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# The Effect of Therapeutic Hypothermia On Heart Rate Variability

**Zachary A. Vesoulis**[1], **Rakesh Rao**[1], **Shamik B. Trivedi**[1], and **Amit M. Mathur**[1]

[1]Division of Newborn Medicine, Edward Mallinckrodt Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri

## Abstract

**Objective—**Heart rate variability (HRV) reflects integrity of the autonomic nervous system. However, no study has investigated the impact of therapeutic hypothermia (TH) on HRV measures in infants with hypoxic-ischemic encephalopathy (HIE). In this study, we evaluate the influence of temperature on measures of HRV for a group of infants with favorable outcomes, as compared to a control group of infants with unfavorable outcomes.

**Study Design—**Term-born infants with moderate-severe HIE underwent standard TH treatment and prospective EEG and ECG recording. Infants with favorable outcome (no seizures, normal/mild EEG scores at 96h, no MRI brain injury, and normal neurodevelopmental scores at 18–24 months) were matched on gestational age, sex and worst encephalopathy score to a group of infants with unfavorable outcomes

Time and frequency domain HRV measures were calculated from 60 min of ECG data obtained at three time points: 24h (hypothermia), 48h (hypothermia), and 96h (normothermia). The effect of time and temperature were evaluated using repeated-measures ANOVA.

**Results—**Sixteen infants were included (8 favorable, 8 unfavorable). For both groups of infants, an increase in the HR, RR and HF power was associated with an increase in temperature, but was not associated with any other HRV measure. In contrast, measures of HRV increased over time, as encephalopathy decreased, for infants with favorable outcomes (reflecting increased cortical-autonomic integration), but not for those with unfavorable outcomes.

**Conclusion—**In general, the effect of hypothermia on measures of HRV is limited to changes in heart rate (bradycardia) and respiratory rate, as opposed to changes in true variability. This supports the hypothesis that persistent changes in HRV are driven by the underlying brain injury and not by the process of TH.

### Keywords

hypoxic-ischemic encephalopathy; therapeutic hypothermia; heart rate variability; outcomes



**Corresponding author:** Zachary A. Vesoulis, MD, 1 Children's Place, St. Louis, MO USA 63100, Phone: 314-286-1524, vesoulis_z@kids.wustl.edu.

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## INTRODUCTION

Heart rate variability (HRV) is the study of beat-to-beat variations in the HR and is a reflection of the integration of the cortical input into the autonomic nervous system.[1] A decrease in variability is thought to reflect decreased vagal input to the sinoatrial node as a result of brain injury. With a worldwide incidence between 1 and 6 out of 1,000 live births, one of most common causes of brain injury in the neonatal period is hypoxic-ischemic encephalopathy (HIE), the result of interrupted placental-fetal circulation for any number of reasons.[2] Infants with HIE have been treated with mild hypothermia (33–34° C) in a number of randomized trials[3,4] with the conclusion that there is decrease in the incidence of mortality (RR 0.75, 95% CI 0.64 to 0.88) and neurodevelopmental disability in survivors (RR 0.77, 95% CI 0.63 to 0.94) for infants with moderate/severe encephalopathy.[5]

Overall, there is limited, and conflicting, human and animal data regarding hypothermia-associated HRV changes, with the finding of preserved HRV parameters in adult humans[6] and pigs[7] and altered HRV characteristics in rats[8,9], although there are challenges extrapolating the rat model to humans.[10] While HRV has undergone limited investigation in infants with HIE as a biomarker for brain injury[11,12], the effect of hypothermia alone on HRV in this population has not been previously reported. In this study, we present the longitudinal HRV characteristics of a carefully selected cohort of infants with normal developmental outcomes in order to delineate the specific effect of therapeutic hypothermia.

## METHODS

### Study design

Infants with moderate-severe HIE were prospectively recruited from the Neonatal Intensive Care Unit (NICU) at St. Louis Children's Hospital for a seizure study (NCT identifier: NCT01027715), the primary outcome of which has been reported elsewhere.[13] All infants underwent a standard whole-body TH protocol which includes 72 hours of mild hypothermia at 33.5°C, continuous video-electroencephalography (cEEG) monitoring with simultaneous amplitude-integrated EEG (aEEG) monitoring, at least one non-sedated brain MRI in the first 14 days of life (scored by a reader blinded to clinical course), and neurodevelopmental testing by a trained and blinded psychologist using the Bayley Scales of Infant Development, Third Edition (BSID-III) at 18–24 months. A comprehensive set of clinical variables was collected including sex, race, arterial cord blood gas parameters, Apgar scores, worst encephalopathy score at cooling (using a modified version of the Sarnat system[14]), need for invasive ventilation, and medication administration. Core body temperature at each time point of interest was recorded using a digital rectal temperature probe. The study protocol was approved by the Washington University Human Research Protection Office.

### EEG recording and analysis

All neonates underwent continuous video-EEG monitoring in accordance with the international 10–20 system of electrode placement using the Stellate Harmonie EEG monitor (Natus Medical Inc, San Carlos, CA). Scalp electrodes (Grass Gold Disc, Natus Medical

Inc., San Carlos, CA) were placed at Fp1, Fp2, F3, F4, Fz, C3, C4, Cz, P3, P4, Pz, T7 (T3), T8 (T4), P7 (T5), P8 (T6), O1, and O2 locations to record EEG activity from frontal, central, parietal, temporal, and occipital areas, respectively. cEEG recording started at the time of study enrollment and continued until rewarming was complete.

cEEG recordings were reviewed for seizures (as defined by Scher et al.[15]) by a pediatric neurophysiologist who was blinded to clinical course. An EEG grade was assigned using a 60-minute period of data at each time point (24, 48, and 96 hours, respectively) using the system described by Murray et al. [16] In this system, a grade between 0 and 4 is assigned based on the background pattern of the EEG. As the EEG pattern progressively deteriorates (loss of synchrony, voltage depression, long periods without activity [interburst interval], loss of sleep-wake cycling), a progressively higher EEG score is assigned, going from essentially normal (score of 0) to a flat trace (score of 4).

## ECG recording

The digital electrocardiogram (ECG) wave output from the patient monitor (Intellivue MP70, Philips Medical, Andover, MA) was sampled at 125-Hz and prospectively collected and archived in a physiology research database (BedMaster Ex, Excel Communications, Jupiter, FL) for offline research purposes.

## Patient selection

Although all infants who have significant enough encephalopathy to warrant admission to the NICU are not healthy by nature, we selected a group of infants from a larger cohort enrolled in a clinical trial[13,17] who initially had moderate-severe encephalopathy but recovered clinically by 48 hours of age, suggesting a favorable prognosis, thereby allowing the possibility of examining the impact of hypothermia and not brain injury on HRV. This favorable outcome was stringently defined as no clinical or electrographic seizures at any time during hospitalization, EEG grade 0 or 1 (representing normal or normal/mild abnormalities) at 96 hours, no injury on MRI and a favorable neurodevelopment status (BSID-III composite scores no more than one standard deviation below the mean (≥ 85) in all domains). A comparison group of injured infants with unfavorable outcomes (defined as presence of seizures, MRI injury and/or neurodevelopmental impairment as measured on the BSID-III composite scores more than one standard deviation below the mean (< 85) in all domains) was derived from the same cohort, matching on gestational age, sex and worst encephalopathy score.

## HRV analysis

In order to capture the impact of hypothermia, HRV characteristics were calculated at three time points: twice during hypothermia (24h and 48h) and once during normothermia (96h). HRV analysis was conducted using Kubios HRV v2.2 (Department of Physics, University of Kuopio, Finland). Three hours of raw ECG data were loaded at each time point and underwent automated R-wave identification. Manual correction was used to remove erroneously detected beats. The resulting R-R tachogram (continuous plot of the interval between successive R-wave) was normalized using cubic spline interpolation in order to allow for spectral calculation without distortion (referred to as the N-N tachogram). Slow

nonstationarities were removed using first order linear detrending. A contiguous sixty-minute segment of the detrended, normalized, error-corrected data was then selected for further analysis in both the time- and frequency-domain.

The time and frequency domain analyses are best thought of as complimentary tools, providing insight into the underlying patterns of heart rate variability. While the time-domain methods evaluate how the signal changes with time, the frequency-domain methods examine how the signal's energy is distributed over a range of frequencies. The primary method for converting a signal from the time-domain (beat-to-beat intervals), in which the ECG is traditionally recorded, to the frequency domain is the Fourier Transform (FT), first described in the 19$^{th}$ century by Jean Baptiste Joseph Fourier. This mathematical approach decomposes a complex, periodic signal (sine and cosine waves) into individual signals at different frequencies, allowing one to measure the strength or power of the signal in discrete frequency bands, also called the power spectrum estimate. An updated version of the Fourier Transform, called the Fast Fourier transform (FFT), was described in 1967 by Peter Welch[18] and improved estimation of the power spectrum by averaging the Fourier Transform over small, overlapping sections of the signal. This methodology has been implemented in a number of software packages and forms the mainstay of digital signal processing.

Three different time domain measures were calculated: the mean interval (mean NN), the standard deviation of the NN interval (SDNN) and the triangular index of the NN interval (TINN). The mean NN and SDNN represent the mean and standard deviation of length of time between normalized heart beats over the 60-minute study period respectively, providing a composite of short and long-term variability. The TINN is a geometric measure of HRV, defined as the length of the base of the triangle defining the sample distribution function. Similar to mean NN and SDNN, it represents overall variability and is relatively insensitive to noise and artifact.

Four different frequency domain measures were calculated: the spectral power (the strength of oscillations) in three bands: (a) very-low frequency (VLF − 0.0033–0.04 Hz, parasympathetic activity), (b) low frequency (LF − 0.04–0.5 Hz, sympathetic activity) and (c) high frequency (HF − 0.5–1.3 Hz, vagal activity) as well as the LF/HF ratio. The total spectral power was calculated using Welch's modified power spectral estimate, which estimates the power spectrum by time-averaging the fast Fourier Transformation of serial, 256-second data segments, with each segment overlapping the previous by 50%. An ensemble average was taken of the resulting spectral density estimates and the power was calculated for each of the defined bands.

## Statistical approach

Given the small sample size and the low likelihood of normally distributed results, univariate comparisons were made using non-parametric tests; Fisher's Exact Test for categorical variables and the Wilcoxon-Mann-Whitney test for continuous variables. The effect of time and temperature on each measure of HRV, heart rate, and respiratory rate was assessed by use of repeated-measures analysis of variance (ANOVA). Analysis was conducted at fixed time points for each subject: 24, 48 and 96 hours of life in order capture the effect of time and temperature. Statistical analysis was conducted using R version 3.2.3 (R Project for

Statistical Computing, Vienna, Austria). Tests were considered statistically significant where $p < 0.05$.

## RESULTS

### Clinical characteristics

Of the sixty-three infants initially recruited for the study, 8/63 (13%) met the defined inclusion criteria for favorable outcome and were matched with eight infants with an "unfavorable" outcome. In general, the two groups were statistically similar at admission. As would be expected, the injured group had a greater proportion of seizures, evidence of MRI injury, and had lower BSID-III scores across all three domains. The full set of clinical characteristics is outlined in Table 1.

### Longitudinal characteristics

Target temperature was achieved at all time points in both groups. During HRV data collection, the infants had measured core temperatures between 32.9 and 33.7° C at hypothermic time points and measured core temperatures between 36.0 and 37.1° C at the normothermic time point. Given the incidence of seizures, it is not surprising that infants in the unfavorable group received more anti-epileptic medications. Infants in the favorable cohort initially demonstrated signs of cerebral dysfunction (as evidenced by a median EEG score of 3 at 24 hours of life) which improved by 48 hours and normalized by 96 hours of life. The unfavorable group displayed a more heterogeneous pattern; some infants demonstrated a normal EEG score by 96 hours of life, while others still demonstrated signs of severe abnormality. A complete listing of longitudinal characteristics is listed in Table 2.

### HRV measures

For infants with favorable outcomes, there was a statistically significant difference between the three time points (24, 48 and 96h) on all time and frequency domain measures of HRV, with a decreased mean NN interval ($p<0.04$), increased SDNN ($p<0.05$), increased TINN ($p<0.01$) and increased spectral power across all three bands. There was a statistically significant difference between hypothermia and normothermia on the heart rate ($p=0.01$), respiratory rate ($p<0.01$), mean NN interval ($p<0.01$), and spectral power in the high frequency band ($p=0.04$) but not the other time or frequency domain measures of HRV.

For infants with unfavorable outcomes, there was not a statistically significant effect of time on any time or frequency domain measures of HRV. There was a significant effect of temperature on heart rate ($p<0.01$) and mean NN interval ($p=0.04$) but not on any other measures. HRV characteristic values and the results of repeated-measures ANOVA analysis are shown in Table 3.

## DISCUSSION

The data from this cohort of infants with moderate-severe HIE treated with TH who had a favorable outcome demonstrate findings in line with expectations, namely that temporary, reversible encephalopathy, but not hypothermia, drives changes in measures of heart rate

variability. This stands in contrast to the group of infants who demonstrated signs of injury and lacked the same evolution in HRV measures over the same time period. As shown in Table 3, HRV parameters increase between 24 and 48 hours for infants with favorable outcomes to similar values measured during normothermia. This change is contemporaneous with improvement in EEG grade and likely reflects increased influence of the CNS in the autonomic nervous system. This finding has a parallel to those of Thoresen et al.[19] and Murrary et al.[16] who demonstrated normalization of aEEG or EEG measures by 48h in infants with a similarly defined favorable outcomes.

Three HRV parameters were affected by hypothermia: mean NN interval, HF power and the LF/HF ratio. The decrease in the mean NN interval is likely the result of the faster HR following rewarming as compared to during hypothermia (a result of an increased basal metabolic rate); the HR remained consistently around 100 beats per minute during hypothermia, increasing by 20% following return to normothermia (Table 3). Bradycardia during TH treatment has previously been associated with better neurologic outcomes[20], possibly a reflection of adequate cerebral perfusion in the setting of nominal metabolic demand (as opposed to the increased metabolism which accompanies injury), and was not surprising to see in this carefully selected cohort.

The increase in HF power (and decrease in LF power) following rewarming at 96 hours, is likely the result of an increased respiratory rate, also increasing nearly 20%, from about 30 breaths per minute to 40 breaths per minute. This increase is likely driven by the increase in cellular respiration with return to normothermia following TH. A respiratory rate of 28–32 corresponds to a respiratory signal in the ECG trace generally below 0.5 Hz while a rate of 37–43 corresponds to a signal above 0.5 Hz, likely causing the shift in spectral power from the LF bin to the HF bin and decreasing the LF/HF ratio in the process.

## Clinical relevance

This finding has important implications for the study of HRV as a predictor of outcome in the setting of hypothermia. There is little doubt that these are critically ill infants who displayed clinical and electrographic signs of cerebral dysfunction, which in turn influenced their heart rate variability. However, other than the decreased heart and respiratory rate, there is no sustained effect of hypothermia on measures of HRV for infants with favorable outcomes. When longitudinal datasets of infants with varying degrees of injury are compared, our data support the notion that differences in HRV values that persist after 48 hours of life are due to cerebral injury or dysfunction and are not a function of hypothermia itself.

In this sense, HRV offers the potential to be a simple, non-invasive, continuous metric providing insight into CNS function. There are a number of possible applications in which this could be used (e.g. during transport or re-warming) when conventional EEG is not possible, feasible or cost-effective. Confidence that hypothermia is not introducing a confounding effect bolsters the application of this approach and increases generalizability.

### Limitations

This study is most significantly limited by the small sample size. Given the strict inclusion criteria in the favorable outcome group (no seizures, normal/mild EEG scores at 96h, no MRI brain injury, and normal neurodevelopmental scores at 18–24 months) a relatively small proportion of the original infants (16/53, 30%) could be included. However, loosening the inclusion criteria would have made it more difficult to distinguish changes in HRV resulting from hypothermia or from brain injury and thereby possibly invalidating the results.

### Conclusion

In infants with moderate-severe HIE, no evidence of brain injury, and normal neurodevelopmental outcomes, HRV parameters measured at 48 hours of life were similar to those measured after rewarming at 96 hours. This supports the hypothesis that differences in HRV that persist beyond 48 hours of life are driven by the underlying brain injury and not by the process of TH. Future studies should focus on validation of these results and further development of HRV as a prognostic tool for outcomes in this population. Availability of this data at the bedside could provide additional information to care providers and family members when goals of care are being discussed.

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## Table 1

Clinical characteristics

| | Favorable N=8 | Unfavorable N=8 | P value |
|---|---|---|---|
| **Gestational age, mean (SD)**, weeks | 38.1 (1.6) | 38.4 (1.9) | 0.71 |
| **Male sex**, n (%) | 5 (63) | 5 (63) | 1.0 |
| **Race**, n (%) | | | |
| Caucasian | 5 (63) | 4 (50) | 1.0 |
| African-American | 3 (37) | 4 (50) | |
| **Arterial cord blood gas pH**, mean (SD) | 7.01 (0.16) | 7.16 (0.19) | 0.07 |
| **Arterial cord blood gas base excess**, mean (SD), mEq/L | −13.19 (7.1) | −10.19 (9.06) | 0.27 |
| **5-minute Apgar score**, median (min-max) | 3.5 (1–9) | 4.5 (1–7) | 0.74 |
| **10-minute Apgar score**, median (min-max) | 4 (1–7) | 5 (1–8) | 0.79 |
| **Worst encephalopathy score**, n(%) | | | |
| Mild | 0 (0) | 0 (0) | 1.0 |
| Moderate | 7 (88) | 6 (75) | |
| Severe | 1 (12) | 2 (25) | |
| **MRI Injury Score**, n (%) | | | |
| No injury | 8 (100) | 4 (50) | 0.07 |
| Mild injury | 0 (0) | 1 (13) | |
| Moderate injury | 0 (0) | 3 (38) | |
| Severe injury | 0 (0) | 0 (0) | |
| **Electrographic seizure**, n (%) | 0 (0) | 3 (38) | 0.47 |
| **Neurodevelopmental outcomes** | | | |
| Cognitive, mean (SD) | 96.8 (10.7) | 83.7 (13.6) | 0.08 |
| Motor, mean (SD) | 95.6 (9.4) | 82.8 (17.8) | 0.10 |
| Language, mean (SD) | 97.3 (14.5) | 88.9 (19.6) | 0.5 |

Univariate comparisons made using Fisher's Exact Test for categorical variables and the Wilcoxon-Mann-Whitney test for continuous variables.

**Table 2**

Longitudinal characteristics

| | Hypothermia (24h) | | | Hypothermia (48h) | | | Normothermia (96h) | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Favorable** | **Unfavorable** | **P value** | **Favorable** | **Unfavorable** | **P value** | **Favorable** | **Unfavorable** | **P value** |
| Core body temperature, median (range), °C | 33.5 (33.4–33.7) | 33.5 (32.9–33.7( | 0.91 | 33.4 (33.3–33.5) | 33.4 (33.3–33.5) | 0.95 | 36.1 (36.0–37.2 | 36.2 (36.0–37.1) | 0.79 |
| Invasive ventilation, n (%) | 6 (75) | 6 (75) | 1.00 | 2 (25) | 4 (50) | 0.61 | 2 (25) | 4 (50) | 0.61 |
| Inotropic support, n (%) | 2 (25) | 2 (25) | 1.00 | 2 (25) | 3 (38) | 1.00 | 0 (0) | 1 (13) | 1.00 |
| Morphine, median (range), mcg/kg/hr | 5 (5–10) | 5 (5–20) | 0.24 | 5 (0–5) | 5 (0–20) | 0.33 | 0 (0–5) | 0 (0–10) | 0.75 |
| EEG grade, median (range) | 2.5 (0–3) | 3 (2–4) | 0.09 | 1 (0–3) | 2.5 (1–4) | 0.12 | 0 (0–1) | 1 (0–4) | <0.01 * |
| Anti-epileptic medications, n (%) | 0 (0) | 3 (38) | 0.20 | 0 (0) | 2 (25) | 0.47 | 0 (0) | 2 (25) | 0.47 |

Temperature and EEG grade data are based on measurements taken at each time point. Categorical data represents the cumulative incidence over the preceding 24h period. Univariate comparisons made using Fisher's Exact Test for categorical variables and the Wilcoxon-Mann-Whitney test for continuous variables.

* denotes statistical significance at $p < 0.05$.

## Table 3

HRV characteristics by time period

| Favorable outcome (n=8) | | | | | |
|---|---|---|---|---|---|
| | **Hypothermia (24h)** | **Hypothermia (48h)** | **Normothermia (96h)** | **Effect of time, P value** | **Effect of temperature, P value** |
| **Mean NN**, mean (SEM), ms | 584.1 (22.2) | 546.9 (20.6) | 483.59 (17.54) | 0.04 * | <0.01 * |
| **SDNN**, mean (SEM), ms | 18.7 (4.4) | 33.83 (5.1) | 32.2 (5.0) | 0.05 * | 0.17 |
| **TINN**, mean (SEM), ms | 113.8 (25.5) | 198.1 (27.1) | 224.4 (40.9) | <0.01 * | 0.10 |
| **VLF power**, mean (SEM), $ms^2$ | 142.7 (60.7) | 443.1 (130.6) | 412.1 (124.7) | 0.03 * | 0.42 |
| **LF power**, mean (SEM), ($ms^2$) | 103.3 (51.5) | 333.3 (94.9) | 397.1 (85.12) | 0.03 * | 0.11 |
| **HF power**, mean (SEM), ($ms^2$) | 13.45 (8.9) | 25.9 (7.7) | 107.7 (37.1) | 0.01 * | 0.04 * |
| **LF/HF ratio**, mean (SEM), | 12.8 (5.8) | 16.6 (5.94) | 6.9 (2.2) | 0.39 | 0.02 * |
| **Heart rate**, mean (SEM), beats per min | 103.0 (6.2) | 104.9 (5.6) | 124.3 (4.8) | 0.10 | 0.01 * |
| **Respiratory rate**, mean (SEM), breaths per min | 34.7 (3.8) | 32.4 (2.4) | 41.9 (1.5) | 0.06 | <0.01 * |

| Unfavorable (n=8) | | | | | |
|---|---|---|---|---|---|
| | **Hypothermia (24h)** | **Hypothermia (48h)** | **Normothermia (96h)** | **Effect of time, P value** | **Effect of temperature, P value** |
| **Mean NN**, mean (SEM), ms | 633.66 (30.2) | 605.9 (27.6) | 493.2 (21.3) | 0.55 | 0.05 |
| **SDNN**, mean (SEM), ms | 23.55 (2.6) | 30.15 (8.0) | 32.7 (6.15) | 0.66 | 0.44 |
| **TINN**, mean (SEM), ms | 161.3 (11.4) | 186.9 (43.8) | 206.9 (32.2) | 0.07 | 0.08 |
| **VLF power**, mean (SEM), $ms^2$ | 139.8 (28.7) | 312.8 (108.5) | 385.9 (131.6) | 0.22 | 0.28 |
| **LF power**, mean (SEM), ($ms^2$) | 218.6 (90.83) | 509.1 (270.9) | 469.7 (156.3) | 0.51 | 0.66 |
| **HF power**, mean (SEM), ($ms^2$) | 16.5 (8.3) | 51.4 (22.3) | 86.1 (30.6) | 0.11 | 0.15 |
| **LF/HF ratio**, mean (SEM), | 16.1 (5.4) | 11.8 (2.9) | 10.1 (2.9) | 0.55 | 0.43 |
| **Heart rate**, mean (SEM), beats per min | 99.1 (5.4) | 100.3 (4.9) | 123.3 (4.8) | 0.15 | <0.01 * |
| **Respiratory rate**, mean (SEM), breaths per min | 31.3 (3.7) | 37 (4.3) | 38.8 (2.9) | 0.15 | 0.35 |

* denotes statistical significance at $p < 0.05$.