The “Neurovascular Unit approach” to Evaluate Mechanisms of Dysfunctional Autoregulation in Asphyxiated Newborns in the era of Hypothermia Therapy

Lina F. Chalak, MD¹,*, Takashi Tarumi, Ph.D., M.A², and Rong Zhang, PHD MSCS.²

¹Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX
²Department of internal medicine at University of Texas Southwestern Medical Center, Dallas, TX

Abstract

Despite improvements in obstetrical and neonatal care, and introduction of hypothermia as a neuroprotective therapy, perinatal brain injury remains a frequent cause of cerebral palsy, mental retardation and epilepsy. The recognition of dysfunction of cerebral autoregulation is essential for a real time measure of efficacy to identify those who are at highest risk for brain injury.

This article will focus on the “neurovascular unit” approach to the care of asphyxiated neonates to review 1) potential mechanisms of dysfunctional cerebral blood flow (CBF) regulation, 2) optimal monitoring methodology such as NIRS (near infrared spectroscopy), and TCD (transcutaneous Doppler), and 3) clinical implications of monitoring in the neonatal intensive care setting in asphyxiated newborns undergoing hypothermia and rewarming.

Critical knowledge of the functional regulation of the neurovascular unit may lead to improved ability to predict outcomes in real time during hypothermia, as well as differentiate nonresponders who might benefit from additional therapies.

Keywords

HIE; hypothermia; rewarming; neurodevelopmental outcomes

Neonatal hypoxic-ischemic encephalopathy (HIE) secondary to birth asphyxia remains a major public health problem that afflicts millions of newborns worldwide and may result in cerebral palsy, mental retardation, learning disabilities, and even death.¹,² Impaired cerebral blood flow is the principal culprit leading to brain injury and is likely to occur as a consequence of interruption of placental blood flow and gas exchange; a state that we will...
refer to as asphyxia to avoid the terminology debates of hypoxic ischemic injury (HIE) and neonatal encephalopathy (NE). The first section of the review will focus on the physiology of cerebral autoregulation in the infant born at term in health and in the presence of HIE. The second section of the review covers the methodology, as well as clinical applications of neuromonitoring in relation to autoregulation and outcomes.

I. Mechanisms of cerebral autoregulation in the neurovascular unit

A successful transition from the fetal to the neonatal environment

CBF increases with postnatal age in parallel with increased cerebral metabolic rates and energy demands in the growing brain. There is tight coupling of brain function, metabolism and blood flow during transition from fetal to neonatal life to support normal development. Autoregulation of CBF refers to maintenance of constant flow over a broad range of cerebral perfusion pressures (CPP) ranging from 25-50 mmHg in newborns.

At term neonatal CBF is 10-20ml/100gm/min and represents approximately 40% of adult values. The fetus has low oxygen tension but compensatory elevations in Hgb F, cardiac output (CO), hematocrits and 2-3DPG which promotes release of oxygen to tissue and offset the low PO$_2$ of the fetus. The most dramatic change in CBF occurs at the time of birth. In the lamb, three fold decrease in CBF occurs in the first 24 hours after birth correlating with increased oxygen content.

What regulates cerebral blood flow?

The “neurovascular unit” consists of specialized endothelial cells interconnected by an elaborate network of complex tight junctions surrounded by basal lamina, astrocytes and neurons. The astrocytes that surround the microvasculature provide the cellular link to the neurons and play an active role in signal transduction pathways and regulating the blood brain barrier.

The endothelium produces several vasoactive factors that regulate CBF, including nitric oxide (NO: endothelium-dependent hyperpolarization factor), eicosanoids, and endothelins. Cerebral autoregulation occurs through arteriolar vasoconstriction with increases, and vasodilatation with decreases in CPP. The stimulus for autoregulatory change in vascular diameter appears to be mediated by endothelial derived signals through NO and/or prostaglandins, and calcium activated K$^+$ channels vasodilation, and endothelin-1 promoting vasoconstriction.

The control of CBF is complex and requires involvement of every single component of the neurovascular unit to accomplish the following:

1. Cerebral autoregulation which maintains constant flow in response to change in CPP.
2. Flow-metabolism coupling which regulates blood flow to match metabolic activity.

_Early Hum Dev. Author manuscript; available in PMC 2015 October 01._
Initial adaptive responses to an asphyxia injury

Asphyxia starts with a fetal insult due to impaired CBF as a consequence of a substantial interruption of maternal and/or fetal placental blood flow and gas exchange. The timing, severity, pattern and duration of the fetal insult as well as the degree of recovery via fetal adaptive mechanisms determine the spectrum of disease, outcomes and possibly the responses to therapy. The fetal circulatory response to hypoxemia and asphyxia is a rapid centralization and autoregulation of blood flow in favor of the vital organs: brain, heart and adrenals, at the expense of almost all other peripheral organs. The latter contributes to multiple organ dysfunction which is considered an integral part of HIE and likely precedes significant brain effects. At the cellular level, the initial reduction in CBF and oxygen delivery initiates a cascade of deleterious biochemical events resulting in a switch to anaerobic metabolism, and ultimately energy failure with depletion of high energy phosphorylated compounds such as ATP and phosphocreatinine. The immediate reperfusion period is demarcated with return of CBF and is characterized by a normal BP and acid base status. More importantly, reperfusion injury occurs with a later increase in CBF between 12 and 24 h, which can last hours to days and is associated with secondary energy failure and final cell death occurs.

Mechanisms of Autoregulation with perinatal asphyxia

The adaptive redistribution of blood flow assures a larger proportion of cardiac output to the brain leading to an initial increase in CBF. This is subsequently followed by exhausted cerebral autoregulation due to final decrease in cardiac output and CPP. Figure.1 presents the hypothetical mechanism by which ensuing hypoxia, hypercapnia and acidosis could lead to loss of autoregulation and possible improvement following hypothermia therapy in patients with good outcomes.

Impaired autoregulation and reperfusion injury in HIE

Neonatal animal studies have confirmed impaired cerebral autoregulation in the face of hypoxia, hypercarbia and acidosis, all of which are integral components of neonatal HIE. Indeed, a linear relationship between CBF and systolic blood pressure was first reported in asphyxiated newborns using Xenon clearance studies. This suggests that changes in arterial pressure are passively transmitted into the maximally dilated cerebral circulation. Specifically, asphyxiated newborns with the most severe brain damage (death and isoelectric EEG) had the highest CBF, loss of autoregulation in response to blood pressure, and impaired vasomotor reactivity to carbon dioxide stimuli. These observations of cerebrovascular dysfunction associated with hyperperfusion have been attributed to lactate accumulation during secondary energy failure causing maximum vasodilation.

Hypothermia therapy and the re-warming challenge

Hypothermia therapy provides neuroprotection via multiple pathways, including the reduction in cerebral metabolism and CBF. The need for a better understanding of cerebrovascular function during re-warming is underscored by studies demonstrating that
exposure to hyperthermia increases neuronal injury.\textsuperscript{36,37} Moreover, brain injury may occur even with modest, brief increases in brain temperature.\textsuperscript{38-40,35,41,42} Rapid re-warming can result in transient uncoupling of cerebral circulation and metabolism with a transient increase in extracellular glutamate and lactate as demonstrated by cardiac bypass studies with 1 hour duration of hypothermia.\textsuperscript{43} Temperature increments are associated with increased heart rate, cardiac output and altered hemoglobin-oxygen affinity that could result in a mismatch between oxygen delivery and consumption\textsuperscript{44} and between CBF and metabolic requirements during re-warming.\textsuperscript{45} For instance, rapid re-warming can exacerbate traumatic axonal injury and impair the cerebrovascular autoregulatory response.\textsuperscript{46,47} Notably, increased metabolic demands and seizures have been described in hypothermic cardiac bypass surgery.\textsuperscript{48} Neonatal clinical trials have avoided rapid re-warming described in surgical and cardiac patients, and have all adopted an empirical 0.5°C/hour rate of re-warming. It is still possible however, that an impaired autoregulatory capacity induced by the severity of the primary insult could also cause more injury at a later stage due to hemodynamic changes in CBF and metabolic demands leading to seizures during the re-warming phase.

In summary, the regulation of CBF is complex and relies on the integrity the neurovascular unit to provide cerebral pressure autoregulation and blood flow-metabolism. The asphyxial injury with acidosis, hypoxia and hypercarbia, as well as the increased metabolic demands during rewarming, which all lead to a varying disturbances in this homeostatic system.

\section*{II. Methodology of Monitoring the “Neuro-Vascular Unit” in an NICU setting}

Despite an era of medical and technological advances, fundamental gaps of knowledge remain such as lack of accessible tools for clinicians to easily determine which infants have intact CBF regulation. In fact, most of the technology reviewed below is limited to research settings.

\textbf{Transcranial Doppler Ultrasonography (TCD)}

TCD provides continuous measurements of CBF velocity in the basal cerebral arteries including the middle cerebral artery (MCA), anterior cerebral artery (ACA) and the posterior cerebral artery (PCA). Changes in CBF velocity represent changes in volumetric CBF if the diameters of the insonated arteries remain relatively constant. CBF velocity waveforms are displayed in real-time and can be used to obtain beat-to-beat changes in systolic, diastolic, and mean CBF velocity. TCD is non-invasive and therefore can be a useful tool to monitor cerebral hemodynamics in newborns with HIE undergoing hypothermia and re-warming.

Using this approach, cerebral autoregulation can be assessed during spontaneous changes in arterial pressure and is referred to as dynamic cerebral autoregulation.\textsuperscript{49} Typically, dynamic cerebral autoregulation is quantified using a transfer function method between changes in arterial pressure and CBF velocity.\textsuperscript{50-52} The metrics of the estimated transfer function gain (the amplitude relationship between changes in arterial pressure and CBF velocity), phase (the temporal relationship between changes in arterial pressure and CBF velocity), and the coherence (the linear correlation between changes in arterial pressure and CBF velocity)
have been used to quantify cerebral autoregulation. More specifically, increases in transfer function gain and reduction in phase have been suggested to indicate impaired autoregulation and vice versa. It also has been shown that dynamic autoregulation is likely most effective at the low frequencies of changes in arterial pressure in the range between 0.002 to 0.20 Hz. Dynamic autoregulation also can be assessed in the time domain using correlation analysis between changes in arterial pressure and CBF velocity. Impaired autoregulation has been observed in the newborns with HIE using invasive PET studies.

One of the major limitations of using Doppler CBF velocity as a proxy of CBF is when the arterial cross sectional area is dynamically regulated such as during seizures when changes in blood pressure are associated with enhanced sympathetic reactivity. In addition, assessment of dynamic cerebral autoregulation using the transfer function method may be appropriate only if changes cerebral hemodynamics are stationary (i.e., time-invariant) under steady-state conditions, while physiologic changes will lead to changes in vascular diameter in most clinical scenarios. Furthermore, the intrinsic nonlinear properties of the cerebral circulation may challenge the validity of transfer function methods.

**Near Infrared Spectroscopy (NIRS)**

NIRS is a non-invasive tool that can be used to measure changes in oxygenated (HbO₂), deoxygenated (Hb), and total hemoglobin (HbT) of brain tissue. Recently developed spatially resolved near infrared spectroscopy also can be used for bedside monitoring brain tissue oxygenation index (TOI) or regional tissue O₂ saturation (rSO₂). TOI or rSO₂ measures the mixed arterial, capillary, and venous O₂ saturation. This constitutes an estimation of changes in cerebral tissue oxygenation (venous 75%, capillary 5%, arterial 20%). The value under physiological conditions, range between 65-80% in newborns. The measurement of TOI or rSO₂ provides indirect measures of CBF under conditions of stable arterial oxygen saturation (SaO₂). In addition, NIRS measurement of HbT can be used to estimate changes in cerebral blood volume (ΔCBV = ΔHbO₂ + ΔHb) Good agreement has been reported between NIRS and TCD autoregulation.

Fractional tissue O₂ extraction (FTOE = (SaO₂ - TOI)/SaO₂) can be used to reflect brain tissue oxygen utilization which is influenced by CBF and SaO₂ and normal reference ranges between 0.2-0.3 in newborns. A normal FTOE suggests an intact coupling between CBF and brain metabolic needs. During restricted blood flow, increases in FTOE are expected to occur to compensate for potential reduction in TOI. Conversely in the presence of constant oxygen delivery, a decrease of FTOE suggests decreased oxygen extraction due to less utilization as seen with cell death. In asphyxiated newborns, before the hypothermia era, high rSO₂ and lower FTOE at 24h reflected secondary energy failure and poor outcomes.

TOI or rSO₂ can be affected by arterial saturation, CBF, cerebral blood volume and cerebral oxygen consumption. Therefore, these variables need to be stable in order to for TOI to reflect accurate steady-state measurements of tissue oxygenation or CBF. Furthermore, changes in skin blood flow may contaminate NIRS signal for assessment of brain tissue oxygenation.
Amplitude-integrated (aEEG) as a measure of neuronal integrity

aEEG is a simple, non-invasive bedside tool that permits continuous evaluation of cortical electrical activity widely used since 1969 and has been extensively reviewed in the neonatal literature. Studies of aEEG by our group as well as by others have reported the usefulness of using aEEG to predict outcomes and detection of subclinical seizures in newborns with HIE. The aEEG therefore can provide quantitative as well as qualitative assessment of brain electrical activity during different phases of hypothermia or rewarming. Together with the TCD and NIRS monitoring of cerebral hemodynamics, aEEG can be used as a useful marker to examine the integrity of the neurovascular unit in newborns with HIE. Complete absence of background cortical electrical activity has been reported when CBF falls below 7ml/100g/min (i.e., ≈50% of normal resting CBF).

III. Clinical applications in newborns undergoing hypothermia & re-warming

A continuous neuromonitoring protocol for newborns with HIE started in 2010 at Parkland hospital in Dallas to determine dynamic autoregulation and to evaluate responses to hypothermia and re-warming. This protocol includes monitoring with a digital data acquisition system (Vital Sync System, Somanetics) that allows input from all bedside monitoring tools, including pulse oximetry, blood pressure, heart rate and NIRS neuromonitoring as well as aEEG qualitative and quantitative analysis using the Brain AnalyZe Research software, which exports the raw data and calculates minute to minute average values for the maximum and minimum electrical activity. Illustrative cases are presented below in Figures 2 and 3 depicting clinical scenarios where neuromonitoring provided insights into mechanism of injury and associated outcomes.

Conclusions

Despite improvements in obstetrical and neonatal care and introduction of hypothermia as a neuroprotective therapy, perinatal cerebral hypoxic-ischemic injury remains a frequent cause of cerebral palsy and mental retardation. The recognition of dysfunctional cerebral autoregulation in asphyxiated neonates may identify those who are at highest risk for brain injury and seizures.

Acknowledgments

Funding Dr. Chalak is supported by NIH Grant K23HD069521 and a Gerber Foundation grant.

Abbreviations

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<tr>
<th>Abbreviation</th>
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<tr>
<td>HIE</td>
<td>Hypoxic-ischemic encephalopathy</td>
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<td>MRI</td>
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REFERENCES


Early Hum Dev. Author manuscript; available in PMC 2015 October 01.


**Key guidelines**

- CBF increases with postnatal age in parallel with increased cerebral metabolic rates and energy demands in the growing brain.

- The control of CBF is complex and requires involvement of every single component of the neurovascular unit. There is tight coupling of brain function, metabolism and blood flow during transition from fetal to neonatal life to support normal development.

- The asphyxial injury with acidosis, hypoxia and hypercarbia, as well as higher metabolic demands of rewarming after hypothermia can all lead to disturbances in CBF autoregulation.

- In high risk HIE infants, ongoing neurological monitoring of the neurovascular unit with NIRS, Doppler and AEEG could provide useful prognostic information.

**Research directions:**

- Fundamental gaps of knowledge remain with respect to development of clinical tools to determine an intact CBF regulation or how to quantify the extent of impaired autoregulation.

- We lack evidence as to whether modulation of autoregulation measures can affect outcomes.

- Future studies to identify sensitive biomarkers of cerebrovascular integrity are highly needed and may guide future targeted neuroprotective therapies to optimize outcomes of neonatal care.
Figure 1.
Hypothetical diagram illustrating 1a: the response of cerebral and systemic hemodynamics to short asphyxia with adaptive responses ensuring a stable cerebral blood flow and 1b: the response of cerebral and systemic hemodynamics to sustained asphyxia with both reduced cardiac output and cerebral blood flow and the possible effect of hypothermia.
Figure 2.
Normal regulation of 2a: cerebral oxygen saturation (rSO$_2$), cerebral fractional tissue oxygen extraction (FTOE), mean arterial pressure (MAP) and 2b: amplitude EEG (aEEG) during 6 hours of hypothermia (left) and re-warming (right). Polynomial line fit was used to show the trend of cerebral and systemic hemodynamic variables over time. Patient maintains normal reference ranges of rSO$_2$ (70-85%) and FTOE (25-35%) as well as continuous aEEG with sleep wake cycles during hypothermia and re-warming. His clinical condition was stable with oxygen saturation of 100% on room air, HR (80 bpm), and MAP (35-45 mmHg). This patient had a normal Bayley III outcome >85 at 24 months.
Figure 3.
Impaired regulation of 3a: cerebral oxygen saturation (rSO$_2$), cerebral fractional tissue oxygen extraction (FTOE), mean arterial pressure (MAP) and 3b: amplitude EEG (aEEG) during 6 hours of hypothermia (left) and re-warming (right). Polynomial line fit was used to show the trend of cerebral and systemic hemodynamic variables over time. Note the persistently increased rSO$_2$ of 95% with a concurrent FTOE of <5%. aEEG revealed a pattern of low voltage discontinuous activity with no sleep wake cycles. Infant had seizures four hours after initiation of re-warming (red arrow). This infant had an abnormal outcome (Bayley III at 24 months: cognitive score 65, motor score 82).