either An Apgar score ≤ 5 at 10 min OR Continued need for ventilation initiated at birth and continued for at least 10 min	
Once infant met either A1 or A2, neurologic examination, Step B was carried out			
Step B. Presence of moderate/severe encephalopathy defined as seizures or signs in three out of six categories.			
No.	Category	Moderate encephalopathy	Severe encephalopathy
1	Level of consciousness	Lethargic	Stupor/coma
2	Spontaneous activity	Decreased	No activity
3	Posture	Distal flexion	Decerebrate
4	Tone	Hypotonia (focal, general)	Flaccid
5	Primitive reflexes:		
	Suck	Weak	Absent
	Moro	Incomplete	Absent
6	Autonomic system:		
	Pupils	Constricted	Skew deviation/dilated/nonreactive to light
	Heart rate	Bradycardia	Variable heart rate
	Respirations	Periodic breathing	Apnea

The neurologic examination is performed by a physician examiner. If the infant met criteria A1 or A2 and criteria B and does not meet exclusion criteria, the infant is eligible. Exclusion criteria are an inability to enrol by 6 postnatal hours, major congenital abnormality, severe growth restriction (birth weight ≤ 1800 g), and refusal of consent by a parent or an attending neonatologist; moribund infants for whom no further aggressive treatment is planned are also excluded. Data from [101].