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# Time Is Brain: Starting Therapeutic Hypothermia within Three Hours after Birth Improves Motor Outcome in Asphyxiated Newborns

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**Key Words**
Hypothermia · Newborn · Time · Outcome · Neuroprotection

**Abstract**
***Objective:*** Therapeutic hypothermia (HT) is the standard treatment for newborns after perinatal asphyxia. Preclinical studies report that HT is more effective when started early. ***Methods:*** Eighty cooled newborns were analyzed and grouped according to when cooling was started after birth: early (≤180 min) or late (>181 min). For survivors we analyzed whether starting cooling early was associated with a better psychomotor or mental developmental index (PDI or MDI, Bayley Scales of Infant Development II) than late cooling. ***Results:*** Forty-three newborns started cooling early and 37 started late. There was no significant difference in the severity markers of perinatal asphyxia between the groups; however, nonsurvivors (n = 15) suffered more severe asphyxia and had significantly lower centiles for weight (BWC; p = 0.009). Of the 65 infants that survived, 35 were cooled early and 30 were cooled late. There was no difference in time to start cooling between those who survived and those who did not. For survivors, median PDI (IQR) was significantly higher when cooled early [90 (77–99)] compared to being cooled later [78 (70–90); p = 0.033]. There was no increase in cardiovascular adverse effects in those cooled early. There was no significant difference in MDI between early and late cooling [93 (77–103) vs. 89 (76–106), p = 0.594]. ***Conclusion:*** Starting cooling before 3 h of age in surviving asphyxiated newborns is safe and significantly improves motor outcome. Cooling should be initiated as soon as possible after birth in eligible infants.


## Introduction

Therapeutic hypothermia (HT) has become the standard treatment for newborns suffering neonatal encephalopathy after perinatal asphyxia [1]. Meta-analysis of the randomized controlled cooling trials (RCTs) has shown that HT significantly improves neurodevelopmental outcome at 18–24 months [2, 3], and the number needed to treat is 6.

As we and others have shown in preclinical studies, HT offers better neuroprotection when initiated immediately after hypoxia-ischemia [4, 5]. The effective neuroprotective time window for HT has been shown to be within the first 6 h after hypoxia-ischemia, as the effec-

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tiveness of HT decreases with delay [5]. The inclusion criteria in the large RCTs were to initiate HT within the first 6 h after birth [6–10]. However, some asphyxiated newborns may well have suffered hypoxia-ischemia antenatally at an unknown time before delivery and outside the effective time window for cooling. This may be one of many reasons why around 50% of cooled newborns still have poor outcomes [11]. In the RCTs, cooling started after approximately 4 h with little variability; therefore, the effect of early or delayed onset could not be investigated [2, 11].

We apply HT during neonatal transport to a centralized cooling center, and all regional hospitals initiate cooling following the same protocol within our network. We assessed how time to start cooling and time to target temperature related to neurodevelopmental outcome at 18–20 months.

## Methods

With ethical permission (CH/2009/3091), anonymized data were prospectively collected between January 2007 and February 2010 and retrospectively analyzed from newborn infants (gestational age ≥36 weeks) with perinatal asphyxia. Infants were born at (n = 58) or transferred to (n = 22) St. Michael's Hospital (Bristol, UK), within 6 h after birth. Infants fulfilled the entry criteria for HT therapy as used in the CoolCap and TOBY trials [7, 10]: Apgar score ≤5 and/or ongoing resuscitation at 10 min, abnormal blood gases with a pH <7.0 or base deficit ≥16 mmol/l were the immediate criteria for perinatal asphyxia, abnormal neurological examination was the second criterion, and moderate or severe voltage changes on amplitude-integrated encephalography or seizures was the third. Seizures were defined clinically or with amplitude-integrated encephalography (CFM, Natus Medical Inc., Seattle, Wash., USA).

Active cooling was performed, using whole body cooling with a manually regulated cooling mattress [Tecotherm, TS Med 200M, Inspiration Healthcare, Leicester, UK (n = 7)], a water-filled cap [Olympic Medical Cool Care System, Olympic Medical, Seattle, Wash., USA (n = 4)] or a servo-controlled cooling jacket [Criti-Cool; MTRE, Yavne, Israel (n = 69)] to a rectal temperature of 33–34°C.

Surviving and nonsurviving newborns were compared regarding markers for severity of asphyxia, birth weight centiles and time to start cooling. Data for gestational age, Apgar score at 10 min, gender, birth weight, worst pH within the first hour after birth, degree of encephalopathy, onset of subclinical or clinical seizures before cooling, blood glucose at birth, survival, time when cooling was started and target temperature was reached, use of inotropic support, and development of persistent pulmonary hypertension (PPHN) were recorded for each newborn.

To assess the effect of time when cooling was started on neurodevelopmental outcome, cooled newborns were grouped into 'cooling early', i.e. HT was started within the first 3 h after birth (≤180 min), and 'cooling late', i.e. HT was started more than 3 h after birth (≥181 min).

Surviving infants were examined using the Bayley Scales of Infant Development II [12] at 18–20 months. We chose Bayley II at 18–20 months since all large cooling trials have examined the children at this time point [2, 11]. Severe disability was defined as any of the following: a mental development index (MDI) <70 and/or a psychomotor development index (PDI) <70 on the Bayley II scales (≥2 SD below the mean) and/or bilateral cortical visual impairment with no useful vision and/or sensorineural hearing loss. Cerebral palsy was diagnosed according to the criteria of Hagberg et al. [13].

*Data Analysis*

Statistical analysis was performed using SPSS 18 (SPSS, Chicago, Ill., USA). Nonparametric continuous data was analyzed with a Mann-Whitney U test for analysis between groups. In the tables, the Wilcoxon median and upper and lower limits of the 95% CI are reported (StatExact 7, Cytel Software, Cambridge, Mass., USA), which corresponds to the nonparametric one-sample test. Multivariate linear regression analysis was performed on all infants with MDI or PDI as the dependent variable. Independent variables were time when cooling was started, time to target temperature, onset of seizures before cooling, place of birth, gestational age, Apgar score at 10 min, gender, birth weight, survival and worst pH within the first hour after birth. A significance value of $p \leq 0.05$ on two-sided testing was considered significant. Median and confidence intervals were calculated on nonparametric data using the Hodges-Lehmann estimator.

## Results

Ninety-four newborns were cooled in the study period. Fourteen were not eligible for further analysis since they did not fulfill the cooling entry criteria as used in the large cooling trials, have a full dataset or were lost to follow-up. Eighty newborns, of which 48 were males, had a full dataset and fulfilled the entry criteria used for HT. Descriptive data for those who survived and those who died (n = 15) are presented separately in tables 1 and 2. Median (IQR) time when cooling started was 180 min (60–285) in survivors and 150 min (60–365) in nonsurvivors (table 1). Time to cooling in 2010 did not differ from time to cooling in 2007. Twenty-two newborns were outborn (15 survivors/6 nonsurvivors). Compared to inborn newborns, time to start cooling and time to target temperature did not significantly differ in outborn newborns.

In survivors, 35 infants started cooling early at a median time (95% CI) of 90 min (60–105) and 31 started cooling late at 310 min (283–332.5; $p < 0.001$). The nonsurvivors started cooling at the same range and distributions of times as the survivors (table 1). Death was not related to the time of starting cooling. In the survivors, 27 of the 35 early cooled newborns started with passive cool-

**Table 1.** Descriptive data of all 80 newborns separated between survivors and nonsurvivors

| Characteristics | Survivors (n = 65) | Nonsurvivors (n = 15) | p value |
|---|---|---|---|
| Median time (IQR) when cooling was started, min | 180 (60–285) | 150 (60–365) | 0.814 |
| Median time (IQR) to target temperature, min | 313 (180–420) | 300 (120–401) | 0.622 |
| Seizures before cooling, n (%) | 44 (67.7) | 12 (80) | 0.351 |
| Gender (male/female), n | 38/27 | 10/5 | 0.561 |
| Median (95% CI) birth weight, g | 3,420 (3,245–3,615) | 3,105 (2,880–3,325) | **0.044** |
| Median (95% CI) birth weight centile | 50.5 (40.8–57.5) | 26.0 (16.1–37.0) | **0.009** |
| Mean (±SD) gestational age, weeks + days | 39+4 (±1.5) | 39+5 (±1.5) | 0.598 |
| Median (CI) Apgar at 10 min | 6.5 (6–7.5) | 4 (2–6) | **0.005** |
| Median (95% CI) worst pH within first hour after birth | 6.99 (6.94–7.04) | 6.83 (6.75–6.94) | **0.006** |
| Low blood glucose at birth (<2.5 mmol/l or <45 mg/dl), n (%) | 11 (16.9) | 5 (33.3) | 0.093 |
| Inotropic support during cooling, n (%) | 35 (53.8) | 15 (100) | **0.001** |
| PPHN, n (%) | 3 (4.6) | 4 (26.6) | **0.007** |
| Outborn, n (%) | 15 (23.1) | 7 (46.6) | 0.067 |

There was a significant difference between the groups regarding APGAR at 10 min, worst pH within the first hour after birth, inotropic support during cooling and PPHN, indicating a larger severity of injury in the nonsurvivors.

**Table 2.** Descriptive data of all 65 survivors

| Characteristics | Time cooling started (≤180 min after birth) (n = 35) | Time cooling started (>180 min after birth) (n = 30) | p value |
|---|---|---|---|
| Median (95% CI) time when cooling was started, min | 90 (60–105) | 310 (283–332.5) | **<0.001** |
| Median (95% CI) time to target temperature, min | 232.5 (172.50–300) | 382.5 (345–430.5) | **<0.001** |
| Seizures before cooling, n (%) | 22 (62.8) | 22 (73.3) | 0.372 |
| Gender (male/female), n | 18/17 | 20/10 | 0.217 |
| Median (95% CI) birth weight, g | 3,418 (3,180–3,675) | 3,433 (3,195–3,743) | 0.737 |
| Median (95% CI) birth weight centile | 50.56 (37.5–63.7) | 50 (35.75–62.45) | 0.911 |
| Mean (±SD) gestational age, weeks + days | 39+2 (±1.7) | 39+6 (±1.3) | 0.323 |
| Median (95% CI) Apgar at 10 min | 6.5 (6–7.5) | 6.5 (5.5–7.5) | 0.727 |
| Median (95% CI) worst pH within first hour after birth | 6.965 (6.915–7.03) | 7.02 (6.94–7.10) | 0.483 |
| Degree of encephalopathy, Sarnat score | | | **0.003** |
| Mild | 1 | 1 | |
| Moderate | 19 | 26 | |
| Severe | 15 | 3 | |
| Method of start cooling, n | | | **0.02** |
| Passive | 27 | 15 | |
| Active | 8 | 15 | |
| Outborn, n (%) | 5 (14.2) | 10 (33.3) | 0.071 |
| Low blood glucose at birth (<2.5 mmol/l or <45 mg/dl), n (%) | 7 (20) | 4 (13.3) | 0.556 |
| Inotropic support during cooling, n (%) | 20 (57.1) | 15 (50) | 0.568 |
| PPHN, n (%) | 2 (5.7) | 1 (3.3) | 0.651 |

There was a significant difference between the groups for time when cooling started and time to target temperature; however, there was no difference regarding markers of severity.

ing compared to 15 of the 30 late cooled newborns (p = 0.02), and the time to target temperature was earlier in the actively cooled group (table 2).

Nonsurviving newborns (n = 15) all had a higher severity grade of encephalopathy, had a significantly lower APGAR score at 10 min (p = 0.005), had a significantly lower pH within the first hour after birth (p = 0.005), had lower blood sugars at birth (p = 0.093), needed significantly more inotropic support (p = 0.001) and had a significant increase in the development of PPHN (p = 0.007;

**Table 3.** Outcome data from all newborns and of the 65 survivors

| | Time cooling started (≤180 min after birth) | Time cooling started (>180 min after birth) | p value |
|---|---|---|---|
| All newborns, n | 43 | 37 | |
| Good outcome (MDI and/or PDI >70) | 26 | 22 | >0.05 |
| Poor outcome (death or severe disability) | 17 | 15 | >0.05 |
| Survivors, n | 35 | 30 | |
| Median (95% CI) PDI | 88 (82–93) | 77.5 (71–84.5) | **0.033** |
| Median (95% CI) MDI | 91 (83–98) | 88 (79–96) | 0.594 |
| Cerebral palsy | 3 | 2 | 0.772 |
| Hearing loss | 2 | 5 | 0.140 |

There was no significant difference in the outcome parameters when comparing all newborns; however, for survivors the PDI was significantly higher in the early cooling group when compared to the late cooling group. MDI, cerebral palsy and hearing loss were not significantly different in survivors.

table 1). They also had significantly lower birth weights (p = 0.044) and birth weight centiles than the survivors (p = 0.009). Among the survivors, subclinical or clinical seizures before the initiation of cooling were seen in 62.8% of newborns in the early group and 73.3% in the late cooling group (p = 0.372).

There was no significant difference between early and late cooling regarding gender, gestational age, APGAR at 10 min, worst pH within the first hour after birth, blood glucose at birth, place of birth, inotropic support during cooling or PPHN (table 2). Newborns from the cooling early group, however, had significantly higher degrees of encephalopathy compared to the cooling late group (p = 0.003; table 2). In both groups, 2 children with mild encephalopathy were cooled due to the clinician's decision involved in the treatment of the newborn.

When comparing all newborns, there was no significant difference in outcome between the cooling early and late groups (table 3). For the survivors, though, the median PDI (95% CI) was significantly higher in the cooling early group [88 (82–93)] than in the cooling late group [77.5 (71–84.5); p = 0.033]. This was despite significantly higher degrees of encephalopathy in the cooling early group. In the early and late group, 2 and 3 infants, respectively, were diagnosed with cerebral palsy. There was no significant difference in median (95% CI) MDI between the early and late cooling groups [91 (83–98) vs. 88 (79–96); p = 0.594; table 2].

MDI or PDI <70 on Bayley II scales occurred in 17 of the 67 infants: 9 from the early cooling and 8 from the late cooling group (table 2). In the early and late cooling groups, 2 and 5 were diagnosed with severe hearing loss, respectively (p = 0.15).

Linear regression analysis showed a significant association between time when cooling was started and PDI (95% CI: –0.513 to –0.028; p = 0.029). However, there was no significant association between time when cooling was started and MDI (95% CI: –0.315 to 0.188; p = 0.617). All other independent variables did not show a significant correlation with the dependent variables MDI and PDI.

## Discussion

This study shows that early initiation of HT before 3 h of age is associated with better motor outcome at 18–20 months in surviving newborns after moderate or severe perinatal asphyxia.

None of the randomized controlled cooling trials were designed to examine the influence of early initiation of HT. The median onset of cooling varied between 4 h and 4.5 h in the first 3 large cooling trials [7–8, 10]. In a secondary analysis of the CoolCap study, it was suggested that there was a trend of better outcome if cooled early [14]; however, no outcome study has significantly shown a better outcome with early cooling. Based on the protocols of the large RCTs, the ILCOR 2010 guidelines state that cooling should be initiated within the first 6 h after birth in eligible newborns [1]. The suggestion of the time window for initiating HT in the first clinical trial was based on a sentinel study in near-term fetal sheep [4]. Gunn et al. [15, 16] showed that starting cooling after 5.5 h was no longer significantly protective in fetal sheep cooled to 34°C for 72 h after experimental asphyxia. We have recently confirmed the same effective time window

for cooling newborn rats [5], using the much used hypoxic-ischemic neonatal rat model developed by Vannucci and colleagues [17]. In addition, we found that hypothermia was only neuroprotective in those rats with moderate, but not severe, insults [5].

No published clinical study has examined longer durations, deeper cooling or different delays in a randomized setting. Two studies are underway in the USA, one is randomizing infants aged 6–24 h to be cooled late or not be cooled (ClinicalTrials.gov identifier: NCT00614744). The other study recruits to a 4-group design within 6 h of age to a cooling temperature of either 33.5 or 32°C lasting either 3 or 5 days (ClinicalTrials.gov identifier: NCT01192776). These studies will answer important questions which we are all awaiting the answer to. At this stage it would not be feasible to randomize between early (<3 h) and late (>3 h) cooling; however, one valid option is to examine the effect of time to cooling in an established cooling cohort with the same outcome as in the trials. We have the benefit of much experience in a single center as all infants since 1998 were entered into either the CoolCap feasibility study or the randomized CoolCap and TOBY trials [7, 10], followed by our registered database. We have increasing concerns regarding potential harm from a delayed start of cooling based on our recent animal work. In a model of severe injury in the neonatal rat, we found that starting after 12 h increased injury. Moderate injury, however, was not exacerbated by late cooling – it was just ineffective [5].

In the data presented here, there was no association between time to start hypothermia and death. Hypothermia starting either early or late did not influence those that died, which is a similar result to our preclinical study. The nonsurvivors in our cohort were more severely asphyxiated compared to the survivors. This supports why even early-onset of HT was not effective in these newborns. When using the primary outcome of combined variables of death or major neurodevelopmental disability as it was used in the large RCTs, there was no significant difference in outcome between the early and late cooling group. However, the primary aim of this study was to analyze if early cooling improves outcome in survivors after perinatal asphyxia and not to analyze if it reduces death of major neurodevelopmental disability.

There are some limitations to our study. First, we did not find an effect concerning time of starting cooling on mental developmental outcome. However, longer-term studies are needed to determine whether this continues into school age as well as if our current finding of significantly improved motor outcome continues into school age. Secondly, although we have shown that there was no significant difference in the number of outborn infants between the early and late cooling group, there were more outborn infants in the late cooling group. Nevertheless, time to start cooling and time to target temperature did not significantly differ in outborn newborns. Time to start cooling, not time to reaching target temperature, was the important therapeutic intervention to improve outcome in survivors. Still, this needs to be verified in a larger patient cohort. Thirdly, if the distribution of insult severity was not the same in the early and late cooling groups, this would influence our results. In the early cooling group, there were 2 patients that had an amplitude-integrated encephalography trace that recovered within 6 h. In the late cooling group, none of the patients recovered that early. Of note, removing these 2 infants from the analysis did not change our findings.

When cooling was first introduced, we were worried about adverse effects of early cooling, in particular hypotension, PPHN and increased acidosis. However, we found that there was no significant increase in the early cooling group and that early cooling is safe.

Different drugs are under investigation to further improve neuroprotection in combination with HT [18]. The most promising ones are xenon [19, 20], erythropoietin [21–23] and melatonin [18, 24], which are currently undergoing preclinical studies, feasibility studies or small randomized controlled studies [21, 25]. Whatever the outcome from combination therapy, it is important to further optimize HT used as the sole intervention.

In conclusion we have shown that starting cooling before 3 h after birth significantly improves motor outcome in newborn term infants cooled after perinatal asphyxia and early cooling was not associated with cardiovascular adverse effects. Cooling should be initiated as soon as possible after birth in eligible infants.


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## Disclosure Statement

We declare no conflicts of interest.

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