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Neuropsychological Outcome following Perinatal HIE: Utility of MR Spectroscopy

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by

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Each person whose signature appears below certifies that this thesis in his/her opinion is adequate, in scope and quality, as a thesis for the degree Master of Arts.

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ABSTRACT OF THE THESIS

Neuropsychological Outcome following Hypoxic-Ischemic Encephalopathy in Neonates: Proton Magnetic Resonance Spectroscopy as Predictor

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Outcome following hypoxic-ischemic encephalopathy (HIE), a neurodegenerative process caused by prolonged asphyxiation during birth, can vary between minimal impairment and cerebral palsy, mental retardation, or death (30%) (McCulloch, Taylor, & Whyte, 1991). Prognosis after HIE is often difficult to establish because traditional predictors, such as Sarnat scores, Apgar scores, and pH, do not always account for adequate variance in outcome. The prognostic utility of MR Spectroscopy in the pathogenesis of asphyxia is promising (Wyatt, 1994). Innovative research indicates that MR Spectroscopy can accurately predict outcome at one year in 91% of neonates with central nervous system injuries (Holshouser et al., 1997). The current study examined neuropsychological functioning following birth asphyxia-related HIE in nine children 3 years 4 months old to 7 years 8 months old (M = 5 years old, SD = 20 months) using ^{1}H-MRS as a predictor of outcome in the following areas: (1) memory and learning; (2) language; (3) visuospatial; (4) attention and executive functioning; and (5) sensorimotor abilities. Results found that both NAA/Cre and NAA/Cho were not correlated with outcome. Elevated Cho/Cre and lactate, however, were the most common finding among more severe HIE, with both metabolites correlated with all outcome areas.

Introduction

Every eight seconds, 1000 babies are born in the United States (Ventura, Martin, Curtin, Menacker, & Hamilton, 2001). A little known fact is that 5 to 6 of those new neonates will experience birth asphyxia that, if prolonged, will result in a destructive neurologic condition known as hypoxic-ischemic encephalopathy (HIE) (Hill & Volpe, 2000; Smith, Wells, & Dodd, 2000; Ventura et al., 2001). This rate corresponds to an estimated 21,780 infants affected annually. While neonatal asphyxiation is associated with a 27% morbidity rate (Hill & Volpe, 2000; Ishikawa, Ogawa, Kanayama, & Wada, 1987); those that do survive have nearly a 50% probability of permanent cortical visual impairment and a 30% probability of developing cerebral palsy, mental retardation, and or seizure disorder (Hill & Volpe, 2000; McCulloch, Taylor, & Whyte, 1991). A more covert but equally troubling finding indicates that, based on the small amount of long-term research currently available, perhaps as many as 50% of these children with moderate HIE will also experience some type of school-related delay (C. Robertson & Grace, 1992).

The key to treating and preventing the adverse effects of asphyxia and hypoxic-ischemic encephalopathy is found through early identification of a neonate at risk for later neurodevelopmental disability (Hill & Volpe, 2000). Providing information and educational materials to parents regarding special neuropsychological problems, services and outcome is the responsibility of both psychologists and physicians. If early intervention is to be provided for these babies, a good method for identifying and assessing the potential level of severity must be implemented.

The physical sciences and medical sciences are just beginning to merge forces in discovering a non-invasive method that may shed light on the underlying mechanisms driving the life threatening neurodevelomental sequelae of birth asphyixia (Wyatt, Edwards, Azzopardi, & Reynolds, 1989). Magnetic Resonance Spectroscopy (MRS) is a radiological procedure that is gaining tremendous recognition due to both its non-invasive methodology and its sensitivity to biochemical markers of hypoxia. Because of the nature of the technique, MR Spectroscopy has enabled identification and assessment to occur within just a few days of injury. The utility of MR Spectroscopy in the pathogenesis of asphyxia is promising (Wyatt et al., 1989). A recent study by Holshouser, Ashwal, Luh, Shu, Kahlon, Auld, Tomasi, Perkin, and Hinshaw (1997) found that long-term outcome following pediatric central nervous system injury was correctly predicted by MR Spectroscopy in 91% of neonates and 100% of infants and children.

Current research, however, has two factors limiting the progression of research on early intervention. First, nearly all research concerning MR Spectroscopy and birth asphyixa has only provided outcome information for the first year or two after birth. Long-term outcome in the study mentioned above was defined as functioning at 6-12 months of age. Secondly, common outcome measures implemented in research often fall short in providing a wealth of both quantitative and qualitative neurodevelopmental outcome information because outcomes tend to be based on fundamental dichotomies of good or poor outcome. While a poor outcome generally refers to a persistent vegetative state or death, a good outcome has a tremendous amount of outcome variability that is often lost in the dichotomy. Intervention and family counseling needs to be able to

address the variability found in a "good" outcome prognosis and assess what that really means for the individual child and family. Extensive neuropsychological examination following the formation of language and cognitive abilities can serve to both elucidate what a "good" outcome really means, and also compliment research with MR Spectroscopy to predict long-term neuropsychological functioning. The current study addresses these limitations by investigating neuropsychological functioning at ages two through seven in conjunction with MR Spectroscopy data as a predictor of outcome in the following areas: (1) memory and learning; (2) language; (3) visuospatial; (4) attention and executive functioning; and (5) sensorimotor abilities.

Hypoxic-Ischemic Encephalopathy

Hypoxic ischemic encephalopathy occurs when a neonate experiences severe asphyxia, a loss of oxygen supply, that results in hypoxemia and ischemia (Hill & Volpe, 2000). At a biochemical level, tissue necrosis develops secondary to ischemia as a result of cerebral blood flow falling below normal levels, which causes a cascade of neuronal depolarization and calcium influx. Furthermore, ischemia leads to a decrease in both intracellular Adenosine Triphosphate (ATP) and phosphocreatine concentrations and increases cerebral lactate concentrations. Alterations in intracellular calcium homeostasis leads to an even greater aggregation of free fatty acids which further increases cell permiability, extracellular potassium, and intracellular calcium (Hill & Volpe, 2000; Ment, Duncan, & Ehrenkranz, 1987).

Neonates asphyxiated at birth have a mortality rate 88 times greater than nonasphyxiated neonates (MacDonald, Mulligan, Allen, & Taylor, 1980). It is important to recognize that asphyxia and HIE are not synonymous syndromes. Instead, HIE is

diagnosed when asphyxia is prolonged and results in severe central nervous system injury. Asphyxia when brief or transient often results in no immediate or long-term injury. However, HIE is diagnosed when asphyxia results in observable neurological deficits. This distinction may be unclear in the literature in that some studies look at only HIE or only asphyxia and criteria for each may vary and be overlapping. Recognizing the inclusion criteria for the study group of each published article is important in understanding the outcome results and how they relate to or differ from outcomes in other similar studies.

Several factors are taken into consideration when diagnosing and assessing the severity of HIE. Typically, HIE is an intrapartem event in full-term neonates that manifests as respiratory difficulties, hypotonia, suppressed or increased reflexes, altered consciousness, and seizures. For the purpose of prognosis, the severity of asphyxia is commonly classified into three grades (or stages): mild (I), moderate (II) and severe (III) (Nelson & Leviton, 1991; Sarnat & Sarnat, 1976). The classification system, referred to as the Sarnat score, was initially designed to assess the severity of the perinatal insult using clinical indictors to predict outcome (Sarnat & Sarnat, 1976). Some of the clinical features of the mild grade include irritability, jitteriness, hyperalertness, normal muscle tone, and tachycardia. Features of the moderate grade are: lethargy, mild hypotonia, seizures, overactive stretch reflexes and Bradycardia. Indicators of the severe grade include: tachycardia and bradycardia, flaccid muscle tone, and absent complex reflexes. Seizures are rare in the mild grade; however, they are a more common event in the moderate grade and most often seen in the severe grade when decerebration is present. Nearly 50% of neonates with HIE experience seizures, which indicates that a high

Table 1. Features of Sarnat Stages/Grades of Hypoxic-Ischemic Encphalopathy in Neonate

	Stage 1	Stage 2	Stage 3
level of consciousness	Hyperalert	Lethargic/obtunded	Stuporous
Neuromuscular Control			
Muscle Tone	Normal	Mild Hypotonia	Flaccid
Posture	Mild Distal Flexion	Strong Distal Flexion	Intermittent decerebration
Stretch Reflexes	Overactive	Overactive	Decreased or Absent
Segmental Myoclonus	Present	Present	Absent
Complex Reflexes			
Suck	Weak	Weak or Absent	Absent
Moro	Strong; low Threshold	Weak;incomplete;high threshold	Absent
Oculovestibular	Normal	Overactive	Weak or Absent
Tonic Neck	Slight	Strong	Absent
Autonomic Function	Generalized Sympathetic	Generalized parasympathetic	Both systems depressed
Pupils	Mydriasis	Miosis	Variable; often unequal; poor light reflex
Heart Rate	Tachycardia	Bradycardia	Variable
Bronchial/Salivary Secretions	Sparse	Profuse	Variable
GI Motility	Normal/Decreased	Increased;Diarrhea	Variable
Seizures	None	Common;Focal/multifocal	Uncommon(excluding decerebration
Electroencephalagram	Normal(awake)	Early: low-voltage Continuous delta and theta. Later: periodic pattern (awake). Seizures: focal 1 to 1.5 Hz spike-and-wave	Early: periodic pattern with isopotential phases. Later: totally isopotential
Duration	Less than 24 hr	Two to 14 days	Hours to weeks

Note. Adapted from "Neonatal Encephalopathy Following Fetal Distress: A Clinical and Electrocencaphalographic Study," by H.B. Sarnat and M.S. Sarnat, 1976, Archives of Neurology, 33, p. 700.

proportion of cases may have experienced more prolonged hypoxic events which are important to identify for early intervention (Perlman & Risser, 1996). A summary of the clinical features of HIE adapted from Sarnat and Sarnat (1976) can be found in Table 1.

While examining the clinical indicators, it is interesting to note the differences in the behavioral presentations of each grade. While mild grades may exhibit normal to increased activity, more severe cases will often demonstrate a poverty of overall movement. Reflexes also demonstrate this increase from normal found in the mild grades and decrease from normal found in moderate and severe grades. Thus, a common finding is that hyperalertness and normal to overreactive reflexes indicate mild encephalopathy, while lethargy, stupor and suppressed reflexes indicate moderate or severe encephalopathy. Additionally, neonates with severe encephalopathy are at a greater risk for brainstem and autonomic dysfunction and increased intracranial pressure (Hill & Volpe, 2000). Other primary clinical and biochemical indicators of asphyxia are: meconium stain, fetal bradychardia, irregular heart rate, high acid-base status of the fetus, low Apgar score, and pathologic placental conditions (Hill & Volpe, 2000).

While the Sarnat score has provided a useful way of classifying the severity of injury, there are tremendous limitations that inhibit the ability to predict outcome based on grade classification. The primary reason for this is that the majority of neonates fall into the moderately injured group (grade II); however, this group also tends to demonstrate the greatest variability in outcome which can range from a good, healthy outcome to death (Peden et al., 1993). As a result, prognosis based simply on severity classification will have a difficult time in identifying the early needs of certain more severely affected neonates that fall into the intermediate range. For example, a study by

Robertson and Finer (1985) reported that of 226 neonates admitted to the neonatal intensive care unit (NICU), 79 were classified as mild HIE, 119 as moderate HIE, and 28 were severe. Additionally, while seizures are useful for prediction of both visuospatial and language functioning at three years of age in mild and severe groups, they did not correlate with outcome in the moderate HIE group. A subsequent study by Robertson and Finer (1989) again found that the greatest variability in receptive language at eight years of age was in the moderate encephalopathy group. As a result, more sensitive indicators of outcome within this intermediate group need to be examined in order to provide the highest quality of care following injury. Although the Sarnat score was one of the first indicators designed to predict outcome, and has been widely supported throughout the literature [Finer, 1983 #52; Shu, 1997 #58; Sweet, 1999 #64; van de Riet, 1999 #63], researchers are focusing on examining alternative and supplementary methods of identification. As a result, in addition to identifying the grade of injury to predict outcome, other methods utilized include: EEG, protein serum, carbon monoxide, nitric oxide, glutamate/glutamine, and interleukin-6 concentrations, glucose metabolism, blood pH, visual evoked potentials, Apgar score, diffusion weighted imaging, magnetic resonance imaging (MRI) and magnetic resonance spectroscopy.

Hypoxic-Ischemic Encephalopathy and Outcome

Several traditional methods have been utilized to diagnose and predict outcome following HIE. In this section, predictors will be reviewed beginning with less commonly utilized biochemical markers while then moving toward examining more robust indicators of cerebral injury and outcome. First, Serum Protein S-100 concentrations in cerebral tissue have been examined. While some research indicates an increase of this

substrate in asphyxiated infants relative to controls, it has not been widely examined (Maschmann, Erb, Heinemann, Ziemer, & Speer, 2000; Nagdyman, Komen, Ko, Muller, & Obladen, 2001; Rosen, Rosengren, Herlitz, & Blomstrand, 1998). Another group of researchers has looked at carbon monoxide (CO) and nitric oxide (NO) concentrations (Shi et al., 2000). The results of their study indicate that both CO and NO plasma levels may be significantly elevated in asphyxiated term neonates. Third, excitatory neurotransmitters have also been investigated as culprits of adverse sequelae following hypoxia (Gucuyener & Atalay, 1999). In this study, researchers found that concentrations of aspartate, and glutamate were significantly increased in cerebrospinal fluid of neonates who experienced asphyxia.

Various studies have examined other biochemical markers such as umbilical cord pH (Toh & Rajadurai, 1999), glucose metabolism (Thorngren-Jerneck et al., 2001), and interleukin-6 (Martin-Ancel et al., 1997). Some research evidence demonstrates that cord blood pH is often inversely related to level of impairment in HIE, with $pH < 7.05$ correlated with more neurological handicaps (Nagdyman et al., 2001; Toh & Rajadurai, 1999). Other research indicates that cerebral spinal fluid concentrations of interleukin-6 have a tendency to increase significantly with increasing severity of HIE. Additionally, interleukin-6 in cerebral spinal fluid is higher in neonates with both adverse outcomes (i.e. cerebral palsy and motor impairment) and brain damage (Martin-Ancel et al., 1997). This biochemical substrate, however, has not been widely researched.

Other investigators have reported that regional cerebral glucose metabolism, measured by positron emitted tomography, is correlated with severity of HIE and outcome at 2 years of age (Thorngren-Jerneck et al., 2001). This study indicated that total

glucose metabolism is inversely correlated with severity of HIE. Average glucose metabolism for mild, moderate, and severe were 55.5, 26.6, and 12.7, respectively. Regarding outcome, neonates who developed cerebral palsy had an average glucose metabolism of 18.1, while neonates with no neurological sequelae had an average glucose metabolism of 41.5 (Thorngren-Jerneck et al., 2001).

One of the more popular and more commonly used indicators and predictors of outcome, due to ease of testing and low cost, are Apgar scores (Martin et al., 1996; Nelson & Ellenberg, 1981; Nelson & Stanley, 1993). One study examined the prognostic utility of Apgar scores to determine the ability to predict persistent handicapping conditions (Nelson & Ellenberg, 1981). These researchers found that low Apgar scores were risk factors for cerebral palsy. Of the neonates followed, 12% with Apgar scores of 0 to 3 at ten, 15, and 20 minutes developed cerebral palsy and mental retardation (Nelson & Ellenberg, 1981). Although Apgar scores are often evaluated as a predictor of what may follow asphyxia, they are often regarded as simply an indicator of what has already occurred (Nelson & Stanley, 1993). Additionally, some researchers have not found good support for utilizing Apgar scores in a prognostic manner (Martin et al., 1996).

Electroencephalography (EEG) is one of the more costly but frequently utilized traditional methods found in the literature to diagnose and predict outcome following HIE. Indicators such as EEG discontinuity, burst suppression patterns, and early serial EEG are often reported as providing meaningful prognostic value (E. Biagioni, Bartalena, Boldrini, Pieri, & Cioni, 1999; Enrico Biagioni et al., 2001; Pressler, Boylan, Morton, Binnie, & Rennie, 2001; Sinclair, Campbell, Byrne, Prasertsom, & Robertson, 1999). One study reported that of those infants with depressed EEG activity during the first eight

hours of life, nearly 57% had either a severe neurological outcome or died by one year of age (Pressler et al., 2001). The 43% that had a normal outcome at one year had recovered normal EEG activity by 24 hours of life. This study indicates that there is a great deal of prognostic value with EEG data if activity is normal during the first eight hours or if depressed activity persists for more than 24 hours. Furthermore, Biagioni et al. (2001) was able to identify a great deal of consistency in predicting outcome between EEG findings and magnetic resonance imaging (MRI).

Several researchers have been able to use MRI to identify cerebral injury and predict neurodevelopmental outcome following perinatal hypoxia (Forbes, Pipe, & Bird, 2000; Keeney, Adcock, & McArdle, 1991; Eugenio Mercuri et al., 2000; Rutherford et al., 1998). An early evaluation of MRI's utility nearly a decade ago stated that poor long-term prognoses are related to both the location of the injury and duration of asphyxia (Keeney et al., 1991). Still a true statement ten years later, researchers have expanded tremendously on their understanding of the damage that results from hypoxia and MRI's role in identifying the impact of the event on cerebral tissue. These early researchers found that lesions of the basal ganglia were highly predictive of poor outcome and just as predictive as diffuse multicystic cavitations (Keeney et al., 1991). This study, however, did not provide further analysis of what a poor outcome is and what neurologic handicap or abnormal neurodevelopmental sequelae MRI is predicting.

A later study by Rutherford et. al. (1998) reported that abnormal signal intensity in the posterior limb of the internal capsule correctly predicted neurodevelopmental outcome 93% of the time following neonatal HIE. In this study, unlike Keeney et al's (1991) investigation, outcome was based on tone, posture, passive and elicited motility,

reflexes, vision and hearing responses. All neonates with an abnormal signal intensity in the posterior limb of the internal capsule either died (44%) or had moderate (11%) or severe (44%) neurodevelopmental delays at one year of age. According to the data in this study, MRI had a sensitivity of 90% and a specificity of 100%. Another group of investigators set out to examine the relationship between MRI and head growth with neurodevelopmental outcome (Eugenio Mercuri et al., 2000). This study found no difference in head circumference at birth of neonates following asphyxia compared with controls. At 12 months of age, however, 48% of asphyxiated neonates were classified as microcephalic and only 3% of the control group were classified as such. Although the study did not directly address the correlation between MRI and neurodevelopmental outcome, it did report that suboptimal head growth predicted poor outcome with a sensitivity of 79% and specificity of 78% (Eugenio Mercuri et al., 2000).

Using diffusion-weighted MRI imaging, Barkovich, Westmark, Bdi, Partridge, Ferriero, and Vigneron (2001) compared T2-weighted MRI images with diffusion-weighted imaging (DWI) and magnetic resonance spectroscopy. Diffusion is the amount of thermal motion that occurs within a fluid. DWI measures the amount of motion occurring within water in cerebral tissue and converts it to an apparent diffusion coefficient so that the rate of diffusion within the individual brain can be compared with that of others. These researchers compared these different radiological procedures from the first day of life for seven infants who experienced perinatal asphyxia. What they found was that while T2 imaging and DWI often underestimated severity of injury on the first day of life, magnetic resonance spectroscopy was acutely sensitive to the first signs of energy failure and cell apoptosis (A. J. Barkovich et al., 2001). It was suggested that

hypoxic-ischemic brain injury might not be apparent on DWI with the first 24 hours because of the reperfusion that occurs prior to secondary injury failure. Thus, early identification is generally not possible with DWI because it is often associated with false negative readings. Magnetic resonance spectroscopy, however, appears to be the most accurate of the three measures in early identification of injury.

Magnetic Resonance Spectroscopy and Outcome

Before reviewing the literature on Magnetic Resonance Spectroscopy (MRS), a brief explanation of what MRS is and how it works should be discussed. Elements, such as phosphorus (^{31}P) and hydrogen (^{1}H) have atomic nuclei which contain an uneven number of nucleons that behave like magnets when placed within a magnetic field (Goplerud & Delivoria-Papadopoulos, 1993). Outside of a magnetic field, the nuclei are randomly aligned. However, when placed within the field, the nuclei align themselves either with or against the magnetic field. When a brief pulse of a specific radiofrequency is applied to the molecules, the nuclei begin to absorb energy, which displaces the aligned nuclei. The nuclei return to their original alignment and lose the additional energy when the input from the electromagnetic frequency ceases. Subsequently, it is this loss of energy that is detected and processed by a computer into resonance and spectral data.

This magnetic resonance signal can provide the data examined in both the ^{31}P and ^{1}H spectrums. Essentially, this non-invasive procedure measures metabolite concentrations with signals stimulated from pre-selected neural tissue. A hypoxic event will essentially lead to a decrease in energy metabolism as a result of the decrease in available oxygen. Since metabolic energy is dependent on specific phosphorus and hydrogen compounds, such as creatine and phosphocreatine, it is this decrease in

metabolic activity that is measured in terms of chemical resonance frequencies emitted (Goplerud & Delivoria-Papadopoulos, 1993; Ross, Ernst, & Kreis, 1995). Additionally, the same atom of hydrogen can have different resonance frequencies when found in different molecules, such as N-Acetylaspartate and choline, or different parts of the same molecule (Huppi & Lazeyras, 2001). A plot of the chemicals measured versus the amount of signal received from each metabolite is a chemical shift or MR spectrum.

Central nervous system (CNS) insults, such as hypoxic-ischemia, typically may cause changes in the following CNS metabolites measured in proton MR spectroscopy: N-Acetylaspartate (NAA), an amino acid which serves as a neuronal marker and is stored in the cell; compounds containing choline (Cho), together with free choline, phosphoryl and glycerophosphoryl choline, creatine consisting of both its precursor, phosphocreatine (PCre), and creatine (Cre); and lactate (Lac) which is a by product of the anaerobic metabolism that occurs in ischemic tissue (Ross et al., 1995). Hypoxic-ischemic insults are related to a decrease in overall N-acetylaspartate (NAA), increases in Choline/Creatine (Cho/Cre) ratios, and decreases in both NAA/Cho and NAA/Cre ratios (Barbara A. Holshouser et al., 1997). Additionally, these events may elevate free-fatty acid and lactate resonances that are not typically present in non-injured tissue (Ross et al., 1995).

Some research has found ^{31}P magnetic resonance spectroscopy (^{31}P-MRS) to have important prognostic value when evaluating HIE. Although the exact mechanism underlying cerebral injury remains unclear, the primary metabolic changes are believed to be a result of the initial hypoxic state with transient improvement during the first 24 hours followed by a secondary decline resulting from impaired energy metabolism

(Martin et al., 1996). Wyatt, Edwards, Azzopardi and Reynolds (1989) have referred to the initial hypoxia as "primary energy failure" and the delayed metabolic impairments as "secondary energy failure" (Wyatt et al., 1989).

Since it is believed that the decrease in the oxidative phosphorylation mechanism is the cause underlying permanent cerebral damage, measuring the ratios of biochemical substrates involved may be helpful in evaluating severity of hypoxia and predicting outcome (Martin et al., 1996). Using ^{31}P-MRS to predict outcome following perinatal asphyxia, Hamilton (1987) compared outcomes of 27 infants with HIE with 18 controls. In the normal control infants, metabolite ratios for phosphocreatine (PCr)/inorganic orthophosphate (Pi) increased with age, as expected, within the normal range. Of the 27 neonates with HIE, 15 had PCr/Pi ratios that were below the normal range. Of the 15 with low PCr/Pi ratios, six developed cerebral atrophy. The remaining nine infants died. The other 12 infants whose PCr/Pi ratios remained in the normal range survived with cerebral atrophy correlating with PCr/Pi ratios (Hamilton et al., 1986). Consistent with Hamilton's (1987) findings, another study found that 68% of infants with PCr/Pi ratios below 95% confidence limits for normal infants died (Azzopardi & Edwards, 1995). Of the remaining 32% that survived, 78% developed motor, sensory or behavioral impairments, commonly reported together with microcephaly (Azzopardi & Edwards, 1995). Furthermore, examining adenosine triphosphate/total phosphorus (ATP/P) ratios revealed that 92% of neonates below 95% confidence limits for this ratio died.

Further support has been found in the literature for utilizing impaired oxidative phosphorylation for predicting outcome following asphyxia (Moorcraft et al., 1991). A more recent study by Martin et al (1996) found a significant correlation between both PCr

and ATP concentrations and severity of encephalopathy. They reported that infants with mild hypotonia, normal sucking reflex, and absence of seizures had consistently greater concentrations of the energy compounds, PCr and ATP, than infants with absent suck reflex, decreased tone, level of consciousness, and presence of seizures. Additionally, PCr and ATP were both highly predictive of neurodevelopmental outcome at 18 months. Unfortunately, neurodevelopmental outcome in this study was categorized into four ordinal groups based on severity of motor deficits rather than data analysis on an interval scale.

Working in a similar way to ^{31}P-MRS, proton magnetic resonance spectroscopy (^{1}H-MRS) has been used to study the biochemical changes that occur following central nervous system injury but instead measures the resonance frequencies of hydrogen (Federico, Simone, & al., 1998; Shu, Ashwal, Holshouser, Nystrom, & Hinshaw, 1997). Some research suggests that ^{1}H-MRS is a preferred measure over ^{31}P-MRS, specifically during the first 24-hours following injury (Hanrahan et al., 1996; Peden et al., 1993). The most important reason for this is the reliability of proton metabolite abnormalities and sensitivity of the ^{1}H-MRS data. The mechanism underlying primary energy failure is believed to involve substrates measured by both ^{31}P-MRS and ^{1}H-MRS (Hanrahan et al., 1996). The difference, however, in the utility of these two studies is that phosphorus recovers back to normal within hours of injury while hydrogen substrates and lactate can remain at abnormal levels for weeks and months (Peden et al., 1993). Secondary energy failure occurs only after phosphorus ratios, previously recovered, begin to show signs of secondary impairment after 24 hours. But at times this period of phosphorus secondary energy failure is temporary and continues for approximately two weeks and then will

generally recover to normal; sometimes total phosphorus will appear reduced indicating permanent brain cell loss (Wyatt et al., 1989).

Wyatt, Edwards, Azzopardi and Reynolds (1989) reported that animal models "confirm that recovery of the phosphorus metabolites and intracellular pH after a severe hypoxic-ischemic episode can be rapid and often complete within an hour or so--though lactate may sometimes persist in the brain tissue after the intracellular pH has returned to normal." Since changes in metabolism are generally not detected by ^{31}P-MRS until 24 hours following injury when secondary energy failure sets in (Wyatt et al., 1989), ^{1}H-MRS's early detection of the primary energy failure, occurring within minutes and hours of injury, can serve to predict severity of both secondary energy failure and poor neurodevelopmental outcome (Hanrahan et al., 1996). The usefulness of predicting secondary energy failure is that it can assist physicians in recognizing infants who will benefit from treatment with neuroprotective agents (Goplerud & Delivoria-Papadopoulos, 1993; Hanrahan et al., 1996). Not only does ^{1}H-MRS deliver earlier identification than ^{31}P-MRS, it also can provide reliable assessment of birth asphyxia weeks to months after birth because proton spectra remain abnormal for much longer than phosphorus spectra (Peden et al., 1993).

Several important points need to be considered when evaluating ^{1}H-MRS data. First, ^{1}H-MRS is exceedingly susceptible to changes in specific metabolite ratios and, unlike ^{31}P-MRS, the amount of lactate in damaged neural tissue can be evaluated. Secondly, in most cases, ^{1}H-MRS data is taken from the occipital gray matter and parietal white matter, with the former being highly affected by global hypoxia (Barbara A. Holshouser et al., 1997). Although activity of cortical tissue is assumed to be involved

and may provide a good indicator of later neuropsychological functioning, interference from skin and bone of the skull do not permit these areas to be studied (Groenendaal et al., 1994). A final point to keep in mind is that metabolite peaks undergo significant changes in the first year or two of life which require normal age-matched controls for comparison of spectrum data in neonatal and infant populations (Kimura et al., 1995; van der Knaap et al., 1990). Normal age-related changes are: NAA/Cho and NAA/Cre ratios increase and the Cho/Cre ratio decreases (Peden et al., 1993).

Influential research by a group at Loma Linda University Medical Center found that specific metabolites consistently predict outcome following a broad-spectrum of acute CNS insults (S. Ashwal, Holshouser, Hinshaw, Schell, & Bailey, 1996; Stephen Ashwal et al., 1997; Auld et al., 1995; Barbara A. Holshouser et al., 1997; Shu et al., 1997). In a series of studies, Auld, Ashwal, Holshouser, Tomasi, Perkin, Ross, and Hinshaw (1995) studied metabolite ratios of thirty infants with acute CNS injury. A poor outcome included all patients that experienced severe disability, vegetative state, or death. Good outcome consisted of all patients who had normal, mild, or moderate disability. They found that lower ratios of both NAA/Cre and NAA/Cho were predictive of poor outcomes as measured by the Glasgow Outcome Scale. While lactate was completely absent in all the good outcome patients, in over 80% of the poor outcome patients the presence of lactate was detected. Using spectroscopy variables alone predicted outcome correctly 81% of the time (Auld et al., 1995).

Further published work on the same sample population by Ashwal, Holshouser, Hinshaw, Schell, and Baily (1996) examined ^{1}H-MRS data from nine children, aged 1 week to 42 months, with cerebral ischemia or CNS malformations secondary to

congenital heart disease. Six of the nine patients were at the level of severely impaired or vegetative state at discharge. Four of these six, however, had spectral data indicating good outcome. Only two of these six had abnormal ^{1}H-MRS readings, predictive of a poor outcome. The four with good prognoses and no lactate signals recovered to a level of mild disability at a 6-12 month follow-up. The two patients that did have the presence of lactate also had reduced NAA/Cre ratios and continued to have severe impairments at the 6-12 month follow-up. The three patients with normal ^{1}H-MRS readings were discharged with either mild or moderate impairments and recovered to levels of normal to mild outcomes at the 6-12 month follow-up (S. Ashwal et al., 1996).

In a third report on this same sample population, Ashwal, Holshouser, Tomasi, Shu, Perkin, Nystrom, and Hinshaw (1997) compared 6 newborns, 19 infants, and 11 children with acute CNS injuries that had elevated lactate signals with 20 newborns, 17 infants, and 24 children with similar CNS involvement but without elevated lactate signals. Newborns with lactate signals had significantly lower NAA/Cho ratios relative to normal controls and higher Cho/Cre ratios relative to lactate absent neonates as well as normal controls. Infants with lactate signals had both lower NAA/Cre and NAA/Cho ratios than lactate absent infants. Children with lactate signals had significantly lower NAA/Cre, NAA/Cho ratios and higher Cho/Cre ratios than lactate absent children and normal controls (Stephen Ashwal et al., 1997).

Neonates with elevated lactate were at higher risk for severe disability, persistent vegetative state, or death. Patients without lactate peaks were more likely to have normal, mild or moderate disability at 6-12 month follow-up. As a result, ^{1}H-MRS data contributed significant prognostic value to outcome, predicting 91% of patients with

lactate elevations would acquire neurodevelopmental sequelae relative to only 9% of those without lactate elevations.

In 1997, Holshouser, Ashwal, Luh, Shu, Kahlon, Auld, Tomasi, Perkin and Hinshaw decided to assess the predictive power of ^{1}H-MRS following acute pediatric CNS injury of 23 neonates, 31 infants, and 28 children using linear discriminant analysis. Various etiologies of CNS insults included meningitis and encephalitis; HIE; accidental and nonaccidental trauma; cardiac arrest and near-drowning; metabolic disorders; and other miscellaneous injuries secondary to vasculitis, sepsis, hypoglycemia, seizures, and Rh incompatibility. Other clinical data analyzed for prognostic value were age; cause of brain injury; occurrence of cardiac arrest; admission Glasgow Coma Score (neonates received 5-minute Apgar scores, Sarnat scores of muscle tone, reflexes and autonomic activity, and electroencephalographic scores instead of GCS); presence of fixed pupils at admission; admission arterial blood gas and blood glucose levels; number of days after the insult and number of days that the patient was unconscious at the time when ^{1}H-MRS was performed; and duration of ventilator support and hospitalization.

Outcome was measured by scores on the Pediatric Cerebral Performance Category Scale, a Glasgow Outcome Scale (GOS) modified for infants and children. Scores were grouped into one of six outcomes: (1) normal (engaged in all age-appropriate activities); (2) mild disability (conscious, alert, and interacted in age-appropriate activities); (3) moderate disability (conscious, and adequate cerebral functioning for most age-appropriate activities); (4) severe disability (conscious and impaired brain functions require dependence on others for support and care); (5) persistent vegetative state and (6) death. GOS groups were then stratified into either good-moderate outcome (normal, mild,

or moderate GOS groups) or poor outcome (severe, persistent vegetative state or death GOS groups).

Consistent with previous findings, spectral data found that lactate peaks were significantly higher in patients with poor outcomes than those with good-moderate outcomes. Eighteen neonates were included in the data analysis. Of the six in the poor outcome group, two that died had no lactate elevations while the other four had significant elevations. Only one neonate exhibited lactate elevations but was in the good-moderate outcome group. Inconsistent results for neonatal patients, the researchers concluded, were most likely due both to the lack of variability in outcome and small sample size. Although none of the NAA/Cho ratios were below two standard deviations of the mean, significantly more neonates with one standard deviation reduction in NAA/Cho ratios were in the poor outcome group. Sarnat scores were the only clinical variable that provided some predictive utility. Using linear discriminant analysis, ^{1}H-MRS data were able to predict outcome in 87% of neonates at 6-12 months follow-up. Prediction was decreased to 83% when clinical predictors were added to spectrum data in analysis. This decrease was attributed to an increase in the false-positive rate (Barbara A. Holshouser et al., 1997).

While data on the Pediatric Cerebral Performance Category Scale may provide fundamental utility in measuring global outcome following a broad-spectrum of acute pediatric CNS insults, more sensitive neuropsychological measures may enhance the quality of outcome data. As a result, the predictive power of ^{1}H-MRS would be better equipped to elucidate just what to expect when a good or poor outcome is predicted, not only broad-spectrum cerebral injury, but also hypoxic-ischemic injury.

^{1}H-MRS and Hypoxic-Ischemic Encephalopathy

The predictive utility of ^{1}H-MRS with HIE has received a modest amount of research attention over the last decade. In one of the first studies addressing neonatal asphyxial injury with ^{1}H-MRS assessment, Peden, Rutherford, Sargentoni, Cox, Bryant, and Dubowitz (1993) examined proton spectrum from asphyxiated infants and correlated these findings with neurodevelomental outcome at one year. Since significant age-related changes occur within the first few years of life, these researchers controlled these changes by including only term and post-term infants (37 to 42 weeks). Studies were conducted between 2 days and 27 days following birth, with a mean of 13.6 days. Assessment included neurological examination, MRI, ^{1}H-MRS, cranial ultrasound scans, and Doppler blood flow studies. HIE was graded according to Sarnat and Sarnat's severity classification. Outcome at one year was divided into three categories based on information from a structured neurological exam and the Griffiths developmental scale: (1) normal outcome, those infants who may or may not have had transient abnormalities prior to outcome assessment; (2) moderate outcome, those infants who had mild abnormalities or neurodevelopmental delay; and (3) severe outcome, those infants with hemi or quadriplegia and/or severe neurodevelopmental delay at outcome assessment.

A region in the parieto-occipital area of the brain was scanned for metabolite shifts. Ratios examined were the same as those in other similar ^{1}H-MRS studies. The researchers compared results from ten HIE neonates with one normal control neonate. The results showed an obvious trend in NAA/Cho ratios with the highest found in the control infant and the lowest found in poor outcome infants. NAA/Cre ratios also indicated an apparent trend with the highest found in the control infant and the lowest

found in poor outcome infants. Cho/Cre ratios did not show a trend between the control infant and the poor outcome infants. Although the study was a small-scale study, the results indicate that ^{1}H-MRS is indeed able to predict outcome more accurately than relying solely on the Sarnat and Sarnat (1969) grade classification. While stage I and III have more determined outcomes, stage II HIE has quite a bit more uncertainty and a greater potential for hidden neuropsychological impairments (Roberston & Finer, 1993). The majority of neonates (eight of ten) in this study were classified as stage II HIE, which alone was unable to predict outcome. With MR spectroscopy, however, the variability is clearly reduced into predictable outcomes that increase a medical professional's ability to provide specialized early intervention (Peden et al., 1993).

A study conducted a year later by Groenendaal, Veenhoven, van der Grond, Jansen, Witkamp, and de Vries (1994) examined both lactate and NAA/Cho ratio peaks and correlated these findings with outcome at 3 months of age for 21 neonates with birth asphyxia. Two neonates were classified as grade I, 14 were grade II, and 5 were grade III. The hypothesis tested was that both the presence of lactate and low NAA/Cho ratios would be indicative of a poor neurodevelopmental outcome. Although outcome was limited to a short period of time, these researchers found a trend in NAA/Cho ratios that were consistent with Peden et al's (1993) findings. In the Groenendaal et al (1994) study NAA/Cho was significantly lower in infants who had a poor outcome than compared with those infants who had normal outcomes. Lactate, however, was found in all five infants who were also classified with grade III (severe) encephalopathy. This finding was in contrast to Peden et al's (1993) study, which did not report any neonate with a lactate peak. The reason for this discrepancy may have been due to the differences in severity of

asphyxia between sample populations. Peden et al's (1993) sample overall had less severe HIE diagnoses than Groenendaal's sample of neonates (Groenendaal et al., 1994).

A third study on the predictive utility of ^{1}H-MRS within 18 hours of injury examined 16 term infants who experienced birth asphyxia and compared them with 10 normal control infants (Hanrahan et al., 1996). Of the 16 infants examined, 7 were grade I, 4 were grade II, and 5 were grade III. Lactate signals were detected in all but one of the asphyxiated infants and four of the control infants, but with a higher proportion found in the injured neonates. Compared to normal controls, there were no significant differences in NAA/Cre and Cho/Cre. Lactate/Cre ratios, however, were found to be significantly higher in neonates with asphyxia (Hanrahan et al., 1996). While the study's focus and concern was primarily on comparison of ^{1}H-MRS to ^{31}P-MRS, there was a modest report of outcome at 8 month of age, which indicated that infants with lactate signals developed multiple handicaps. However, this report of long-term outcome was presented for only one infant.

In a follow-up study, this same team of researchers, Hanrahan et. al (1999), published a report on the outcome at one-year for 31 neonates. In this study they reported that nine had an adverse outcome, which was defined as death or severe neurodevelopmental impairment. Lactate/Cre elevations predicted adverse outcome at one-year of age with a sensitivity of 66% and specificity of 95% (Hanrahan et al., 1999). Although these researchers only reported on lactate/Cre ratios, the strong relationship between metabolite ratios and outcome is clearly explicated in their data.

A fourth group of researchers examined the same question regarding metabolite peak-area ratios abnormalities, but investigated two different regions of the brain

following birth asphyxia and compared these findings with one-year outcome (Penrice et al., 1996). In addition to examining metabolite abnormalities, normal perinatal maturational changes were also investigated with a large group of control infants. The study examined 19 infants with asphyxia and 35 control infants. Of the infants with HIE, 5 were Sarnat grade I, 12 were grade II, and 2 were grade III. Overall, 58% (n = 11 out of 19) of HIE infants had an increase in Lac/NAA in the thalamus, the occipitoparietal region or both relative to controls. In the occipitoparietal region of asphyxiated infants, Lac/Cho was the only metabolite ratio that was significantly increased relative to control infants. In the thalamic region, however, Lac/NAA, Lac/Cho, and Lac/Cre all were significantly increased relative to control infants. For the eight infants with Lac/NAA ratios from either region above the 95% confidence limits, one had minor impairments, five infants had major neurodevelopmental impairments, and two were dead at the time of the one-year follow-up. For the seven infants who had Lac/NAA ratios within the 95% confidence limits, four were classified as normal and three had minor impairments at the time of follow-up. Accordingly, the study reports that Lac/NAA elevations were found to be predictive of major disabling impairment or death at one-year outcome (Penrice et al., 1996).

This same team of researchers later investigated the same question regarding the prediction of outcome using ^{1}H-MRS posed by the previous studies (Amess et al., 1999), but instead of focusing on differences in brain regions, they compared the prognostic power of ^{1}H-MRS to a structured neonatal neurological assessment. The study included 28 asphyxiated infants and 18 control infants, each of which received spectroscopy scans within 48 hours of injury. The results indicated that, again, Lac/NAA ratios accurately

predicted adverse outcome at one-year follow-up with a positive predictive value of 92% and a specificity of 93%. While a structured neurological assessment provided useful prognostic information, it was found to have a high false-positive prediction rate.

Another study by Barkovich and colleagues (1999) examined 31 asphyxiated, term neonates using ^{1}H-MRS and compared this data with outcome on the Bayley Infant Development Scale at 12 months. These researchers also reported that elevated lactate and decreased NAA were good indicators of neurodevelopmental outcome in the asphyxiated sample population (J. Barkovich et al., 1999).

While most spectroscopy data is collected within days of injury, there is evidence that metabolite information collected up to a month following injury can offer prognostic value (Hanrahan et al., 1998). In one study designed to address this issue, Hanrahan et al (1998) found that, concurrent with notions regarding secondary energy failure, lactate peaks were still detectable later than one month after birth in seven of eight neonates with abnormal neurodevelopmental outcomes at one year of age. Additionally, no lactate was found one month following injury in any infant with a normal outcome at one year. Interestingly, a minute amount of lactate was detected in one of the six controls studied one month after birth. This infant, however, was reported to have a normal neurodevelopmental outcome at one year of age.

Hypoxic-Ischemic Encephalopathy and Neuropsychological Outcome

When there is head injury during the first few months or years of life, the long-term consequences will differ significantly from adult head injuries that occur within a mature and well-developed nervous system. But even injuries that occur in early childhood can result in drastically different neurodevelopmental sequelae than injuries in

the neonatal period. Neurodevelopmental literature suggests that early childhood injuries may disrupt the developmental sequence and result in deficits in foundational skills that delay or inhibit acquisition of more complex skills (Spreen, Risser, & Edgell, 1995). Since the neonatal brain is in a period of cell proliferation and migration, this process complicates the impact hypoxic injury has on neuropsychological development and can often produce the more adverse behavioral outcomes that emerge in later childhood (Spreen, Risser, & Edgell, 1995). Some researchers report that HIE injuries can subsequently redirect, or hinder neuronal cell migration altogether (Rorke, 1992). As a result, head injuries in later childhood may have better outcomes because the foundational skills have already been established and the brain can adapt better than if the skill is unlearned. However, it is important to keep in mind that the degree of plasticity, regardless of age of injury, is also determined by the nature, site and severity of the hypoxia (Spreen, Risser, & Edgell, 1995). So even though CNS insults that occur in infancy may generally lead to greater deficits than those that occur in early childhood, the severity and diffuseness of the injury is important in determining potential resiliency or plasticity. In summary, neonates that experience HIE do run a significant risk of developing neuropsychological impairments in the areas of memory and learning, expressive and receptive language skills, visuospatial processing, attention and executive control, and sensorimotor skills.

Memory and learning. While there is clear evidence that memory impairments can be found in individuals who have experienced a broad range of CNS injury, there is a dearth of experimental support of memory deficits in HIE. A review of the literature identified three studies, one that looked specifically at HIE-related memory deficits, one

not clearly specified as addressing HIE and memory and the other is a case study of hypoxic-ischemic induced memory impairment (Korkman, Liikanen, & Fellman, 1996; Maguire, Vargha-Khadem, & Mishkin, 2001; Roberston & Finer, 1993). The first study used psychoeducational testing to examine the long-term sequelae of HIE (Roberston & Finer, 1993). The study found that while the mild (stage I) encephalopathy group performed similar to the normal control group on memory tasks and the severe (stage III) HIE group had more profound neurological and memory deficits, the nondisabled moderate (stage II) HIE children performed below both mild HIE and controls on memory and short-term recall tasks. The significance of this is that while nondisabled moderate HIE children may present both medically and behaviorally identical to mild HIE children, there are striking differences neuropsychologically.

In another study, Korkman, Liikanen, and Fellman (1996) examined the neuropsychological consequences of birth asphyxia at an average of seven years of age and found results unlike what was reported by Robertson et al (1993). Unexpectedly, Korkman et. al. (1996) reported that not only did children with birth asphyxia perform better than very low birth weight children in memory and executive functioning, they performed just as well as healthy control children. While the findings are somewhat disconcerting given the evidence of previous research, two important points need to be taken into consideration when reviewing experimental evidence of potential neuropsychological deficits following HIE. First, while many studies that evaluate long-term sequelae of birth asphyxia tend to include only infants with documented HIE, some studies, like Korkman et al (1996), had less stringent criteria for group inclusion. The birth asphyxia group in this study included both 13 children with diagnosed HIE and an

additional 23 other children with no documented HIE. The 23 were included because they met the following criteria: umbilical cord pH less than 7.05, positive hypoxanthine and positive arginine vasopressin, both nontraditional indicators of asphyxia severity not found to correlate with outcome. As a result, a majority (63%) of the sample population included not only children with birth asphyxia histories that are less severe than reported in other published studies, but these 23 children most likely had not endured a hypoxic-ischemic injury similar to either the 13 children with HIE or with children in other studies where HIE is the inclusion criteria. A larger number of children with HIE in the sample may have resulted in clearer impairments which would have been more consistent with Robertson et al's (1993) findings.

A second point to keep in mind is exclusion criteria that may also limit the severity of injury in the sample population. In Korkman et al's (1996) study, children who were disabled were not included in analyses. In Robertson et al's (1993) study, however, children with disabilities were included. Thus, in Korkman et al's (1996) analysis, six children were excluded from the birth asphyxia group because of either mental retardation or cerebral palsy. The significance of this exclusion on the outcome was astonishing, and was noted by the authors. The researchers reported that "after excluding the six severely disabled children, the birth asphyxia group was not impaired relative to the control group" (Korkman et al., 1996). In light of this insight and awareness of the sample population, the findings are not so unexpected. Korkman et. al. (1996) concurred that the limited severity of injury in their sample could sway neuropsychological and intellectual outcome.

The third study was a case presentation of a 22-year-old male, Jon, who was born at 26 weeks gestation with selective bilateral hippocampal damage secondary to perinatal hypoxic-ischemia (Maguire et al., 2001). The study was primarily concerned with whether Jon's memory retrieval network activation for episodic and semantic information was similar to six healthy control subjects. Jon exhibited severe impairments in episodic memory even though he demonstrated excellent semantic memory (Maguire et al., 2001).

While the researchers were interested in hippocampal activation during autobiographical (episodic) memory retrieval, they also were interested in neuroimaging evidence for neural reorganization that may reflect plasticity following early hypoxic-ischemic injury. The study found that largely medial and left-lateral brain regions (medial frontal cortex, left temporal pole, left hippocampus, left parahippocampal gyrus, left middle temporal gyrus, left temporoparietal junction and retrosplenial cortex) mediated both Jon's and controls' memory retrieval; however, Jon exhibited activation of homologous brain regions on the right. This bilateral activation was a unique and clear indication of neuronal plasticity and recruitment following early hypoxic-ischemic insult.

In summary, Robertson and Finer (1993) reported that memory impairments correlate well with the severity of HIE; Korkman et al (1996), however, reported that memory and executive functioning in children with either HIE or only birth asphyxia was similar to normal children when those with mental retardation and cerebral palsy were excluded. Robertson and Finer (1993) also made a clear distinction between children who may appear medically and behaviorally similar may not perform similarly on tasks of memory functioning and other neuropsychological areas. Lastly, Maguire et. al. (2001)

reported that structural memory locations can be affected by HIE and impair memory performance for episodic but not semantic memory.

Language development. More extensive work has been directed at the language development of these children than was found for memory retrieval. The earliest examination of language acquisition in children with severe perinatal asphyxia was conducted in 1981 at the Department of Child Health at the University of Manchester (D'Souza, MCartney, Nolan, & Taylor, 1981). During that period speech impairments and language delays were frequently reported as somehow related to hearing impairments. But some researchers weren't convinced of this and decided to investigate how severe perinatal asphyxia may influence vocabulary, language content, structure of language, and articulation.

D'Souza, McCartney, Nolan, and Taylor (1981) examined 26 asphyxiated neonates born between 1973 and 1976, with 11 less than 37 weeks and 5 less than 10^{th} percentile for gestational age, and found some interesting results. At a two to five year follow-up, while hearing was normal for all children except one, the results for speech and language were startling. Using the Reynell Developmental Language Scales and Edinburgh Articulation Test (EAT), 10 of the 25 children were below normal limits in at least one language area. Five had delayed expressive and receptive language, three old enough to be given the EAT all had severe speech articulation defects, and two had no speech secondary to cerebral palsy (D'Souza et al., 1981). While the researchers attempted to explain the language deficits in the context of probable middle-ear disease or physical disabilities, they were unable, for most of the children, to give reasons for the language delays and speech impairments. Although they clearly stated the importance of

early detection for improved quality of life at the end of the report, they did not give any indication in their review that hypoxic-ischemic injury may have been the predisposing event that interfered with normal neuronal proliferation and migration.

A second study conducted in 1985 found similar results (Charlene Robertson & Finer, 1985). Using the Peabody Picture Vocabulary Test (PPVT) as a measure of receptive language and mean length of utterances (MLU) recorded as morphemes as a measure of expressive language, the study examined 167 survivors of HIE at 3.5 years of age with no control group. The researchers reported that receptive and expressive language of children with severe HIE were impaired relative to moderate HIE children; and both receptive and expressive language of moderate HIE children was also delayed relative to the mild HIE children. Children with mild HIE were generally average in language skills except for those that displayed hypotonia and suppressed reflexes at birth. These children exhibited greater impairment in language acquisition. Even within the total sample of 167 children, an abnormal examination at hospital discharge was found to be predictive of delayed receptive and expressive language development. While the study clearly stated that children with mild HIE generally fall into the average range of language functioning and severe HIE children fall into the most impaired range, it was much more difficult to give an accurate prognosis for moderate HIE children regarding language development.

In a follow-up study of 149 of these same children at 5.5 years of age, the researchers now obtained comparison information from two control groups (Roberston & Finer, 1988). One comparison group was comprised of 71 graduates in the same cohort from a neonatal intensive care unit (NICU) with a history of encephalopathy and

followed exactly like the study population. The other comparison group was comprised of 188 peers with uneventful birthing histories. When compared to mild HIE, NICU graduates, and normal peers, more moderately impaired HIE children displayed over a 12-month delay in expressive language skills reported on the Zimmerman Verbal Abilities Subtest. While the moderate HIE group also exhibited more impairment in receptive language skills on the PPVT, this was not statistically significant (Roberston & Finer, 1988).

In a third follow-up study when the children were 8 years of age, Robertson et al (1989) reported that, overall, children with any previous HIE (n=145) obtained receptive language standard scores significantly below those in a normal peer group (n=155). There was no significant difference, however, between HIE children without major physical impairments (all mild and some moderate encephalopathy) and normal peers in receptive language abilities scores on the PPVT. A fourth study from Robertson et. al. (1992) further confirmed these findings and accurately predicted outcome of a second cohort based on the outcome of the previous cohort (C. Robertson & Grace, 1992). Contrastingly, Korkman et. al. (1996) reported no difference in language skills in 36 five to nine year olds following birth asphyxia when compared to 45 normal healthy controls. An interesting finding was that when language deficits did occur in Korkman et al's (1996) study, they tended to co-occur with visuo-motor deficits such that if visuospatial functioning was impaired language was also generally impaired as well. The implications of how the findings conflict with Robertson and Finer's (1989) findings have been addressed in the above section on memory and executive functioning.

In summary, D'Souza et. al. (1981) reported that while both receptive and expressive language skills were significantly impaired following HIE, hearing deficits were unable to account for the language delays. Furthermore, between 1985 and 1989, Robertson and colleagues conducted series of studies examining language at ages three, five, and eight that corresponded to D'Souza (1981) and colleagues' findings in that severity of encephalopathy correlated with later receptive and expressive language impairments. A more recent study by Korkman and colleagues (1996) provided contrasting results, which indicated that language performance for less severe asphyxia was relatively similar to healthy control children.

Visuospatial processing. Visuospatial processing in children following HIE is frequently complicated by damage to many areas within the visual system (E. Mercuri et al., 1997). First, visual loss secondary to anterior visual pathway dysfunction (i.e. optic atrophy, optic nerve hypoplasia, refractive errors, nystagmus, strabismus, retinopathy, amblyopia, myopia, hyperopia, astigmatism, esotropia, and exotropia) has been reported in infants and children with documented HIE (Goggin & O'Keefe, 1991; Luna, Dobson, Scher, & Guthri, 1995; McCulloch et al., 1991; Roland, Jan, Hill, & Wong, 1986; van den Hout et al., 2000; Van Hof-van Duin & Mohn, 1984). For example, one early study reported visual deficits, such as nystagmus, impaired visual acuity, visual field size, and blindness in nearly 90% of the sample population (Van Hof-van Duin & Mohn, 1984). Another more current study indicates ophthalmological abnormalities in over 42% of children with perinatal insults (van den Hout et al., 2000). In order to control their influence, these pregeniculate pathway deficits are important to rule out when assessing higher level visuospatial functioning.

Secondly, visual loss secondary to cortical visual impairment (CVI) also complicates and interferes with accurate quantification of visuospatial processing. CVI, formerly known as cortical blindness (Roland et al., 1986), is a type of vision loss unexplained by ocular findings, and is associated with dysfunction of the optic radiations, visual association cortex and striate cortex, which are all located posterior to the geniculate bodies and make up the posterior visual system (Luna et al., 1995; Roland et al., 1986). Primary symptoms found in CVI include restricted visual field findings and impaired visual acuity (Luna et al., 1995). Since the optic radiations and visual cortex are watershed regions between the posterior communicating artery and middle cerebral artery, they are extremely susceptible to hypoxic-ischemic states (Roland et al., 1986). According to several published reports, birth asphyxia is the major cause of permanent CVI (Luna et al., 1995; McCulloch et al., 1991). While vision loss due to CVI makes examining visuospatial processing difficult, if not impossible, some reports conclude that the majority of HIE survivors recover with little or no visual impairment from damage to these areas (McCulloch et al., 1991). This conclusion, however, does not rule out the possibility that subtle visuospatial deficits may persist, even in apparently "recovered" survivors. Whether or not visuospatial deficits are related to or independent of CVI has not been determined.

While visual impairments have been studied and reported extensively in the literature, higher cortical visuospatial processing has not received equal widespread attention. The majority of research addresses the impact of asphyxia on cortical visual impairment (Casteels et al., 1997; Chen, Weinberg, Catalano, Simon, & Wagle, 1992; McCulloch et al., 1991; Roland et al., 1986; van den Hout et al., 2000), but only a

handful of studies have examined visuospatial processing. Robertson et al (1985) reported on the Visual-Motor Integration Test (VMI) as an indicator of precision and coordination between visual input and motor output in children 3.5 years of age. Not surprisingly, the study reported a correlation between an abnormal examination at discharge and lower scores on the VMI for the total population of children with HIE. Additionally, stage of HIE also correlated well with performance in visual-motor tasks. This study did not, however, report a control group. Robertson et. al. (1988) subsequently published a second study three years later examining 5.5 year olds with either mild or moderate encephalopathy and compared them with the two groups mentioned above in the language development section. In this study they found that, again, neurological symptoms indicative of neonatal encephalopathy at discharge for any stage of HIE correlated with a significant delay in performance on the VMI. Additionally, children with moderate encephalopathy had significantly lower scores than mild, NICU graduates and normal peers (Roberston & Finer, 1988).

The third study by Robertson et al (1989) again reported that all children with a documented history of HIE (n=145) performed significantly worse on visual-motor integration tasks than children in the control group (n=155). But when examining children without obvious impairment (i.e. cerebral palsy, deafness, convulsive disorder, and cognitive delay), there was no difference in visual-motor integration from normal peers. A fourth study further confirmed the delays found in visual-motor integration and was able to predict outcome of a second cohort based on the outcome of the previous cohort (C. Robertson & Grace, 1992). Again, however, Korkman et. al. (1996) reported that while a control group of healthy children outperformed an asphyxia group on the

VMI and visuomotor precision test of the NEPSY, the difference was not significant. Additionally, as reported above, visuospatial deficits tended to co-occur with language deficits.

In summary, visuospatial abilities are mediated by injury in other regions of the visual system. Inadequate functioning in either the visual pathways anterior (ophthamological deficits) or posterior (CVI) to the lateral geniculate nuclei have been commonly reported in children with a history of HIE and can lead to interference of visuospatial processing abilities. As a result, visual loss secondary to these lesions is important to rule out prior to assessing visuospatial capabilities. Information available regarding visuospatial performance does indicate that both hospital discharge examination and HIE severity correlated with performance on the VMI. Korkman, however, found that children with less severe forms of HIE performed similar to control children. Interestingly, when children with more severe neurological impairments were excluded from analysis in both Robertson et. al.'s (1989) and Korkman et. al.'s (1996) examinations, HIE children's visual-motor integration performance was no different from the control groups.

Attention and executive control. Surprisingly, there appears to be less literature that addressed HIE and attention and executive control (i.e. self-regulation, selective and sustained attention, planning, and flexibility in thinking). Only three studies specifically addressed attention and concentration abilities. Robertson et al (1993) reported that of the three stages of HIE, the group most at risk for future academic and neuropsychological difficulties because of attention impairments are the nondisabled moderate encephalopathy survivors. The reason they are considered most at risk is because these

children appear medically identical to normal children and children with a history of mild HIE, but neuropsychologically they are different. In this report, these researchers indicated that these children perform consistently worse in tasks that require attention than both normal and mild HIE children (Roberston & Finer, 1993). Korkman et. al. (1996), however, reported that while sustained concentration was essentially similar for normal and HIE children, normal children did outperform HIE children on tasks involving inhibition and control on the NEPSY, though the difference was not significant.

A more recent report reviews the altered biochemistry in the striatum that accompanies hypoxic-ischemic injury and the role it plays in the development of Attention Deficit Hyperactivity Disorder (ADHD) (Toft, 1999). The author attempted to give an explanation for ADHD based on the increased sensitivity of striatal neurons to perinatal insults, however, there was only animal research provided in support of the author's hypothesis. However, the author did recognize and acknowledge the loose association of animal hyperactivity following injury to human ADHD. Regardless, the hypothesis proposed is that elevated striatal lactate secondary to hypoxia "may impair the formation of frontostriatal circuits at a critical stage of development and may play a role in the pathogenesis of the behavioral disturbances observed in a proportion of children with a history of perinatal adverse events" (Toft, 1999). While the report did not specify any experimental support connecting ADHD to birth asphyxia, the hypothesis is noticeably compelling and the author appeared interested in finding empirical support for this connection.

In summary, Robertson and colleagues (1993) reported that nondisabled children with a history of moderate HIE are at a greater risk for future academic and

neuropsychological difficulties, secondary to unidentified attention deficits, relative to both disabled moderate and severe HIE children and less impaired mild HIE children. In contrast, Korkman et. al. (1996) reported that while there was some impairment of inhibition and executive control in an asphyxia group, these children performed similar to normal children on tasks of sustained attention. Furthermore, Toft (2001) published a recent study indicating that HIE may mediate the pathogenesis of ADHD. While the review outlines a theory that is intended to help explain ADHD, the implications of the theory are only partly related to attention and executive control issues. Thus, it is important to keep in mind that ADHD is different then executive function or just attention.

Sensorimotor. There is very little research that directly addresses the relationship between perinatal asphyxia and sensorimotor impairments. One study directly addressed sensorimotor functioning, such as fine motor speeds, and imitation of rhythmic and sequential hand movements. In this study, Korkman et. al. (1996) reported findings that indicate children with a history of less severe forms of birth asphyxia and HIE performed at an equivalent level as normal healthy children on sensorimotor tasks of the NEPSY. Implications of these researchers' findings are discussed in the above section on memory and learning.

Exploratory Covariate Main Effect: Time Since Injury

There is no evidence in the HIE literature that directly addresses the amount of time that passes following the injury and how this may co-vary with neuropsychological impairment. This may be partly do to the lack of research in general on long-term outcome and also partly because the injury occurs at a very unique time in life, birth.

Because of the nature of this period of vast developmental changes it is very difficult to track and retest functioning across time from birth to early childhood on specific neuropsychological tasks. Or there may be no report of covariance of time because it may have no affect on impairment, which would result in rather consistent and stable deficits across different age groups. For early childhood head injuries, however, there is some indication from the literature that neuropsychological impairments do improve over time, especially within the first two years, even though performance deficits will persist relative to normal children with uncomplicated birth histories (Fay et al., 1994). Thus, it will be interesting to see whether the amount of time that has passed since the hypoxic-ischemic injury co-varies with future outcome performance on diverse neuropsychological tasks between different age groups.

Problem statement

In summary, there is evidence that specific neuropsychological modalities are affected by early perinatal hypoxic-ischemic states. Furthermore, some areas are affected more than others, specifically, visuospatial and language processing regions. Many attempts have been made to predict basic good/poor outcome of these children, one to two years following discharge, using measures from Apgar scores to cord blood pH level to Sarnat scores to EEG and MRI. These endeavors have been able to predict with some accuracy the fundamental dependency level of the children, such as functionally independent or dependent on caretaker, at an average of two-years follow-up, but not at finer or different levels of functioning between these two endpoints. Neuropsychological functioning in memory and learning, language, visuospatial and sensorimotor processing and attention and executive functioning, is important for academic success but has been

given very minimal research consideration with this population at three to twelve years of age. Additionally, research assessing the prediction of neuropsychological outcome following HIE has little to offer in the way of identified predictors with good specificity, sensitivity and correlational value, especially with nonimpaired moderate encephalopathy survivors (Robertson et al., 1989). Other research, however, using ^{1}H-MRS has revealed promising results in the prognostic utility of ^{1}H-MRS when examining neuropsychological functioning with both adult head injury patients (Friedman et al., 1999) and other pediatric CNS injuries (Brenner, 2001). Thus, the current study proposes to examine neuropsychological outcome following HIE in children three to twelve years of age using ^{1}H-MRS as a predictor.

Hypotheses

1) In the current study it is hypothesized that traditional predictors such as Sarnat score, Apgar score, EEG, discharge status and cord blood pH will correlate with and account for a significant proportion of variance in neuropsychological functioning in three- to twelve-year old children with documented histories of HIE. Neuropsychological areas examined will be memory and learning, language, visuospatial abilities, attention and executive control, and sensorimotor skills.

2) It is hypothesized that metabolite abnormalities measured with ^{1}H-MRS will correlate with and account for a significant proportion of variance in neuropsychological outcome. Specifically, both NAA/Cho and NAA/Cre are hypothesized to positively correlate with impairments in memory and learning, language skills, visuospatial abilities, attention and executive control, and sensorimotor skills. Additionally, both Cho/Cre and lactate are

hypothesized to negatively correlate with impairments in memory and learning, language skills, visuospatial abilities, attention and executive control, and sensorimotor skills.

3) It is hypothesized that metabolite abnormalities, such as decreased NAA/Cho and NAA/Cre, elevated Cho/Cre and the presence of lactate will add to the predictive value of traditional prognostic indicators such as Sarnat score, Apgar score, EEG, and blood cord pH when evaluating long-term neuropsychological outcome. Neuropsychological functioning that will be assessed includes memory and learning, language, visuospatial abilities, attention and executive control and sensorimotor skills.

Methods

Participants

Children with documented histories of HIE born between March 1989 and November 1998 at Loma Linda University Medical Center who were originally enrolled in a study regarding the prognostic utility of ^{1}H-MRS were contacted to participate. Children who have been lost to follow-up, or are in a persistent vegetative state, severely disabled or dead were excluded from the study.

Procedures

Children who were diagnosed with HIE at birth between March 1994 and November 1998 and are consistent with study inclusion and exclusion criteria were contacted by telephone to participate in the study (for Telephone Script see Appendix B). At the time of the telephone contact, parents were told that participants will be given a $10 gift certificate to "Wal-Mart" for participation in the study, however participation was voluntary. At the time of the appointment, parents were asked to read and sign an informed consent form indicating the purpose and objectives of the study (see Appendix B). Assessment of neuropsychological functioning with the NEPSY Developmental Neuropsychological Assessment was then conducted at Loma Linda University Department of Psychology Kids FARE or in the Pediatric Neurology Clinic at Loma Linda University Faculty Medical Offices. All clinical indicators, including ^{1}H-MRS, Apgar score, Sarnat score, EEG, GOS and pH were obtained from Loma Linda University Medical Center Pediatric Neurology Department and Radiology Department neonatal spectroscopy study database.

Instruments

Proton Magnetic Resonance Spectroscopy (^{1}H-MRS). ^{1}H-MRS was used for the evaluation of both the presence of lactate and the metabolite ratios NAA/Cho, NAA/Cre, and Cho/Cre in the occipital region of neonates. Metabolite data was collected between 1 and 49 days after birth (x = 9.22, SD = 9.91). Neonates were transported to the MR scanner once they were determined medically stable by neonatal intensive care unit (NICU) personnel. Sedation with chloral hydrate (40 to 80 mg/kg/dose) was administered when necessary and monitored by NICU personnel. Studies used a circularly polarized head coil in a conventional 1.5T whole body imaging system (Magnetom SP400, Numaris 2.3, Siemens Medical Systems, Erlangen, Germany). MR imaging was used to visualize optimal placement for the spectroscopy study within the brain region of interest. Localized water-suppressed proton spectra were collected using stimulated echo acquisition mode sequence with a repetition time of 3,000 ms, echo time of 20 ms, middle interval time of 30 ms, 1,024 data points and 128 acquisitions and attained in an 8 cm^3 volume of gray matter in the occipital region and repeated with white matter in the parietal region. Corrections of eddy-current-induced phase shifts were made by obtaining eight additional acquisition spectrum at each voxel without water suppression. Localized shimming and optimization of the gaussian pulse amplitude for maximum water suppression was adjusted before the spectra were acquired. Study time average was 60-70 minutes per neonate.

NEPSY Developmental Neuropsychological Assessment (Korkman, Kirk, and Kemp, 1998). Neuropsychological information was obtained by administration of

the NEPSY, an instrument that assesses children ages 3 through 12 years of age (Korkman, Kirk, & Kemp, 1997). The test consists of 27 subtests that evaluate 5 functional domains: (1) memory and learning, (2) language, (3) visuospatial processing, (4), attention and executive control, and (5) sensorimotor abilities. The five domain scores each have a mean of 100 and a standard deviation of 15.

Standardization of the NEPSY was based on a sample of 1,000 children ages 3 to 12 years of age. One hundred children were stratified into each of the ten age groups. Each age group had 50 males and 50 females and the median age for each group was the fifth month. Sample selection was determined to be representative of children living in the United State according to race/ethnicity, parent education and geographic region according to the US Census Bureau. The NEPSY is reported to have good content validity based on its ability to measure and assess neuropsychological functioning of children in the five domains that are consistent with A.R. Luria's neuropsychological theory. Revisions of content were made on the basis of the impact of neurological disease on the developmental of memory and learning, language, visuospatial skills, attention and executive control, sensorimotor abilities (Korkman et al., 1997). Construct validity reveals moderate positive correlations between domain scores for children 3-4 years of age and low to moderate positive correlations for children 5-12 years of age. Test authors report that moderate correlations for older children without specific neuropsychological deficits are expected. Subtests between domains have lower correlations than subtests within domains. Correlation between the five NEPSY domains and the Wechsler Preschool and Primary Scale of Intelligence-Revised (WPPSI-R) Full Scale IQ are low to moderate positive correlations ($r = .26$ to $.57$). Correlations of the five NEPSY domains

with the Wechsler Intelligence Scale for Children –III (WISC-III) Full Scale IQ are low to moderately positive correlations (r = .25 to .49). While all NEPSY domains correlated to some extent with the Conners' Continuous Performance Test, with sensorimotor and visuospatial domains obtaining the highest correlations (sensorimotor r = -.28 to .36 and visuospatial r = -.43 to .31) (Korkman et al., 1997).

Results

Descriptives

Forty-nine neonates born at Loma Linda University Medical Center with documented HIE and one control neonate received ^{1}H-MRS between March 1989 and June 1999, and were eligible for inclusion in this study. Descriptive information for the sample population can be found in Table 2.

Twelve neonates died at the hospital leaving 37 patients who were contacted for follow-up participation in the current study. During this contact, 4 (10%) more were excluded from the study for the following reasons: 2 had died and 2 were severely impaired/PVS. Of the remaining 33 contacted for participation, 16 (48%) agreed, 2 (6%) refused to participate, and 15 (45%) were lost to follow-up (i.e. no contact information was available). Of the16 that agreed and made appointments, 9 (56%) were assessed and 7 (44%) did not show for their appointment time. Three (42%) of the no shows rescheduled and 100% of them did not show for the second appointment.

A control patient was included in all correlational data analysis in view of the fact that all clinical values for the control patient were within two standard deviations of the mean for the HIE group. Examination of bar charts and histograms indicates that the control patient was continuous with clinical participants on all variables and thus will serve to contribute to the power of analysis by increasing sample size. Additionally, correlational data analysis examines the relationship between variables along a continuum. Thus, including the control patient in the data analysis will contribute to what we know about those relationships. Descriptive data for those that participated with HIE in relation to the control can be found in Table 2.

Table 2
Descriptive data for sample population and sample that participated.

	Sample Population		Sample Participated		
	N = 49		N = 8 (*control =1)*		
Clinical Variable	Mean	SD	Mean	SD	p
Gestational Age	37.41	3.736	35.625 *(41)*	6.116	.297
Age at H-MRS	8.98	9.72	11.5 *(4)*	15.43	.694
Days Hospitalized	32.73	33.85	36.00 *(5)*	29.34	.958
Days Ventilator	11.77	15.92	12.88 *(0)*	16.55	.948
5 minute Apgar	**5.00**		**5.00** *(9)*		.337
pH	7.083	.235	7.113 *(-*)*	.289	.770
EEG	**2.00**		**2.00** *(2)*		.467
NAA/Cre	.873	.277	.893*(.960)*	.356	.785
NAA/Cho	.628	.247	.631 *(.877)*	.291	.709
Cho/Cre	1.508	.543	1.472 *(1.094)*	.335	.517
Lactate	.52	.50	.50 *(0)*	.53	.324

Italacized = control patient values
* = value not available
Bold = median value

For all nine participants gestational age ranged from 26 to 41 weeks (M = 36.222, SD = 5.995). Age at ^{1}H-MRS reading ranged from 3 to 49 days old (M = 10.67, SD = 14.65). Hospitalization ranged from 5 to 98 days (M = 32.56, SD = 29.32). Only one patient that participated was comatose following injury. This neonate was in a coma for 33 days. Seven (78%) received respiratory support. Number of days on ventilator support ranged from 0 to 44 days (M = 11.44, SD = 16.06). Five minute Apgar scores ranged

from 1 to 9. Blood cord pH ranged from 6.74 to 7.44 (M = 7.113, SD = .289). EEG readings ranged from 0 to 3. Three neonates were classified as Sarnat grade I, four were grade II, none were grade III, and two did not receive Sarnat classification. Metabolite ratios, taken from a voxel in the occipital region, were available in eight of the nine patients. Ratios were as follows: 1) NAA/Cre ranged from .244 to 1.388 (M = .901, SD = .331), 2) NAA/Cho ranged from .189 to .947 (M = .662, SD = .283), and 3) Cho/Cre ranged from 1.094 to 2.068 (M = 1.425, SD = .337). Lactate signals were present in four neonates. Status at discharge, according to the GOS, was normal for three neonates, mild for three, moderate for one, severe for one, and one did not have GOS status. Age at the follow-up examination ranged from 3 years 4 months old to 7 years 8 months old (M = 5 years old, SD = 20 months).

To determine whether the sample that participated is representative of the total sample, an independent t-Test was run to examine differences in continuous clinical variables according to participation status. An alpha level of .05 was used for all statistical analysis. Analysis for equality of variance between those that participated, did not participate (including: no show, refused and lost to follow-up) and those that died revealed significant differences on GOS, $t(46) = -2.576$, $p = .013$. No significant differences were found on any of the other clinical variables. Further analysis was conducted using a Chi-Square analysis to examine difference in GOS between those that participated, died and were lost follow-up was also significant, $\chi^2(10, N = 46) = 43.305$, $p = .000$), $\gamma = .785$. Results indicated that those that have died had a significantly higher GOS score (M = 5.85, SD = .55) when compared with those that participated (M = 2.00, SD = 1.07). Because Sarnat classification is also a categorical variable, a Chi-Square was

used to analyze whether or not there was a systematic relationship between Sarnat classification and participation status. Results revealed that participation did vary in relation to Sarnat classification, with those that participated having a higher proportion of grade I than those that died, $\chi^2(4, N=50) = 12.225$, $p = .016$. Those that did not participate (ie no show, refused, lost to follow-up) did not differ from those that participated on any of the variables, including Sarnat classification and GOS.

Data Screening

The data were first examined for missing data, homoscedasticity, univariate outliers, and normality. With regard to missing neurological data, ^{1}H-MRS data from the occipital region was not conducted for one of the patients for unknown reasons. Additionally, two other participants were missing lactate data, two were missing cord blood pH and one missing Sarnat score, one was missing GOS status, and another was missing five-minute Apgar. In regard to missing neuropsychological data, the Memory Domain score was missing for one child with mild cerebral palsy and limited expressive language due to heavy loading of this domain on oral-motor functioning. Because missing data appeared to be randomly distributed, these cases were deleted from statistical analysis when these variables were utilized. For correlation analyses, the assumptions of homoscedasticity of variance and linearity did not appear to be violated.

To assess univariate outliers and representative nature of those that participated relative to those that did not, boxplots for each of the continuous variables were analyzed according to participation status. Outliers were defined as any value greater than 2.5 standard deviations from the mean. Accordingly, one outlier was identified for number of days in coma for those that participated. This individual patient, who also did not have

^{1}H-MRS data, was the only participant that experienced a comatose state. Examination of the histograms for this variable and other clinical variables indicated that this patient was discontinuous with the rest of the sample for this variable only. All other clinical values were within 2.5 standard deviations. While this one case does pose a threat to the representative nature of the sample, correlational analyses conducted with and without this particular patient did differ slightly for EEG, but not five-minute Apgar, Sarnat, pH, or GOS. Thus, results regarding traditional indicators will include this participant. There were no outliers in any of the neuropsychological outcome variables. To visually assess outliers, boxplots for each neuropsychological area examined are listed below in figures 1 through 5.

Figure 1

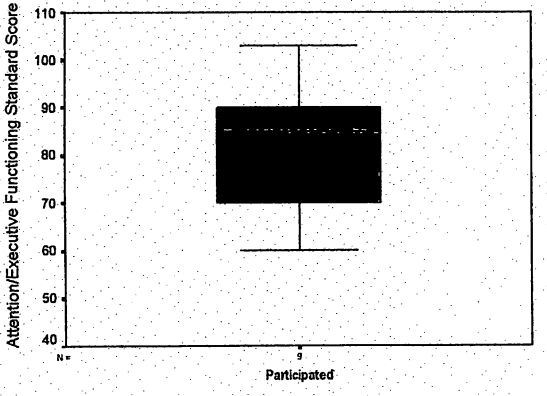


Figure 2

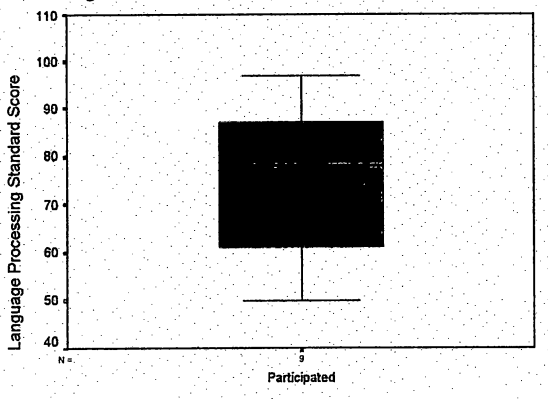


Figure 3

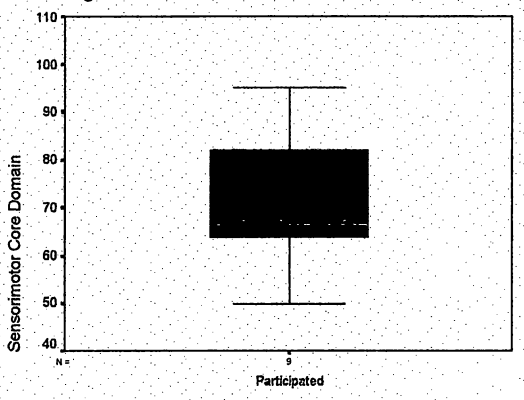


Figure 4

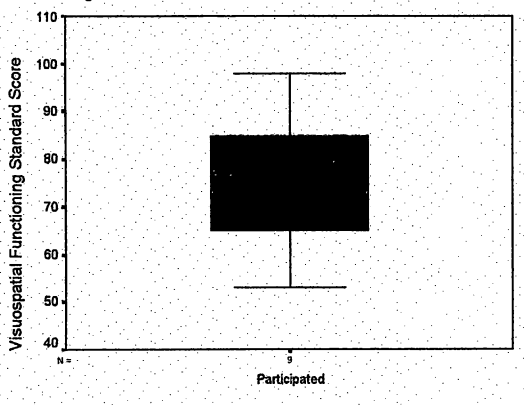
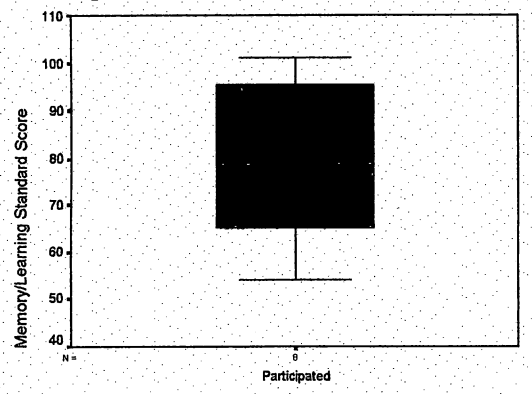


Figure 5



To assess normality, each of the clinical and outcome variables was assessed for univariate normality. Examination of histograms for the sample population revealed

normal distributions for each of the clinical variables. Histograms of the sample that participated revealed that distributions did not follow a normal bell-curve shape. However, this was expected given the small sample size (N=9). Because clinical variables for those that participated did not differ significantly from those that did not participate, with the exception of GOS and Sarnat for those that died, lack of normal distribution poses a threat only in regards to power and not representation of the sample population. Additionally, neuropsychological outcome variables appeared to have a slight positive skew. This was expected, however, due to the fact that lowest standard scores obtainable on the NEPSY created a floor effect, which skewed the distributions slightly. As a result, this does not pose a threat to normality.

Description of neuropsychological outcome variables for the nine participants can be found in Table 3. Results indicate that attention and executive functioning for patients who experienced HIE are below the expected level. Language, sensorimotor, visuospatial and memory and learning were all well below the expected level of performance.

Table 3
Descriptive Data for Neuropsychological Outcome variables

	NORM POPULATION		SAMPLE		
Neuropsychological Domain	Mean	SD	Mean	SD	p
Attention/Executive Function	100	15	82.00	15.50	.008
Language	100	15	75.11	16.98	.002
Sensorimotor	100	15	72.22	14.75	.000
Visuospatial	100	15	75.89	14.95	.001
Memory/Learning	100	15	79.13	17.47	.012

Hypothesis 1- Relation of Neuropsychological Functioning to Clinical Variables

The first hypothesis was that traditional predictors such as Sarnat score, Apgar score, EEG, discharge status and cord blood pH will correlate with and account for a significant proportion of variance in neuropsychological functioning in three- to twelve-year old children with documented histories of HIE. Neuropsychological areas included memory and learning, language, visuospatial abilities, attention and executive control, and sensorimotor skills. It was hypothesized that Sarnat, EEG, discharge status measured by GOS, and pH will negatively correlate with neuropsychological areas. Additionally, it was hypothesized that Apgar score at five minutes will be positively correlated with each neuropsychological area. Based on available literature and understanding of the impact hypoxia has on the watershed regions, it was hypothesized that correlations will be strongest for visuospatial and sensorimotor functioning.

Table 4
Correlation Matrix: Traditional clinical indicators and neuropsychological functioning

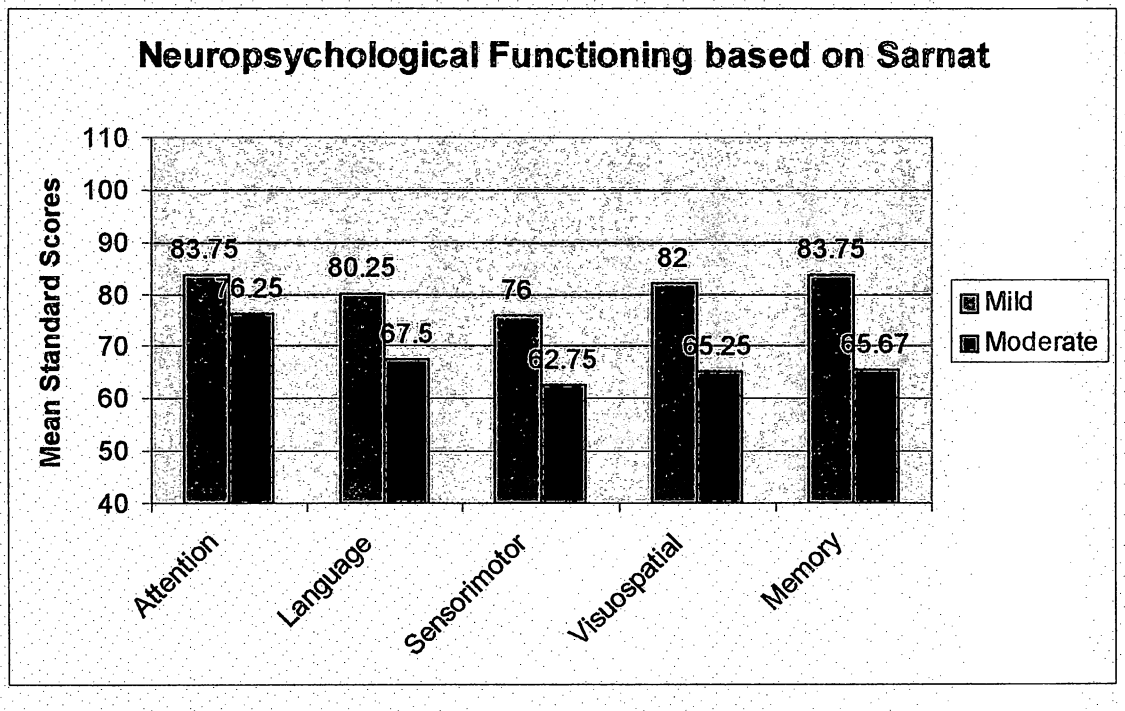
	Attention	Language	Sensorimotor	Visuospatial	Memory
Sarnat	-.262	-.385	-.551	-.629	-.594
Apgar	.169	.219	.248	.122	.506
EEG	-.473	-.725*	-.580	-.630	-.659
pH	.117	.363	-.186	.190	.319
GOS	-.829*	-.349	-.199	-.443	-.329

*Significant at the .05 level

Results are summarized in Table 4. Sarnat score, which is a categorical variable, in this sample included four grade I and four grade II. To examine the relationship between Sarnat classification and neuropsychological outcome, an Independent t-Test

was used. Results indicated that there was not a statistically significant difference between Sarnat grade and any neuropsychological outcome variable. The effect sizes for attention and executive functioning indicated a great deal of overlap in performance (γ = .50). For memory, language, sensorimotor and visuospatial functioning, however, modest to large effects sizes were observed. Language processing skills were better in Grade I (M = 80.25, SD = 17.5) than grade II (M = 67.5, SD = 17.82), γ = .85. Sensorimotor functioning was also better in grade I (M = 76, SD 12.73) than grade II (M = 62.75, SD = 10.34), γ = .88. There was a large effect between grade I and grade to in visuospatial performance, with better visuospatial skills found with Sarnat grade I (M = 82, SD = 13.64) when compared to grade II (M = 65.25, SD = 10.01), γ = 1.12. For memory and learning there was a large effect size indicating significantly better performance on memory functioning for patients with Sarnat grade I (M = 83.75, SD = 13.67) than those with Sarnat grade II (M = 65.67, SD = 15.30), γ = 1.21. Because Sarnat is a dichotomous variable, correlations were also derived. These can be found in Table 4. For each outcome area assessed, there was a trend for higher Sarnat classification to be correlated with lower neuropsychological performance, however, none were statistically significant. These relationships can be found in Figure 6.

Figure 6



Neither five-minute apgar scores nor blood cord pH correlated with any neuropsychological variables. EEG was negatively correlated with language processing suggesting that as EEG data became increasingly abnormal, language functioning began to decline ($r(9) = -.725$, $p = .027$). Figure 7 is a scatterplot of the relationship between EEG and language processing. Attention, sensorimotor, visuospatial, and memory functioning were also negatively correlated with EEG, but did not reach statistical significance. Discharge status as measured by GOS was negatively correlated with attention and executive functioning ($r(8) = -.829$, $p = .011$). Figure 8 is a scatterplot of the relationship between GOS and attention. Correlations with other neuropsychological domains were negative, however, they did not reach statistical significance. Results can be found in Table 4.

Figure 7

Relationship between EEG and Language Processing

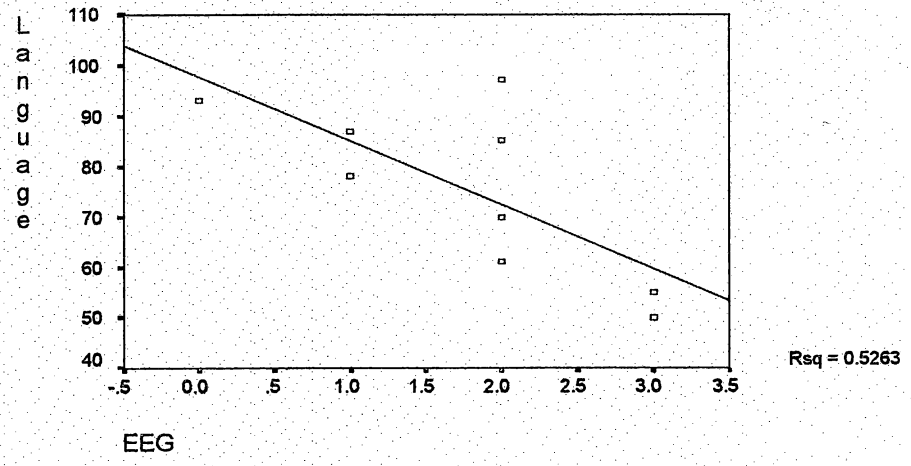
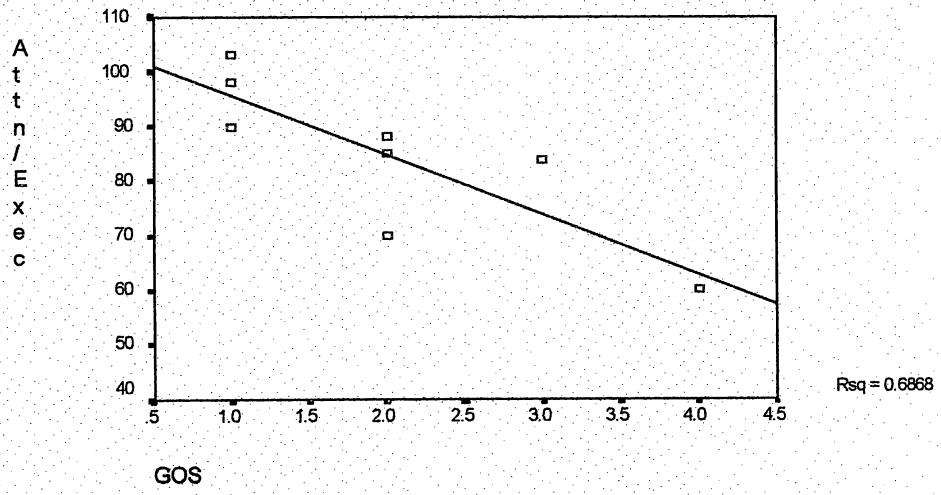


Figure 8

Relationship between GOS and attention



Hypothesis 2-Relation of Neuropsychological Functioning to ^{1}H-MRS Variables

The second hypothesis was that metabolite abnormalities measured with ^{1}H-MRS will correlate with and account for a significant proportion of variance in neuropsychological outcome. Specifically, both NAA/Cho and NAA/Cre are

hypothesized to positively correlate with impairments in memory and learning, language skills, visuospatial abilities, attention and executive control, and sensorimotor skills. Additionally, both Cho/Cre and lactate are hypothesized to negatively correlate with impairments in memory and learning, language skills, visuospatial abilities, attention and executive control, and sensorimotor skills. Because metabolites are measured in the occipital region, it was hypothesized that correlations will be strongest for visuospatial and sensorimotor functioning.

Table 5
Correlation matrix between ^{1}H-MRS indicators and neuropsychological functioning

	Attention	Language	Sensorimotor	Visuospatial	Memory
NAA/Cre	-.069	-.284	-.094	-.089	-.277
NAA/Cho	.190	.050	.266	.281	.183
Cho/Cre	-.626	-.739*	-.721*	-.825*	-.790*
Lactate	-.849*	-.789*	-.722*	-.873*	-.821*

*Significant at the .05 level
**Significant at the .01 level

Results for the second hypothesis can be found in Table 5. There were eight participants with complete ^{1}H-MRS data. Lactate in the occipital region was negatively correlated with all five neuropsychological outcome variables. In other words, as lactate levels increased, attention/executive, language, sensorimotor, visuospatial and memory functioning decreased. A scatterplot of lactate in relation to neuropsychological outcome can be found in Figure 9. Furthermore, Cho/Cre was negatively correlated with all five neuropsychological outcome variables. All with the exception of attention and executive functioning were significant. As a result, as Cho/Cre levels increased, performance in

language, sensorimotor, visuospatial, and memory abilities decreased significantly. A scatterplot of Cho/Cre in relation to outcome can be found in Figure 10. Neither NAA/Cre nor NAA/Cho were correlated with any outcome measure. Scatterplots for each of these variables can be found in Figures 11 and 12, respectively.

Figure 9

Relationship between Lactate and Outcome

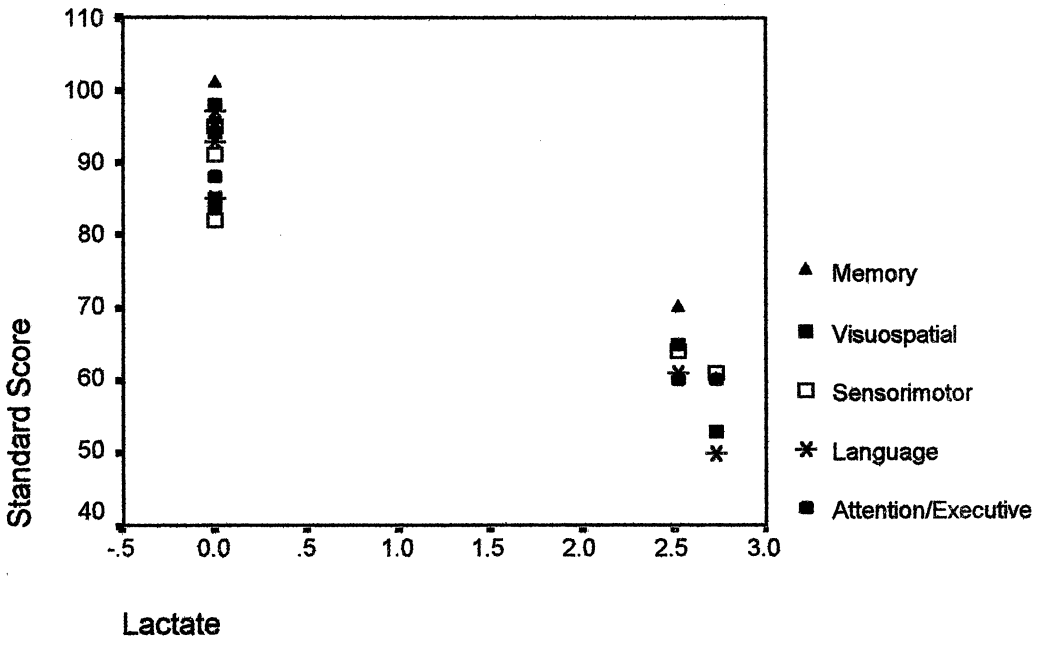


Figure 10

Relationship between Cho/Cre and Outcome

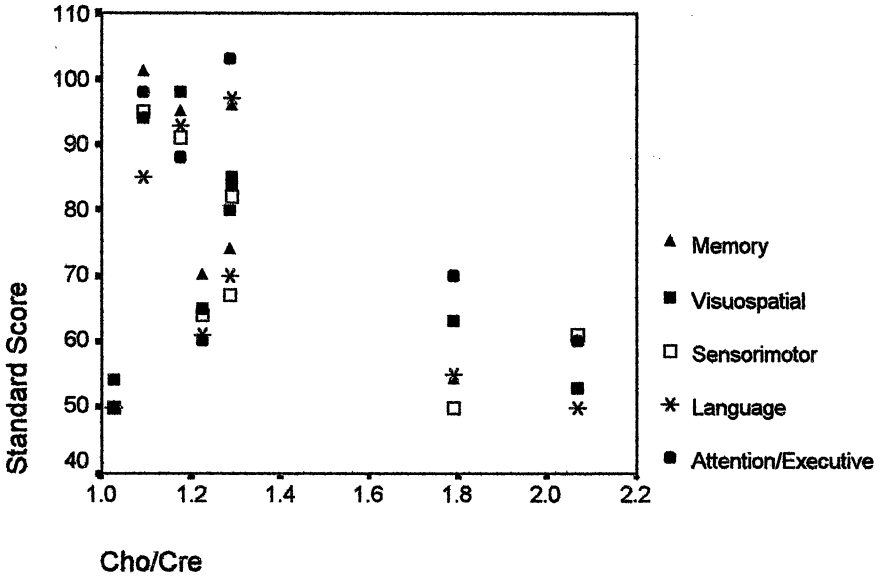


Figure 11

Relationship between NAA/Cre and Outcome

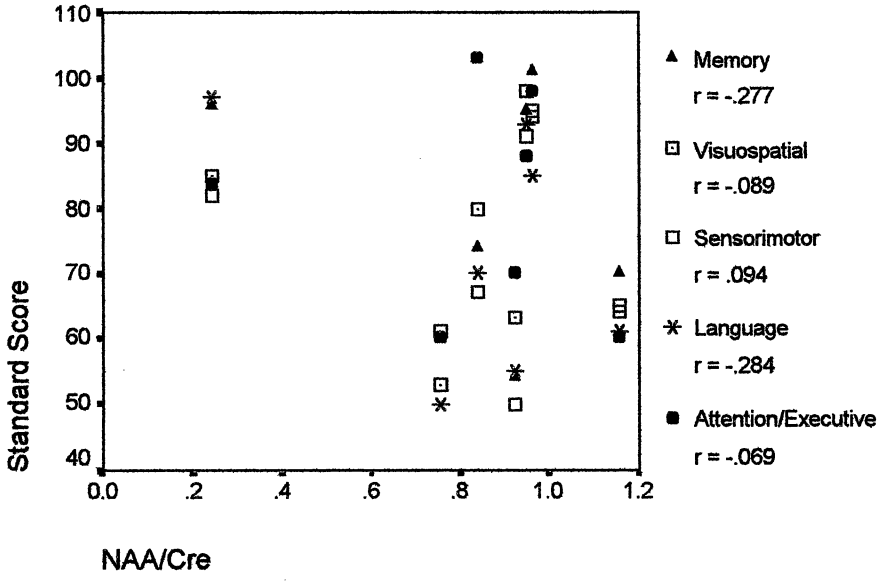
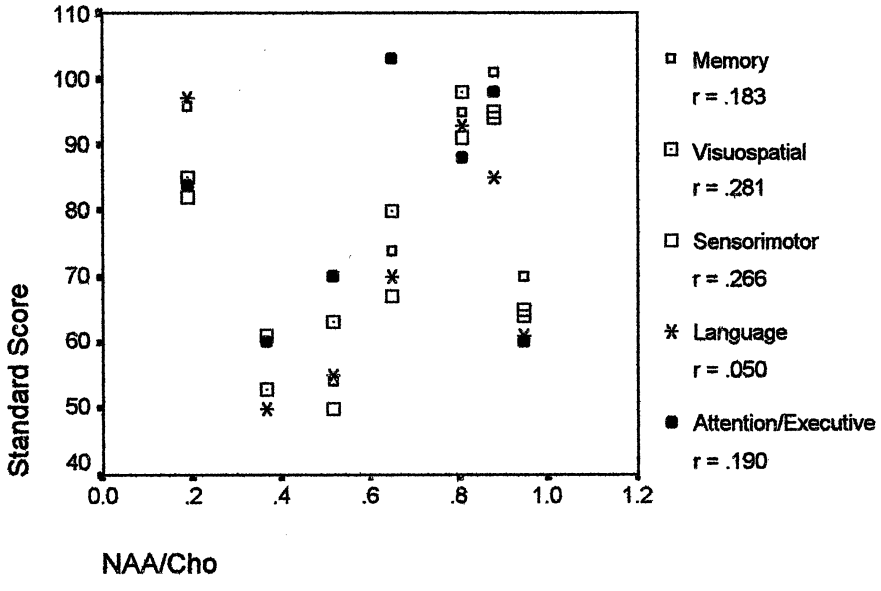


Figure 12

Relationship between NAA/Cho and Outcome



Hypothesis 3 – Relation of outcome to both traditional and ^{1}H-MRS predictors

The third hypothesis was that metabolite abnormalities, such as decreased NAA/Cho and NAA/Cre, elevated Cho/Cre and the presence of lactate will add to the predictive value of traditional prognostic indicators such as Sarnat score, Apgar score, EEG, and blood cord pH when evaluating long-term neuropsychological outcome. Neuropsychological functioning that was assessed included memory and learning, language, visuospatial abilities, attention and executive control and sensorimotor skills. Do to the large number of participants with missing values for predictor variables and small sample size, a hierarchical regression model partialling out traditional clinical indicators first to determine unique contributions metabolite data has on explained variance within neuropsychological outcome could not be completed.

Addendum

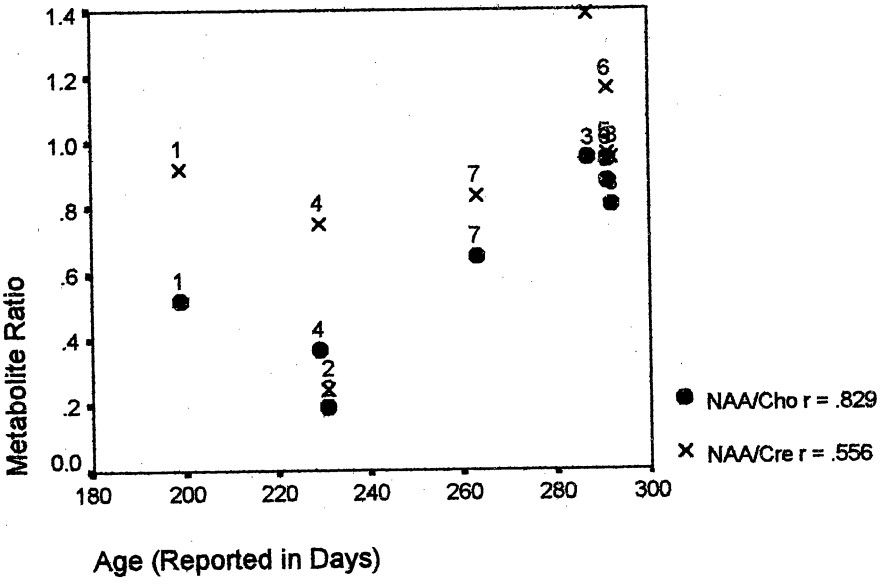
Due to the small number of participants, an addendum was added to the study protocol, which included those patients with severe impairments and PVS. The two severely impaired patients were contacted to participate. Due to the severity of impairments, these patients were unable to participate in standardized testing with the NEPSY. As an alternative, a Developmental Questionnaire designed to assess the basic functional physical and verbal level of the patients was sent in the mail. One of the two questionnaires was returned. Data from the questionnaire was analyzed qualitatively. Qualitative information confirms that higher Cho/Cre and lactate signals indicates greater neuropsychological impairment.

During data analysis it was discovered that gestational age at the time MRS data was collected correlated with NAA/Cho ($r(8) = .829$, $p = .011$) and correlated mildly with NAA/Cre ($r(8) = 556$, $p = .153$). This finding is consistent with previous research that indicates an increase in both ratios with age (Kimura et. al., 1995; van der Knaap et. al, 1990). This relationship can be seen in Figure 13.

As a result, removing the effects of gestational age at MRS was believed to strengthen the ability to examine the relationship between both metabolite ratios and outcome. However, due to the small sample size, it was not possible to covary out age at ^{1}H-MRS.

Figure 13

Metabolite Ratios and Age at MRS



Discussion

The brain consumes approximately 20% of the entire body's oxygen supply. When hypoxia results in a decrease of oxygen, there is generally widespread cerebral damage that follows. Previous research indicates that nearly 50% of children who experience birth-related HIE develop delays in academic performance (C. Robertson & Grace, 1992). As a result, early identification and intervention is of great importance (Hill & Volpe, 2000). However, traditional clinical predictors do not sufficiently capture the complex biochemical impact HIE can have on later neuropsychological development. Additionally, their ability to explain the variability in outcome is lacking, often with unacceptable false-positive rates (Barbara A. Holshouser et al., 1997).

Recent research using ^{1}H-MRS has been instrumental in identifying early biochemical markers of injury and reveal significant correlation with neurological outcome. However, previous reports on outcome have been limited to six to twelve months of age. As a result, while some articles have reported academic delays, these delays have not been examined in relation to biochemical predictors. Thus, the current study assessed the utility of measuring metabolite ratios at birth to predict neuropsychological development in pre-school and school age children.

In the current study, children with documented HIE performed below the expected level in all five neuropsychological areas, unlike the birth asphyxia group in Korkman et al.'s (1996) report. Sensorimotor functioning, which measures the ability to integrate visuospatial and proprioception skills, was the most severely impaired. Language development and visuospatial skills were also quite vulnerable to hypoxic ischemic injury. It is important to reiterate the difference in inclusion and exclusion

criteria between these two studies. While Korkman et al (1996) included 36 children, only 13 of which had met the criteria for HIE, the current study included only children with documented histories of HIE. Furthermore, while Korkman et. al (1996) excluded children with disabilities, such as mental retardation or cerebral palsy, the current study included children with disabilities by qualitatively examining outcome in relation to clinical indicators. The differences in sample selection between the two studies make comparison exceedingly difficult. If the current study had included more children who did not meet the HIE criteria, results may have been similar to Korkman's.

Clinical Variables

The first study hypothesis related to traditional clinical measures of HIE and their relationship to neuropsychological outcomes. Clinical measures of import, as supported by previous research, included EEG, GOS, pH, apgar and Sarnat classification. Previous research indicates significant differences between Sarnat grades and neuropsychological outcome. Studies that have examined the neurological development report that the mild and moderate groups generally have similar and overlapping neurological outcomes. Interestingly, studies that have looked at the neuropsychological development indicate that there are significant differences between the mild and moderate groups. While the current study did find significant differences between the mild and moderate groups, these were not statistically significant possibly due to the small sample size. Even so, the magnitudes of the effect sizes support the practical significance of these differences.

With nearly every neuropsychological area examined, grade II performance dropped 1 SD below grade I. Sarnat appeared to have a greater effect on memory and

learning than visuospatial functioning. Patients with Sarnat grade II (i.e. moderate HIE) dropped, on average, 18 points (>1 SD) below Sarnat grade I (i.e. mild HIE) in memory performance. Furthermore, Sarnat grade II patients scored, on average, 17 points below grade I patients on visuospatial skills and 13 and 14 points below in language processing and sensorimotor performance, respectively. Performance on attention, language, and sensorimotor functioning was better for patients with Sarnat grade I compared to grade II, however, these differences elicited a great deal of overlap in performance.

It is possible that the relative sensitivity that memory and visuospatial functioning have to hypoxia stems from their locations within the cortex. A great deal has been reported indicating that tissue within the boundaries between the anterior and middle and posterior cerebral artery blood supply are the most vulnerable to hypoxic insults (Volpe, 2001). One area within the watershed region between the anterior and middle arteries, the hippocampus, is reported to be responsible for encoding, storing and retrieving information in memory. Since the visual system has projections throughout the brain, several areas may contribute to and be involved with visuospatial deficits following asphyxia. First, portions of the primary visual cortex and optic radiations are located in the watershed region between the posterior and middle cerebral artery supply, which may partly explain why impairments in visuospatial skills are consistently noted. Secondly, it is also possible that the visuospatial deficits observed are a result of the relative vulnerability regions of the primary visual cortex in the occipital lobe also have to global hypoperfusion (Volpe, 2001). Thirdly, periventricular leukomalacia (PVL), hemorrhagic lesions in the posterior parietal region, is commonly reported in premature neonates (Roscigno, 2002), and also related to deficits in visuospatial functioning (Sans et al.,

2002). Because of the immature status of a premature brain, changes in cerebral blood flow and pressure, such as those found during hypoxic-ischemic events, place these infants at a significant risk for developing PVL and visuospatial deficits (Volpe, 2001).

It is important to note that although patients with grade I outperformed grade II, these patients still performed below the expected level in all areas when compared to the normative population. Thus, although they may appear less affected, patients with grade I continue to have delays. Furthermore, both groups had the greatest impairment in sensorimotor functioning, suggesting that pontine, cerebellum and frontoparietal cortical areas may be more susceptible to injury than others. Some research suggests that the posterior limb of the internal capsule, white matter tracts that carry afferent sensory information, is also more susceptible to injury (A. J. Barkovich et al., 2001), which may shed light on the sensorimotor deficits. Furthermore, pontosubicular necrosis, apoptosis of the pontine gray matter and the subiculum of the hippocampus, has been frequently reported following perinatal asphyxia and hypoxemia (Ahdab-Barmada, Moossy, & Painter, 1980; Aso, Scher, & Barmada, 1990; Mito, Kamei, Takashima, & Becker, 1993; Rossiter, Anderson, Yang, & Cole, 2002). As such, sensorimotor impairments might be partly explained by neuronal necrosis of these brainstem areas.

Previous research regarding the prognostic utility of apgar scores has been mixed with some reporting them as good predictors of cerebral palsy (Nelson & Ellenberg, 1981), and others reporting them as a very poor indicator of outcome (Martin et al., 1996). In the current study, there were positive relationships between apgar and outcome suggesting that five-minute apgar scores were higher for patients with better neuropsychological performance. These trends, however, were no more consistent than

chance. This is not surprising given previous mixed reports of apgar's utility in predicting outcome.

The same is true for cord blood pH. Previous research is inconclusive regarding the ability to examine pH levels and predict outcome. In the current study, there was no relationship between pH and outcome. It is suggested that this may be partly due to the timing of pH measurement. If taken following administration of medications to counteract the damaging effects of cardiac arrest, measurements may be inaccurate and unrelated to the severity of arrest.

EEG, on the other hand, has been previously reported as a good indicator of neurological outcome (Pressler et al., 2001), and was also found to be a good predictor of neuropsychological outcome in the current study. EEG was negatively correlated with each area of neuropsychological functioning, suggesting that as EEG became more abnormal performance on the neuropsychological evaluation decreased. While statistical significance was attained only for language processing performance, with 52% of the variance in language explained by EEG, other areas of functioning attained practical significance. EEG accounted for 43% of the variance in memory and learning, 39% in visuospatial performance, 34% in sensorimotor, and 22% in attention and executive functioning. EEG may be a more robust clinical variable because it is a more direct measure of brain activity than say pH, apgar, GOS, and Sarnat. Because brain wave activity is directly measured from cortical neuron activity, there is a greater likelihood that it would be a better indication of neurocognitive processing abilities than the other clinical variables. Additionally, location of abnormal EEG patterns could suggest areas of impairment ad hoc. In the current study, EEG data was a categorical variable stratified

into normal, mild, moderate, and severely abnormal. As a result, location of abnormal patterns could not be identified and correlated with outcome. Furthermore, while several children presented with mildly abnormal EEG's, the actual location and type of abnormality was not available in the data. These children also showed the greatest variability in outcome. The reason for this may be related to brain location of the abnormal activity. One child may have exhibited mildly abnormal brain waves in the temporal lobe, while another may have been found in the occipital lobes. As a result, one child may have more language impairments in relation to the abnormal temporal lobe activity than the other child who may have demonstrated very little language impairment, although both demonstrated mildly abnormal EEG's. As a result, it would be important to examine in more detail the location and type of abnormal EEG activity recorded in relation to neuropsychological functioning.

Although GOS is not a traditional indicator but an outcome measure (measured at six to twelve months of age), it was interesting to see how well it correlated with some of the neuropsychological outcome measures and not others. Of interest is the high correlation between GOS and attention and executive functioning. For the eight patients with GOS data, it seems that the ability of each infant to connect with and attend to the physician was somewhat consistent and predictive of the ability to attend and focus as a preschool and school age child. It is likely that physicians can infer a child's ability to attend from behavior observed during infancy. Simultaneously, it may be more difficult to infer language, sensorimotor, visuospatial and memory functioning from these same temperament characteristics. Furthermore, the ability to attend to the environment may be more susceptible to hypoxia than other neuropsychological areas.

Additionally, the finding that age at time of testing was not correlated with outcome suggests that neuropsychological development does not change as a function of time since injury. Furthermore, this could indicate that neuropsychological deficits observed at an early age are relatively stable over time. Because of this finding, early diagnosis and intervention is important for the academic success of each individual child with HIE.

^{1}H-MRS Variables

The second study hypothesis was to examine the relationship between H-MRS metabolites and neuropsychological outcome. Based on previous research findings, it was expected that neuropsychological functioning would be impaired when there was a decrease in NAA/Cre and NAA/Cho. Additionally, based on previous research, it was anticipated that neuropsychological functioning would be impaired when Cho/Cre and lactate were elevated. The current study did not find correlations with either NAA/Cre or NAA/Cho for any area of neuropsychological outcome. It did, however, find strong correlations between both lactate and Cho/Cre and neuropsychological outcome.

As for NAA/Cre and NAA/Cho, it could be that both of these ratios are less specific indicators of neuronal injury than lactate and Cho/Cre. Previous research has reported mixed results with NAA/Cre and NAA/Cho. Some studies report lower levels of both ratios in infants with poor outcomes (Peden et al., 1993), while other studies found that NAA/Cre levels in infants with HIE were no different than healthy controls (Hanrahan et al., 1996; Penrice et al., 1996). The reason for the conflicting results is not clear. Several studies have reported that NAA/Cre and NAA/Cho change as a function of age (Kimura et al., 1995; Penrice et al., 1996; van der Knaap et al., 1990). What these

studies indicate is that both NAA/Cre and NAA/Cho increase with age. It might be that since these metabolite ratios vary as a function of age, results would depend on the amount of statistical control applied in each study (or the variability in gestational age plus days to ^{1}H-MRS) when examining the relationship between NAA/Cho and NAA/Cre and outcome. In the current study, gestational age ranged from 26 to 41 weeks and days to ^{1}H-MRS ranged form 3 to 49 days. Additionally, in the current study, gestational age at ^{1}H-MRS was correlated with NAA/Cho, $r(8) = .829$, $p = .011$, but not NAA/Cre $r(8) = .556$, $p = .153$, although there was a clear trend of increasing NAA/Cre with increasing age. If the effects of age could have been controlled statistically, NAA/Cho and NAA/Cre might have elicited greater correlations with outcome. However, sample size limitations did not permit this type of analysis to be conducted.

Another possibility for a lack of correlation could be related to the influence one case has in a small sample. This one patient was born at 26 weeks gestational age and also elicited the lowest values for both NAA/Cre and NAA/Cho. Discussed also in the study's limitations section, this one patient did not fit the trend of the other eight patients and may have subsequently thrown off all statistical analysis. Indeed, with this one patient excluded, NAA/Cho had positive correlations with outcome, with the proportion of variance explained ranging from 10% for attention to 48% for memory. Correlations with NAA/Cre were positive but did not account for a large amount of variance.

While there are fairly good norms available for term neonates and infants, there is less known about what are appropriate levels for the preterm neonates. Since references ranges were unavailable for this gestational age, it was exceedingly difficult to interpret these values in light of the good neuropsychological outcome for this patient. Age

appropriate changes may partially explain why this child performed well in spite of demonstrating the lowest NAA/Cre and NAA/Cho ratios in the sample. As such, development of reference ranges for preterm neonates would be clinically useful.

Conversely, Cho/Cre and lactate were highly correlated with outcome. These two variables were able to predict neuropsychological outcome better than other traditional clinical indicators. Previous research reports that lactate is the most consistent biochemical indicator of neurological outcome (S. Ashwal et al., 1996; Stephen Ashwal et al., 1997; Barbara A. Holshouser et al., 1997; Shu et al., 1997). In the current study, lactate was found to be a good predictor of neuropsychological outcome. In fact, not only was lactate significantly correlated with each of the five outcome areas examined, it was the only indicator predictive of a good outcome for one child. While most other clinical indicators for this one patient suggested a poor prognosis, including apgar, GOS, and discharge status, this patient did not demonstrate the presence of cerebral lactate. Furthermore, this child was in a coma for approximately 33 days and the parents were counseled on removing life support. In spite of this, a full recovery was made and the child's neuropsychological performance was in the normal range. Since lactate is the by-product of metabolic activity occurring in the absence of oxygen, the detection of this substrate indicates that the severity of hypoxia was sufficient enough to deplete neural cells of oxygen (Volpe, 2001). In any case, because lactate was highly correlated with each neuropsychological outcome area, this suggests that the presence of lactate may be an ominous finding.

Cho/Cre was also found to be highly predictive of neuropsychological outcome, with four of the five outcome areas statistically significant. In each case, higher levels of

the ratio were correlated with greater impairments. Cho/Cre was correlated with attention, but it was not as strong a correlation as with other areas. Because ratios were taken from the occipital region, this may suggest that attention and executive functioning has little to do with occipital lobe functioning. It is interesting to note that both lactate and Cho/Cre had the strongest correlations with measures that tapped into visuospatial processing abilities (visuospatial subtests), which is consistent with the location of the primary visual cortex within the occipital lobes. Furthermore, development of visuospatial abilities occurs sometime during the first year. It is likely that HIE injuries at birth can subsequently redirect or hinder cell migration. As a result, a hypoxic insult to cells that have not fully developed specialized skills, such as visuospatial processing, may have permanent affects on those skills. This idea is supported by research indicating that skills that have already been established appear to be more resilient to injury (Spreen et al., 1995).

Limitations

There were several limitations in the current study. Most were related to small sample size. First, there was not a large enough sample size to run multiple regression analyses. As a result, comparison of the unique contributions to variance H-MRS makes in relation to traditional predictors was not possible. Secondly, the sample size also adversely affected the amount of power available to reject the null hypothesis with most of the correlations.

Additionally, due to the small sample size, the impact each case has on statistical outcome is exponential. While no metabolite data was greater than 2 standard deviations from the sample mean, there was one patient who obtained some of the lowest values for

NAA/Cre and NAA/Cho but also had some of the highest scores on the outcome measures. When compared to control values, this patient was nearly 3 SD below the mean (2 SD for sample mean) for NAA/Cre and 3 SD below the mean (1.5 SD for sample mean) for NAA/Cho and was in the average to low average range of neuropsychological functioning. Because of sample size limitations, the impact this one case has on the statistical outcome is remarkable.

It is also possible that a restricted range for some metabolite variables worked against statistical power to find good solid correlations with outcome variables. NAA/Cre data was generally within 1 SD of the mean, with the exception of one patient 2 SD below. Four patients were within 1 SD above and one patient 1.5 SD above the mean, two patients were within 1 SD below and one patient was 2 SD below. Given this, there was not a great deal of variability within this small sample. Furthermore, there was not a clear association between this metabolite ratio and neuropsychological outcome. While the two that were 1 SD below the mean performed well below the expected level on the neuropsychological evaluation (in the expected direction), the one patient 1.5 SD above the mean did not perform much better (counter to hypothesis). Furthermore, the patient 2 SD below the mean outperformed each of these and was within average to low average range of neurodevelopment (counter to prediction). Additionally, while there was a great deal of variability in outcome for the four patients who were 1 SD above the mean, there was very little difference in their NAA/Cre ratios to account for this variability. As a result, lower NAA/Cre did not correlate with impaired neuropsychological performance.

For NAA/Cho data, four patients were 1 SD above the mean, three were within 1 SD below, and another approximately 1.5 SD below. Of the four above the mean (high

NAA/Cho), two had neuropsychological functioning within the average (in the expected direction), while the other two performed well below the expected level (counter to prediction). Of the four patients below the mean, two were well below the expected level of neuropsychological functioning (in the proposed direction), one within the average range and the other with functioning ranging from attention in the average range to sensorimotor well below the expected level. This qualitative analysis reveals that while four patients demonstrated outcome in the average range, only half of these had appropriately high NAA/Cho ratios. Simultaneously, of the four patients that had impaired neuropsychological performance, only half of these demonstrated decreased levels of NAA/Cho. As a result, the relationship between NAA/Cre and NAA/Cho and neuropsychological outcome is inconsistent, which lead to difficulty in observing a clear correlation. These findings suggest that either there is no relationship between NAA/Cho and outcome in this sample or that the association is obscured by confounding variables, such as age at H-MRS. It is quite possible, given the conflicting findings in the literature as well, that there are other complex multifactorial biochemical issues that have not yet been addressed.

Finally, regarding study limitations, the study included both preterm and term neonates. There is a great deal of literature suggesting that the neurological sequelae differ for term and preterm neonates, with the latter more susceptible to periventricular leukmalacia. As a result, including both term and preterm in the current study may have potentially confounded the results.

Implications

Given the results of previous research regarding the prognostic utility of ^{1}H-MRS, it is clear that both lactate and Cho/Cre are robust indicators of neuronal injury following perinatal HIE. Furthermore, the current study also attests that these biochemical markers, identifiable within hours following injury at birth, are miniature windows into the future of these children. Neuropsychological development in the preschool and school age children was predicted, with a high degree of accuracy, by whether or not lactate was present at birth. Because lactate has been reported in other research as a robust measure of outcome (S. Ashwal et al., 1996; Stephen Ashwal et al., 1997; Barbara A. Holshouser et al., 1997; Shu et al., 1997), the results of the current study regarding lactate are believed to be generalizable. The same is true for Cho/Cre, although this was not as robust in predicting later neuropsychological outcome. It is clear that a systematic change occurs when there is a decrease in oxygen. The relationship between both NAA/Cre and NAA/Cho and age related changes poses interesting statistical challenges to accurate measurement of these metabolites in the face of hypoxia. With a larger sample, it may be possible to control the effects of age more accurately so that the impact hypoxic-ischemic insult has on outcome can be more precisely quantified.

Future Research

While previous studies have reported that changes in NAA/Cre and NAA/Cho are fairly good indicators of short-term neurological outcome, the current findings suggest that these ratios are not correlated with long-term neuropsychological outcome. In the future, studies should focus on determining whether or not initial declines in NAA/Cre

and NAA/Cho following injury are adequate markers of long-term neuropsychological impairments or if their impact on cortical functioning becomes minimized over time.

Future research should also take into account developmental changes these metabolites undergo during the first few years of life and attempt to curtail the impact this will have on analysis. In addition to developmental changes, the patterns of injury in term and preterm infants tend to differ remarkably. As such, it would be important for future researchers to examine these infants separately. Additionally, future researchers should make certain that they have sufficient statistical power to make their results generalizable.

It would also be interesting in future research to obtain intellectual and achievement measures to examine the occurrence of learning disabilities and determine if these correlate with biochemical changes in different regions of the brain. As a result, voxel location in the brain as well as multiple voxel would shed light on how different some neuronal regions function relative to others and correlate this with neuropsycholgoical functioning. It would also be important to examine the prognostic utility of short versus long echo times in MR spectroscopy. While traditional methods have relied on short echo times, more current research is pointing to the usefulness of longer echo times. A recent study suggests that longer echo times (270msec) with neonates less than one month of age were more predictive of outcome than shorter echo times (20 msec). Additionally, longer echos were better able to detect lactate at any age (B. A. Holshouser, Ashwal, Shu, & Hinshaw, 2000). Furthermore, it is important to further elaborate on the indications of lactate and Cho/Cre on neuropsychological

outcomes with larger sample populations with birth asphyxia and even other instances of hypoxia in children such as carbon monoxide inhalation and cardiac arrest.

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APPENDIX A

Loma Linda University
Department of Psychology
Student Research in Kids F.A.R.E. Laboratory

Informed Consent
For
Neuropsychological Functioning in Children with a
History of Hypoxic-Ischemic Encephalopathy

poses and Procedures

ur child is invited to participate in a research study of neuropsychological functioning children who have experienced birth trauma that resulted in a decrease of oxygen pply to the neonate. The purpose of this research study is to see how children have overed, developmentally, following an injury like the one your child experienced. ditionally, the study will be looking at medical record data to see how well it works in dicting how well children recover.

rticipation in the study will take approximately 1 to 2 hours, during which time your ld will be asked to perform a number of tasks. Some tasks require your child to answer estions with verbal responses. Visual tasks require your child to respond by pointing verbally responding. Other tasks require your child to respond using a piece of paper d pencil. Or you may be asked to fill out a questionnaire that asks questions about your ild's current level of functioning.

sks

iis study is not likely to cause you or your child physical discomfort. If, however, /she becomes restless, agitated or tired due to the length of time involved, we would be ppy to take a break (upon request), re-schedule or quit. Or you may choose not to fill it the questionnaire.

enefits

ou may find the information about your child's current level of functioning to be lpful. Participation can provide the opportunity for free referral and recommendation formation, if necessary. We anticipate that your child's participation will contribute to ir understanding about the recovery of skills as well as areas that may benefit from tervention with children.

____ Initial

____ Date

rticipants' Rights

rticipation in the study is voluntary. As parents, you are invited to be in the room ring testing. You or your child's decision not to participate at any time during the ɔcess will be respected and will not affect present or future care through Loma Linda iiversity. All you need to do is tell the experimenter that you do not wish to continue. that time, we will give your child a $10 gift certificate, and will be happy to discuss ; or her performance on completed tasks.

iestions and Concerns

ɛase feel free to ask questions concerning the study at any time during or after rticipation. Questions you may have following participation can be directed to Kiti eier, Ph.D. at the Department of Psychology, Loma Linda University, (909) 558-8577. you wish to contact an impartial third party not associated with this study regarding y question or complaint you may have about the study, you may contact the Office of tient Relations, Loma Linda University Medical Center, Loma Linda, CA 92354, one (909) 558-4647 for information and assistance.

ɔnfidentiality

l of the information gathered during you and/or your child's participation in this search study is confidential. Any identifiable information obtained will be removed ior to data entry and analysis so that complete confidentiality can be maintained. Any ritten or published document resulting from this study will not disclose your child's entity without your permission.

osts/ Reimbursement

ıere is no cost to you for your child's participation in this study, and as a token of our ɔpreciation, your child will receive a $10 gift certificate to Wal-Mart for participating in e study.

____ Initial

____ Date

ormed Consent Statement

signing below, you are agreeing to the following statements:

I have read the contents of the consent form and have listened to the verbal explanation given by the investigator.
My questions concerning this study have been answered to my satisfaction.
I hereby give voluntary consent for me and/or my child to participate in this study.
Signing this consent document does not waive my rights, nor does it release the investigators, institution, or sponsors from their responsibilities.
I may call Kiti Freier, Ph.D. at (909) 558-8577 if I have additional questions or concerns.
I have been given a copy of this consent form.

_____ _____

rent's Signature Date

_____ _____

rticipant's Signature Date

ave provided a verbal explanation to the child and parent regarding the study and have swered any questions they have or will have to the best of my ability.

_____ _____

vestigator's Signature Date

APPENDIX B

Loma Linda University
Student Research in Kids F.A.R.E. Laboratory

Written Script for Telephone Contact
for
diction of Neuropsychological Outcome following Hypoxic-Ischemic Encephalopathy
Using MR Spectroscopy

llo, my name is Dr. Kiti Freier. I am calling for Loma Linda University Children's spital Department of Neurology. The reason I am calling is to ask whether you would willing to allow your child to participate in a research project that is being conducted. e focus of the research is on the developmental outcome of children with a form of th asphyxia similar to what your son/daughter experienced. This study will attempt to ntify outcome more accurately by looking at specific areas of development such as mory and learning, language, attention, visual organization, and motor functions.

e are also interested in determining if a child's outcome following birth asphyxia can predicted based on medical records, tests and procedures that are conducted and lected at the time of injury. Ideally this research will identify medical information and ocedures that are helpful in assisting parents and professional in understanding tential developmental outcomes.

rticipation if voluntary and if you agree to allow your child to participate, he/she will evaluated at the Loma Linda University Kids F.A.R.E. laboratory by research sistants under the supervision of a licensed psychologist. The entire assessment will e no longer than 1 to 1 1/2 hours. To show our appreciation, at the end of the aluation your child will receive a $10 gift certificate to Wal-Mart as well as a written ort summarizing their performance.

ould you like to schedule an appointment for a researcher to contact you about the tails of the study to see if you wish to have your child participate? At that time, if you ree, we will proceed with the evaluation.