The Role of Proton Magnetic Resonance Spectroscopy in Neonatal and Fetal Brain Research

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The biochemical composition and structure of the brain are in a rapid change during the exuberant stage of fetal and neonatal development. ^{1}H-MRS is a noninvasive tool that can evaluate brain metabolites in healthy fetuses and infants as well as those with neurological diseases. This review aims to provide readers with an understanding of 1) the basic principles and technical considerations relevant to ^{1}H-MRS in the fetal-neonatal brain and 2) the role of ^{1}H-MRS in early fetal-neonatal development brain research. We performed a PubMed search to identify original studies using ^{1}H-MRS in neonates and fetuses to establish the clinical applications of ^{1}H-MRS. The eligible studies for this review included original research with ^{1}H-MRS applications to the fetal-neonatal brain in healthy and high-risk conditions. We ran our search between 2000 and 2023, then added in several high-impact landmark publications from the 1990s. A total of 366 results appeared. After, we excluded original studies that did not include fetuses or neonates, non-proton MRS and non-neurological studies. Eventually, 110 studies were included in this literature review. Overall, the function of ^{1}H-MRS in healthy fetal-neonatal brain studies focuses on measuring the change of metabolite concentrations during neurodevelopment and the physical properties of the metabolites such as T_1/T_2 relaxation times. For high-risk neonates, studies in very low birth weight preterm infants and full-term neonates with hypoxic–ischemic encephalopathy, along with examining the associations between brain biochemistry and cognitive neurodevelopment are most common. Additional high-risk conditions included infants with congenital heart disease or metabolic diseases, as well as fetuses of pregnant women with hypertensive disorders were of specific interest to researchers using ^{1}H-MRS.
Evidence Level: 1
Technical Efficacy: Stage 2

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Magnetic resonance spectroscopy (MRS) is a noninvasive imaging tool that is particularly useful for monitoring brain biochemistry in pathological conditions. MRS can provide valuable information on neurochemical changes that occur in healthy newborns, those at risk for brain injury, or for evaluation of neurological disorders. However, MRS studies can be challenging from an acquisition and quantification perspective in the fetal and neonatal period, given the rapid developmental changes in the immature developing brain. This 2-part review sets out to 1) describe the basic principles and technical considerations of MRS in fetal-neonatal neuroimaging and 2) provide readers with a comprehensive overview of the role and application of ^{1}H-MRS in the study of the fetal-neonatal brain with an emphasis on future directions that improve its accuracy, reliability, and consistency for data acquisition in both research and clinical situations.

Basic Principle

MRS apply the same basic principle as magnetic resonance imaging (MRI) to utilize MR scanners to detect

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radiofrequency (RF) signals from nuclei possessing nuclear spin. The signals arising from nuclei spin in different local chemical environments are separated along the frequency axis due to the difference in the resonant frequency known as chemical shift. For in vivo MRS, the most observed nucleus is the hydrogen proton (^{1}H), followed by carbon (^{13}C), phosphorus (^{31}P), sodium (^{23}Na), and xenon (^{129}Xe).[1,2] Due to a proton's high gyromagnetic ratio, high natural abundance and presence in most molecules in the body, ^{1}H-MRS is the most popular modality for studying neuro-metabolite concentrations in clinical applications. It detects molecules other than water characterized by their distinct chemical shifts for metabolite modeling. It has been commonly used to detect endogenous tissue metabolites. The minimum detectable metabolite concentration is in the order of 0.5–1 mM.[3–5] The area under the resonance peak is proportional to the number of protons of different resonances within one molecule and to concentrations of the metabolites. Other contributing factors including *J*-coupling and echo time (TE) also impact the overall peak areas. Since most in vivo metabolites contain protons, the high sensitivity of ^{1}H-MRS induces an inherent limitation of overlapping metabolite resonances in a narrow chemical shift range, especially for upfield metabolites. The considerable number of overlapping metabolite resonances can make the quantification challenging at clinical field strength.

Quantification

Following data acquisition, spectral quantification is the next most critical step. It is performed directly after preprocessing are complete. To estimate the spectral peak areas of different metabolites, existing methods such as linear combination modeling (LCM), peak fitting, and peak integration have been implemented in many popular fitting algorithms including LCModel,[6] Tarquin,[7] FSL-MRS,[8] and Osprey.[9] A community consensus has recently coalesced and has recommended the use of LCM fitting to quantify metabolite spectra[10] due to its advantage in fitting each metabolite as an individual function called a basis spectrum. In LCM, the acquired spectrum is modeled as a weighted sum of metabolite basis functions (Fig. 1), and the basis set can be generated either by a set of single-metabolite phantoms or by numerical simulation.[11,12] Common metabolites of interest will be introduced in the following section.

Metabolites of Interest

Quantitative ^{1}H-MRS has a broad range of preclinical and clinical research applications. In the brain, changes in metabolite levels can be observed in neurodevelopment, neurodegeneration, neurological diseases, or psychiatric disorders.[13] ^{1}H-MRS also can be a potential tool to detect biomarkers for brain tumors although the prevalence is very low in neonates.[14–16] Metabolites

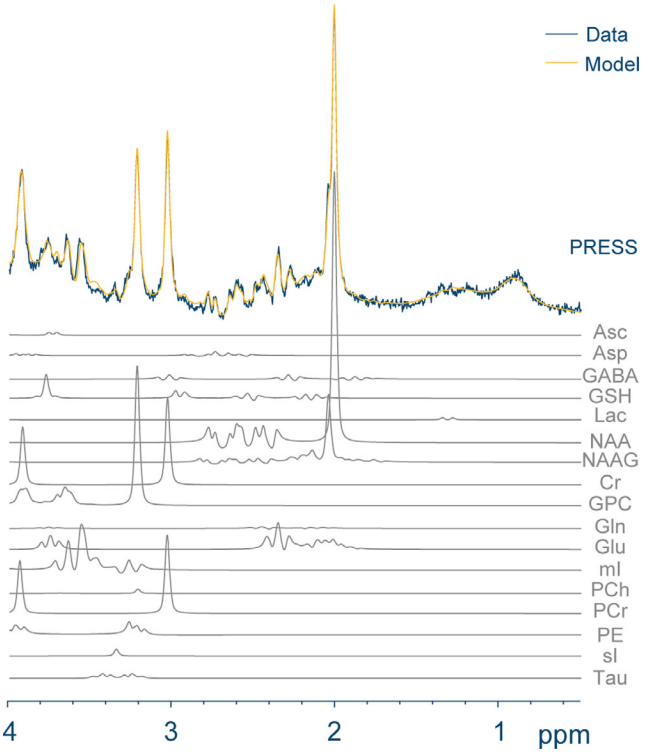

FIGURE 1: A representative basis set applied to model spectral data acquired from a healthy neonate using Osprey. Seventeen metabolite basis functions were included and presented. Linear combination modeling was performed to model the in vivo spectrum (blue) and the resulting fits were shown (yellow). Asc = ascorbic acid; Asp = aspartic acid; GABA = gamma-aminobutyric acid; GSH = glutathione; Lac = lactate; NAA = N-acetyl aspartate; NAAG = N-acetyl aspartyl glutamate; Cr = creatine; GPC = glycerophosphocholine; Gln = glutamine; Glu = glutamate; mI = myo-inositol; PCh = choline-containing compounds; PCr = phosphocreatine; PE = phosphorylethanolamine; sI = scyllo-inositol; Tau = taurine.

of interest with high concentrations including N-acetyl aspartate (NAA), creatine (Cr), myo-inositol (mI), glutamine (Gln), and glutamate (Glu) (collectively as Glx), and choline (Cho) can be observed and quantified from spectra acquired using unedited short-TE localization sequences.[17] NAA, Cr, and Cho have been the most common and conventional metabolites of interest to be investigated since the beginning of in vivo research to study brain biochemistry using ^{1}H-MRS. NAA is an important biomarker for neuronal health and neurodevelopment. It has been often used as an indicator of neuro fiber integrity. Cho has the highest level of concentration during the prenatal, decreases rapidly during early neurodevelopmental stage and becomes stable after birth.[18] Some evidence shows that an increased level of Cho exerts neuroprotective effects to the injured brain and improve brain recovery.[19] Cr is often used to monitor altered energy metabolism as it is a central energy marker and responsible for energy storage and transfer. It is relatively stable in most conditions, so it has been used as an internal reference for comparison to other metabolites.[20] Some metabolites are presented at detectable levels (of the order of 1 mM) but cannot be

resolved due to the limited chemical shift dispersion and overlying signals of more concentrated metabolites at similar resonance frequencies. *J*-difference editing is the most common approach for spectral editing which has been widely implemented in many editing schemes.[21–25] These editing schemes distinguish resonances of low-concentration metabolites from overlying signals. γ-aminobutyric acid (GABA) is one of the most popular metabolites being studied using these editing schemes.[26] It serves as the main inhibitory neurotransmitter in the mature brain. Noteworthy for this current review, it is an excitatory neurotransmitter in the fetus and newborn.[27] Other metabolites of target in edited MRS included glutathione (GSH, another excitatory neurotransmitter besides GABA and Glu),[28] ascorbic acid (Asc, also known as vitamin C),[29] aspartic acid (Asp), lactate (Lac, can also be measured using unedited MRS at TE 144 or 288 msec),[30] phosphorylethanolamine (PE),[31] ethanol (EtOH, an exogenous compound),[32] 2-Hydroxyglutarate (2HG, a biomarker for a subset of gliomas)[14] etc. Characteristics of each individual and clinically relevant compound have been previously described in detail.[33,34]

Technical Challenges

MRS comes with technical challenges, and the wider research community has been working to improve the overall quality of the data acquisition extensively. Early MRS consensus papers have described issues and potential solutions that improve acquisition, shimming and processing to tackle artifacts in data acquired from the general population.[35] In fetal and neonatal ^{1}H-MRS, there are the added difficulties associated with involuntary motions, poor shimming due to the smaller brain volume and the dynamic change of the brain structure and unknown T_1/T_2 relaxation times in brain tissues and metabolites as obstacles that must be addressed to maintain the data quality.

Motion

Motion is one of the most challenging issues to overcome in fetal-neonatal MRS and it can have a pronounced effect that inevitably degrades the quality of data acquisition. Spectra with severe motion will result in spectral distortions, increased linewidths, lower signal-to-noise ratio (SNR; Fig. 2a), unwanted sampling of tissue outside the region of interest (eg, lipid contamination from the skull due to head movements; Fig. 2b), and incoherent averaging and subtraction artifacts due to chemical shifts. This is particularly important for neonatal and fetal scans performed in a non-sedated environment because involuntary head motions happen frequently during natural sleep. Current consensus has recommended three common approaches that can be employed for mitigating motion, including 1) subject immobilization, 2) retrospective correction, and 3) prospective real-time correction using tracking methods. Visual inspection or automatic outlier detection algorithm can be applied to remove corrupted transients. Prospective corrections including acquisition of rapid navigator images between repetitions and optical tracking can precisely monitor subject motion to update the acquisition volume in real-time.[36] Some studies in the older pediatric population applied image-based navigators to mitigate motion artifacts on sick children during MRS acquisitions.[37,38] However, many other consensus recommendations that deal with motion artifacts have not been applied on neonatal and fetal scans.

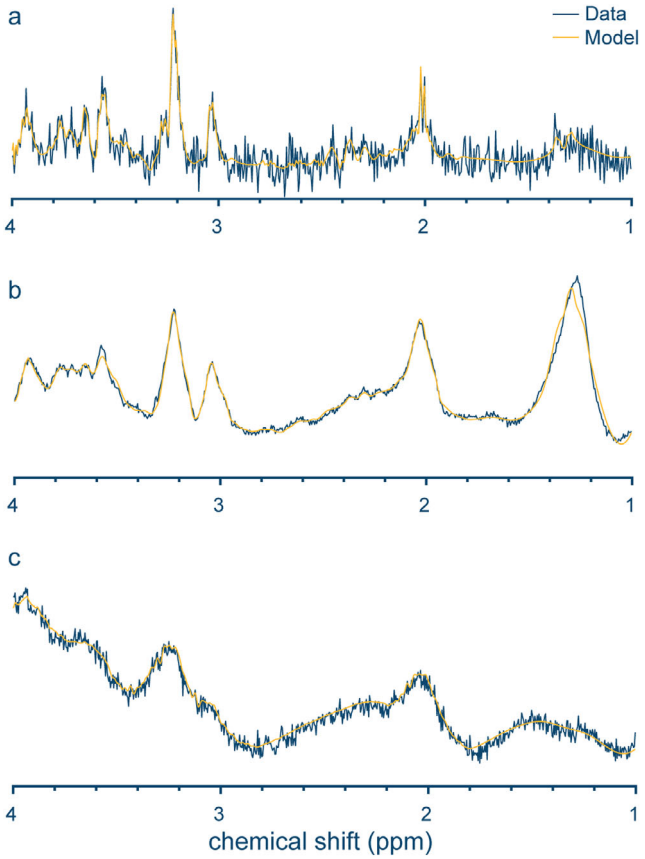

FIGURE 2: A representative in vivo spectrum with (a) low signal-to-noise ratio, (b) lipid contamination, and (c) bad shimming. Data were acquired in the right frontal lobe of neonates using PRESS 3 T (TE/TR: 35 msec/1500 msec; transients: 128).

Shimming

Shimming or B_0 field homogenization reduces field spatial variation using shim coils. It has been a common issue for MRI and MRS. However, MRS is particularly susceptible to bad shimming (Fig. 2c) because it relies on precise measurements of metabolite peaks within a small voxel especially in the developing and irregular brain shape of infants. Impact of B_0 inhomogeneity on localization accuracy leading to incorrect slice profile and spatial position for voxel localization that reduces SNR, broadens spectral width and induces spectral distortions.[39] Real-time higher-order (>first) shim provides greater ability to correct the complex variations due to the increase of spatial complexity with higher order in the linear combination of spherical harmonic functions. In neonatal and fetal scans, it is particularly difficult to achieve a good shim due to the relatively small brain volume and the non-

uniform shape of the targeted brain regions. In addition, fetal scans are further complicated by the spatial location which further deteriorates the shim given the distance of fetal brain tissue relative to coil placement and additional layers of intervening tissue (amniotic fluid, placenta, maternal uterus, and subcutaneous tissues). Repeated or manual shimming and repositioning of the mother may improve the shim quality. However, the effort does not always lead to success.

T_1/T_2 Relaxation

In fetuses and neonates, the T_1 and T_2 relaxation times of white matter and gray matter rapidly change due to the dynamic progression of myelination.[40] T_1 relaxation of white matter increases relative to gray matter and vice versa for T_2 relaxation between the second and third trimester of gestation through the first months of life for infants[41] (Fig. 3). Due to the rapid change of T_1 and T_2 relaxation times, brain regions and tissue segmentations can be challenging in fetuses and neonates. Brain templates generated using the fetal and neonatal populations allow more accurate brain region segmentations.[42,43] For ^{1}H-MRS, metabolite measurements are impacted by some degree of variation due to the tissue compositions of the voxel (i.e., the amount of white matter, gray matter, and cerebrospinal fluid within the MRS voxel). An accurate tissue segmentation within the voxel and the correct values of T_1/T_2 relaxation times of the tissues for fetus and neonate allow tissue corrections that account for the metabolite contribution from different tissue types.[44]

To quantify using LCM, metabolite T_1/T_2 relaxation times can be applied to correct any signal loss during decay. These values were reported in early studies using multiple TEs and TRs in healthy and preterm infants. T_2 relaxation times were higher in NAA, Cr, Cho, and mI in neonates when compared to the adult populations; T_1 relaxation had a similar regressive effect on Cr and mI as a function of age in infants. The inverse was true for NAA and Cho. For preterm neonates, T_1/T_2 relaxation times were reported in post-conceptional age of 37.8 weeks in which results were comparable but noticeably different from those in healthy neonates[45–47] (Table 1). However, in most LCM tools, T_1 relaxation times are assumed to be constants across age and T_2 relaxation corrections are applied using values obtained from adult populations. These data suggest that those assumptions may not be valid during the early developmental stages, nor across disease states.[48–50]

Frequency and Phase Drifts

In fetal-neonatal scans, random translational head motion leads to extra phase shifts[51] in addition to the impact of frequency drifts that deteriorate editing efficiency and co-editing contributions which ultimately induce subtraction artifacts. The overall spectral quality reduces if no correction is being made especially for edited MRS. Prospective real-time movement and frequency corrections mainly apply in MRS of adult population are rare in pediatric studies. Future application of them in fetal-neonatal scans would improve data acquisition by minimizing the impact from frequency and phase drifts.

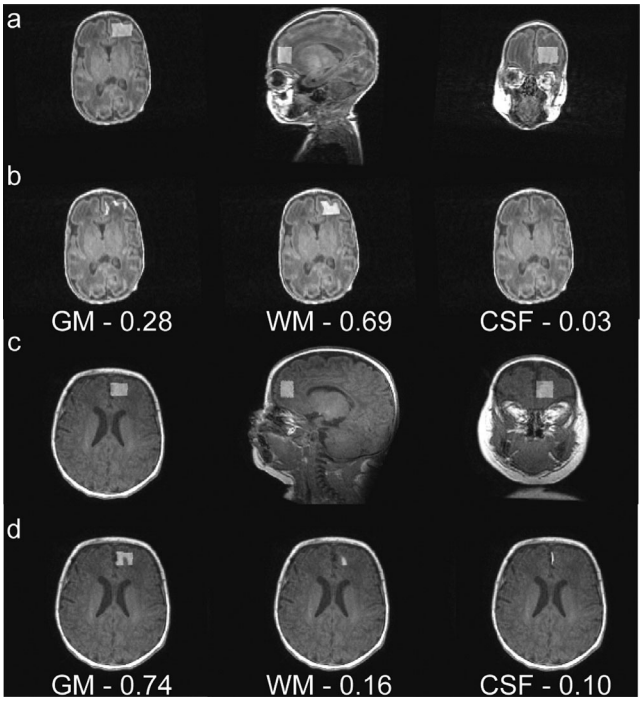

FIGURE 3: T_1-weighted brain images of (a) a preterm neonate (female, GA at MRI—32 weeks) and (c) a healthy term neonate (female, GA at MRI—45 weeks). Panels (b) and (d) are the segmentations of the MRS voxels. As shown in panel (d), contrast between white matter and gray matter is less differentiated as myelinated white matter becomes brighter compared to unmyelinated white matter in panel (b), resulting 16% white matter segmentation in the white-matter-rich right frontal lobe compared to 69% in panel (b) in which the myelination process has been initiated. The rapid changes of T_1/T_2 relaxation times due to the dynamic progression of myelination that induces the lack of contrast between white matter, gray matter and cerebrospinal fluid may led to inaccurate tissue segmentations within the MRS voxel and T_1/T_2 correction for quantification of metabolite concentrations.

Methods

^{1}H-MRS has frequently aided in the investigation of early fetal-neonatal brain development. Previous reviews in neonates and children cover topics for unique metabolites,[52] specific MRS modalities,[53] editing sequences,[34] and older pediatric populations.[54,55] While MRS technologies improve, new tools and new sequences emerge leading to new implementations, improvements, and applications that previous literature may not have covered. To keep members of the MRS community up to date, there is a need to renew

TABLE 1. Clinical Data and Protocol for Studies in T_1/T_2 Relaxation Times (msec) for Healthy Preterm and Term Neonates

	N	Brain Regions	TR (msec)	TE (msec)	Field Strength	Vendor	Sequence	Major Findings (msec)
Kreis et al 1993	77	Mid. and parieto-occipital cortex	1500/3000/5000	30/40/60/90/135/270	1.5 T	GE	STEAM	NAA/Cr/Cho/ml: T_1: 930/1620/1320/1520 (higher Cr/mI and lower NAA/Cho in infants compared with adults) T_2: 524/228/431/301 (all higher in infants compared with adults)
Toft et al 1994	22	Striatum	1600/6000	20/46/92/272	1.5 T	Siemens	STEAM	NAA/rCr/Cho/Inositol: T_2: 324/216/398/76
Kugel et al 2003	84	Basal ganglia	1884/2000/6000	25/136/272	1.5 T	Philips	PRESS	NAA/Cr/Cho/mI/Lac: T_1: 1171/1388/1217/1336/1820 T_2: 499/224/273/68/1022

our knowledge and give an update to the previous review literature based on long-standing established research.[54,56]

Searching Methods

The literature review in PubMed used the following keywords: proton magnetic resonance spectroscopy neonate OR proton magnetic resonance spectroscopy fetus NOT review NOT meta-analysis NOT books NOT documents. The period for publication included in this review was the year 2000 to the year 2023. This was to keep the content and discussion of the manuscript relevant. This review also accounts for landmark publications in the 1990s that made significant findings from the early phase of neonatal MRS usage for clinical applications. These landmark publications were selected for their impact (citations >100) relevant to healthy term or preterm neonates, neurodevelopment, neurological diseases, cardiopulmonary, and metabolite conditions. A separate section of fetal MRS had been included to summarize emerging data on the role of MRS in the developing brain.

Exclusion and Inclusion

PubMed provided 366 results (280 publications between 2000 and 2023 and 86 between 1990 and 1999) using key words mentioned previously. After excluding original studies that did not involve any fetuses or neonate groups within the first 4 weeks of life; in vitro, non-proton MRS, non-neurological/brain, and animal studies were also excluded. Ninety-five papers between 2000 and 2023 and 15 landmark papers between 1990 and 1999 were included in this literature review. They were categorized and presented in three main groups: 1) MRS in healthy neonates, 2) MRS on neonates with neurological disease and illnesses, and 3) MRS on living fetuses. The steps used to identify, include, and exclude papers for this review are presented in a flowchart (Fig. 4). The following sections provide its readers with a comprehensive overview of ^{1}H-MRS for recent research and clinical applications in the fetal-neonatal developing brain.

MRS in Healthy Neonates

Dynamic Change of Brain Metabolites Concentration During Early Neurodevelopment

One of the most predominant roles of fetal-neonatal ^{1}H-MRS is to study the dynamic change of brain metabolites in the developing brain during the early stage of infancy. NAA is one of the most abundant metabolites in the human brain.[54] Several studies showed a positive correlation of NAA concentrations to the increasing gestational age of neonates in the cerebellum, frontal lobe, basal ganglia, and thalamus.[46,57–62] Since NAA is an

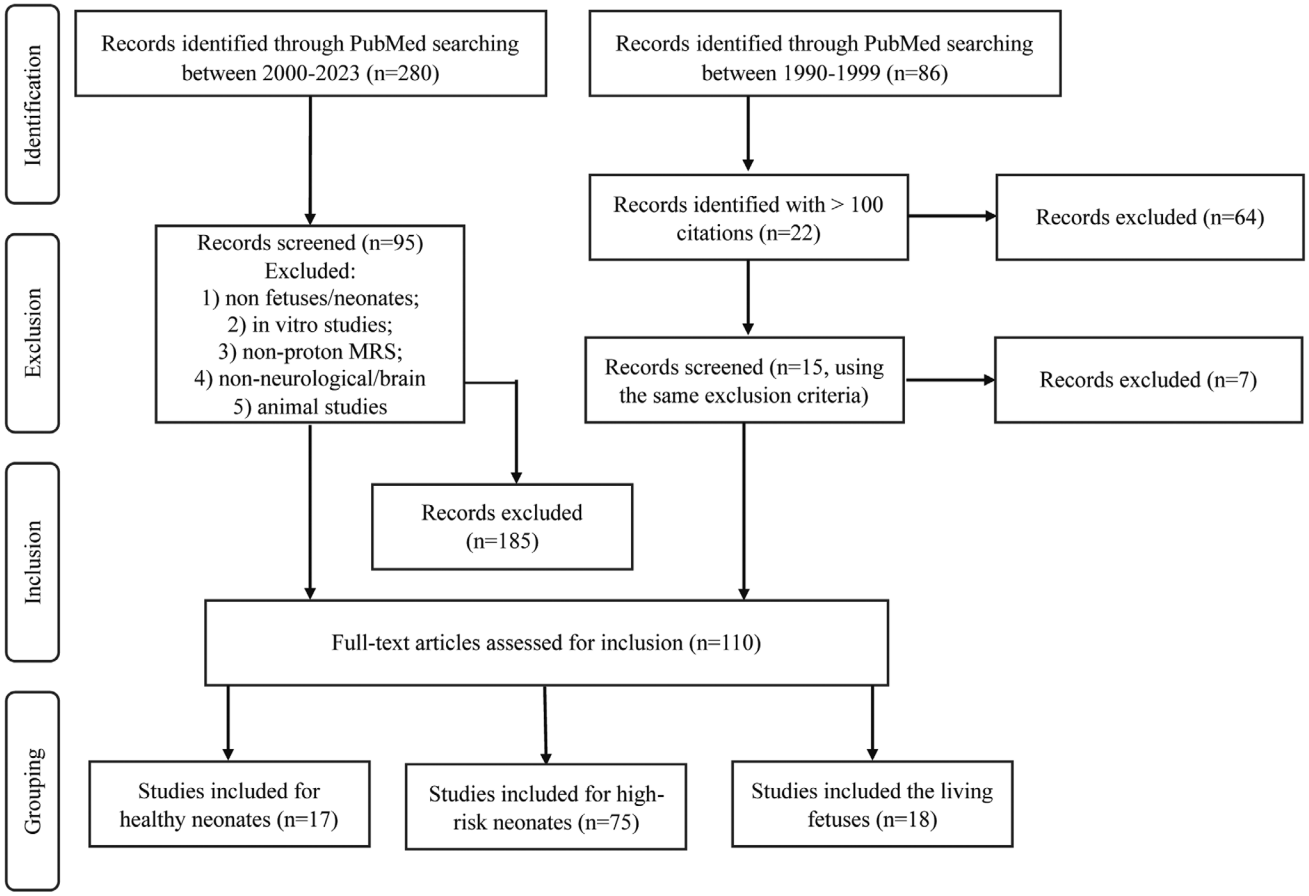

FIGURE 4: A flowchart for the steps of searching relevant literature from identification to exclusion and inclusion followed by grouping for analysis.

important biomarker for neuronal health and neurodevelopment during infancy, measurements of NAA using ^{1}H-MRS have been used as indicator for healthy brain maturation. For other metabolites, two studies reported on Tau as it increased in cerebellum[57] and decreased in white matter of centrum semiovale along with brain maturation.[63] Two studies reported an increase of tCr and a decrease of inositol/Cr as a function of advancing gestational age[46,58]; reduced Glx was reported in centrum semiovale in preterm neonates compared with full-term neonates.[63] These results generated the brain biochemical profile in healthy neonates during their early stage of neurodevelopment and provided references for metabolite comparisons between healthy and sick neonates with neurological diseases or brain lesions.

Recently, advanced edited sequences[23–25] were implemented to study low-concentrated metabolites such as GABA in addition to the conventional high concentration metabolites as mentioned. A longitudinal study reported common brain metabolites as well as GABA from the right basal ganglia in a group of preterm neonates at 37–46 and 64–73 postmenstrual weeks.[64] GABA measurements were contradicted based on the reporting method. GABA/Cr ratio decreased significantly as a function of age but not in GABA/Cho ratio and water-scaled GABA measurement. A similar contradiction was found in a cross-sectional study. Water-scaled GABA+ (i.e., GABA+ macromolecules) of infants in the basal ganglia and cerebellum were significantly lower than those in children. Contradictory results were reported for measurements in Cr ratios as preterm and term infants had a significantly higher GABA+/Cr ratio in the cerebellum compared with children.[65] The discrepancy between water-scaled and Cr ratios measurement may be the result of the differences of Cr concentrations, due to the changes in the early stage of neurodevelopment in the basal ganglia and cerebellum between newborns and children, in which the inconsistent denominator dominated the calculation. Reporting methods must be used carefully especially for fetal-neonatal measurements because metabolites that are thought to be stable could change rapidly over the early stage of neurodevelopment. Results could be biased for longitudinal studies that reported their measurements in metabolite ratios due to the significant changes in the denominator of the calculations. Besides GABA, a systemic approach to measure low concentration metabolites including GSH and Glx in the neonatal population has been investigated using the advanced Hadamard encoding and reconstruction scheme.[66] However, the application of the advanced editing sequences has been very limited in fetal-neonatal studies given its ability to measure many important in vivo brain

molecules including but not limited to GABA, GSH, Glx (Glu + Gln), Lac, Asc, Asp etc.

Regional Differences in Brain Metabolites

Numerous studies to date have reported on regional brain metabolite concentrations, particularly in the cerebellum, basal ganglia, and gray/white matter of the frontal lobe. One study reported regional differences in brain metabolite measurements between healthy and preterm newborns.[67] The overall NAA, Cho, and Cr concentrations were significantly higher in the cerebellum in term infants compared to preterm infants after adjustments for postmenstrual age. Glx/Cho ratio in the right basal ganglia, GABA+, Cho, and GABA+/Cho ratio in the right frontal lobe were overall increased in term neonates compared with preterm neonates.[67] In the cerebellum, there was a relative increase in GABA+, Glx, Cho, Glu, Gln, GSH, and myo-inositol compared to concurrent measures in the basal ganglia. Conversely, the basal ganglia had higher levels of NAA and Cr. In terms of Cho ratios, GABA+, Glx, NAA, and Cr were highest in the basal ganglia.[60] For more advanced editing schemes, Hadamard-encoded MRS on healthy neonates was first reported in 18 term-born neonates (median postmenopausal age: 41 weeks, range 39–47 weeks) to study GABA+, GSH, and Glx.[66] When a T_2 correction was applied to the measurements, GABA+/water and Glx/water ratios were significantly higher in the thalamus than the anterior cingulate cortex; when non-water-scaled and non-T_2-corrected measurements were reported, Glx/tCr and GSH/tCr ratios were significantly lower in the thalamus compared to the anterior cingulate cortex. Any metabolite measurement based on tCr ratio were heavily driven by the tissue content in different brain regions resulting in a similar influence as reported by Tomiyasu et al.[65] Of note, most existing algorithms perform relaxation correction using T_1/T_2 relaxation times of white matter, gray matter, and metabolites from average adult population as the correction factors. These factors in fetus and neonates have yet to be measured which may cause biased results if the correction factors are implemented incorrectly.

Sex-Specific Differences in Brain Metabolites

In another study, GABA+, Glx, and Cho were reported to be higher in the right frontal lobe in male preterm neonates compared to females.[68] This discovery suggested that sex differences may play a role in the contribution of ex-utero brain metabolic concentrations and biologic variability due to infant sex. This should be considered in all early-life studies using MRS.

Overall, the key metabolites, including NAA and tCr increase while mI and tCho decrease in the fetal-newborn period. Conflicting results have been reported for GABA and Glx as the modeling of low concentration metabolite peaks are complicated by the low SNR fetal-neonatal spectra, underlying macromolecule background signals and subtraction artifacts. Further studies are needed to investigate GABA and Glx changes in the developing brain. Similarly, studies to date have revealed regional and sex-specific differences in metabolite concentrations, all of which should be considered when evaluating early fetal-neonatal MRS studies. A summary of findings in metabolite concentration changes in healthy term and preterm infants are listed in Table 2. In healthy term and preterm infants without brain injuries, ^{1}H-MRS plays a role in studying neurochemical profiles during the early stage of rapid neurodevelopment. It helps to set a benchmark for the dynamic change of metabolite concentrations during healthy brain maturation.

MRS in High-Risk Neonates

Research has investigated the brain's metabolite changes in preterm infants without structural brain injury in comparison to full term healthy newborns. Findings overall are comparable across different studies except for the low concentration metabolites such as GABA and Glx.[52,69–72] Discrepancies are most likely in relation to the factors like data quality in the low-concentrated metabolite, the selection of relevant modeling methods, reporting methods as being "absolute" or "metabolite ratios," and measures in different brain regions during data acquisition. Apart from healthy neonates, ^{1}H-MRS has been applied to monitor neurochemical changes in sick neonates and their neurodevelopmental outcomes after infancy. The following sections summarize applications of ^{1}H-MRS in neonates with complications or brain diseases.

Very Low Birth Weight

Preterm neonates at very low birth weight (VLBW) are of specific interest to investigators who study neurochemical profiles using MRS. The NAA/Cho ratio in the preterm VLBW cohort shows significantly decreased in the frontal cortex, hippocampus, and subventricular zone compared to those in healthy controls at term-equivalent age.[69] Persistent differences in metabolites (lower Cr, higher Glx/Cr, and higher Cho/Cr) were observed in VLBW preterm children at age 3–4, with related differences in poorer language expression, early executive function, and verbal intelligence quotient (IQ) compared to children born term healthy controls.[71] Preterm infants small for gestational age due to placental insufficiency were compared to those appropriate-for-gestational-age. However, no significant differences were found.[70] This study supported the increase of NAA/Cho as a function of age in the gray matter whereas mI/Cho was decreased in both small- or appropriate-for-gestational-age groups. A similar study reported a decrease of NAA in preterm with intrauterine growth restriction compared to preterm with healthy weight.[72] This is of specific interest as preterm and LBW infants are at risk for disrupted brain metabolism with the abrupt cessation of maternal-placental transfer of key nutrients. ^{1}H-MRS can provide dynamic measures of how these growth delays influence brain development as deceased NAA has been frequently reported which is often used an indicator for healthy brain growth while lower Cr levels may reflect the potential

TABLE 2. Brain Metabolite Concentrations in Healthy Term and Preterm Neonates Without Significant Brain Injury

	Preterm (N)	Term (N)	Brain Regions	Field Strength	Vendor	Sequence	Measures	Major Findings
Akasaka et al 2016	0	17	Frontal lobe	1.5 T	Hitachi	PRESS multi-voxel	/Cr ratio /Cho ratio Longitudinal at 42 PCW and at 3, 6, 9, and 12 months after.	Correlation relationship with PCWs: Positive: NAA/Cr, NAA/Cho Negative: Cho/Cr, Ins/Cr
Basu et al 2019	53	0	Cereb.	1.5 T	GE	PRESS	i.u. /Cr ratio	Correlation relationship with PMAs: Positive: NAA, Cho, and Cr Negative: Cho/Cr
Basu et al 2020	38	0	RFL	3 T	GE	MEGA-PRESS	i.u.	↑ GABA+, Glx, Cho in male infants compared with female
Basu et al 2022	0	58	Cereb. RBG, RFL	3 T	GE	MEGA-PRESS	i.u. /Cho ratio	GABA: Cereb. > RBG > RFL Glx: Cereb. > RBG > RFL Cho: Cereb. > RBG > RFL GSH: Cereb. > RBG > RFL NAA: RBG > RFL > Cereb. Cr: RBG > Cereb > RFL GABA+/Cho: RBG > RFL > Cereb. NAA/Cho: RBG > RFL > Cereb. Cr/Cho: RBG > Cereb. > RFL
Basu et al 2023	75	48	Cereb. RBG, RFL	3 T	GE	MEGA-PRESS	i.u. /Cho ratio	↑ Glx/Cho, GABA+, Cho, GABA+/Cho (adjusted for PMA)
Huppi et al 1991	12	8	Cerebellum	1.5 T	GE	STEAM	mM[a]	↑ NAA, Tau
Koob et al 2016	16	16	CSO	1.5 T	Siemens	PRESS	/H_2O ratio	↑ Tau, tCr, Glx
Kreis et al 2002	9	16	CSO, thalamus, occipital GM	1.5 T	GE	PRESS	/H_2O ratio	Metabolite concentrations were significantly different in various brain regions.
Maria et al 2021	0	18	ACC, left thalamus	3 T	Philips	HERMES	i.u. /tCr ratio	Glx/tCr: ACC > Thal. GSH/tCr: ACC > Thal. GABA+: Thal. > ACC Glx: Thal. > ACC
Tanifuji et al 2017	20	20	Right basal ganglia	3 T	GE	PRESS	i.u. /Cr ratio /Cho ratio Longitudinal study	↑ NAA, Glx, Cr, NAA/Cr, Glx/Cr NAA/Cho, Glx/Cho ↓ Ins, GABA/Cr, Cho/Cr, Ins/Cr Ins/Cho (not significant for GABA and GABA/Cho)

TABLE 2. Continued

	Preterm (N)	Term (N)	Brain Regions	Field Strength	Vendor	Sequence	Measures	Major Findings
Toft et al 1994	9	13	Striatum	1.5 T	Siemens	STEAM	/H_2O ratio	↑ NAA, PCr + Cr ↓ Inositol
Tomiyasu et al 2013	60	19	BG, CSO, cerebellum	3 T	Siemens	PRESS	mM	↑ tCr, tNAA, Glx, mI (cereb.) ↓ mI (BG) (as a function of PCA)
Tomiyasu et al 2017	28	10	BG, cerebellum	3 T	Siemens	PRESS, MEGA-PRESS	/H_2O ratio /tCr ratio	↑ GABA+/H_2O ↑ Normalized GABA+ (compared with 12 children age of 10.2 years)
Tomiyasu et al 2022	108	0	Deep gray matter, CSO	3 T	Siemens	PRESS	/tCho ratio	↑ tNAA/, tCr/, Glx/tCho in GM ↑ tNAA/tCho in CSO ↓ mI/tCho GM (as a function of PMA)

"↑" Indicates increased metabolite concentrations as a function of age between preterm and term neonates or between neonates and children unless specified for other conditions (vice versa for ↓). ">" Indicates higher metabolite concentrations between the brain regions. ACC = anterior cingulate cortex; Cereb. = cerebellum; CSO = centrum semiovale; GM = gray matter; i.u. = institutional units; LFC = left frontal lobe; PCA = postconceptional week; PMA = postmenstrual age; RBG = right basal ganglia; RFL = right frontal lobe; Thal. = thalamus.
[a]Using total creatine concentration from human brain autopsy at similar age as an internal endogenous marker.

decrease of brain energy metabolism in neonates with VLBW. A more comprehensive summary of clinical data and protocols used for VLBW infants are provided in Table 3.

Relationship Between Brain Biochemistry and Cognitive Neurodevelopmental Outcomes

Associations between ^{1}H-MRS brain metabolite concentrations acquired from preterm neonates and their relationship to cognitive outcomes have been reported as well. The most common findings were an association between decreased NAA concentrations to cognitive and neurodevelopment decline and delay regardless of the use of different neuro-developmental testing. These tests include: the Bayley Scales of Infant and Toddler Development-III (BSIDIII) scores, Ages and Stages Questionnaire-2 (ASQ-2), Differential Abilities Scale (DAS-II) etc.[69,73–77] NAA/Cho ratio in the thalamus was reportedly much lower in preterm neonates with a mild developmental delay compared with preterm infants with normal development.[74] For neonates with low social economic status, there was a negative association reported between Cho/Cr ratio in the left centrum semiovale and motor assessment scores at age 3 years.[78] The presence of Lac in preterm neonates was associated with lower fine motor and cognitive scores at 18–24 months life.[73,75] Collectively, these data suggest that ^{1}H-MRS measurements in preterm infants may serve as important biomarkers for cognitive neurodevelopmental outcomes. Clinical data and protocol for association of preterm neonates and cognitive outcomes are listed in Table 4.

Hypertensive Disorders of Pregnancy

Hypertensive disorders of pregnancy (HDP), including chronic hypertension, preeclampsia-eclampsia, preeclampsia superimposed on chronic hypertension, and gestational hypertension similarly may disrupt the transfer of key metabolites to the developing fetus and result in compromised perinatal transition.[79] The Research was performed on a group of preterm neonates of HDP mothers. NAA/Cho and NAA/Cr ratio in the bilateral thalami were significantly higher in the HDP group compared with the non-HDP group. Interestingly, results suggested preterm infants exposed to HDP may experience a utero accelerated brain maturation and increased neuronal activity,[80] in which relates to one of their previous population-base studies that suggested preterm infants with HDP have lower odds of adverse neurodevelopmental outcomes compared to the non-HDP preterm infants.[81] However, their arguments were solely based on the findings of the altered NAA measurements in the bilateral thalami region. As both preterm birth and HDP can affect levels of brain metabolites, covariances related to preterm birth such as defects or delayed maturation of the respiratory system

TABLE 3. Brain Metabolite Changes in Very Low Birth Weight (VLBW) Preterm Infants

	VLBW Preterm (N)	Term (N)	Brain Regions	Field Strength	Vendor	Sequence	Measures	Major Findings
Bapat et al 2014	31	12	Subventricular zone, hippocampus, right frontal lobe	3 T	Philips	PRESS	/Cho ratio /mI ratio	↓ NAA/Cho in all three regions-of-interest in VLBW infants
Phillips et al 2011[a]	16/12	7/8	ACC, left frontal lobe	3 T	Siemens	PRESS	i.u. /Cr ratio /Cho ratio	↑ Cho/Cr and Glx/Cr and ↓ NAA/Cho and Cr in VLBW children compared to controls at 3–4 years
Roelants-van Rijn et al 2004b	14/26	0	Basal ganglia	1.5 T	Philips	PRESS	/Cho ratio	No significant difference in metabolite concentrations between AGA and severely SGA infants.
Simoes et al 2017[b]	48	26	Frontal lobe	3 T	Siemens	PRESS	/Cr ratio	↓ NAA in prematurely born intrauterine growth restriction than in prematurely born but adequate for gestational age infants. ↑ NAA in prematurely born but adequate for gestational age compared with term adequate-for-gestational-age infants

ACC = anterior cingulate cortex; AGA = appropriate for gestational age; SGA = small for gestational age.
[a]Phillips et al (2011) has two preterm groups at different GAs and two corresponding control groups.
[b]Simoes et al (2017) compared early onset preterm intrauterine growth restriction (P-IUGR) group with 1) preterm adequate-for-gestational-age (P-AGA) and 2) term adequate-for-gestational-age (T-AGA) groups.

TABLE 4. Association Between Preterm Infant Brain Biochemistry and Neurodevelopmental Outcomes

	Preterm (N)	Controls (N)	Age at Follow-Up	Neuro Developmental Test	Brain Regions	Field Strength	Vendor	Sequence	Major Findings
Bapat et al 2014	31	12	18–22 mo.	BSID-III[a]	Subventricular zone, hippocampus, RFL	3 T	Philips	PRESS	NAA/Cho in subventricular zone and RFL ~ Bayley mental scores
Gire et al 2022	69	N/A	24 mo.	ASQ-2[a]	FL, PO	1.5 T/3 T	Multiple scanners	PRESS	FL: ↓ NAA/Cho ~ total ASQ and communication score ↑ Lac/Cr motor skills
Hyodo et al 2018	33	16 terms	18 mo.	Kyoto-Scale	BG	3 T	Siemens	PRESS	↓ NAA/Cho ~ mild developmental delay
Hart et al 2014	33/38	N/A	19 mo.	BSID-III[a], Amiel-Tison	FL/PL	1.5 T	Philips	PRESS	NAA/Cho ~ adverse outcome Lac ~ lower fine motor and lower cognitive composite scores
Van Kooij et al 2012	58	N/A	24 mo.	BSID-III[a]	Cereb.	3 T	Philips	PRESS	Mother's educational level and NAA/Cho have positive correlation with cognitive scores.
Illapani et al 2022	59	N/A	36 mo.	SES[a], BSID-III[a], DAS-II[a]	CSO	3 T	Siemens	PRESS	Cho/Cr ~ motor development SES ~ Cho/Cr and motor outcome
Kendall et al 2014	43	N/A	12 mo.	BSID-III[a]	PL	1.5 T	Siemens	PRESS	NAA/Cho and Cho/Cr ~ cognitive and motor composites

"~" Indicated significant relationship or association between variables. BG = basal ganglia; CSO = centrum semiovale; Cereb. = cerebellum; FL = frontal lobe; mo. = months; PL = posterior lobe; PO = parieto-occipital; RFL = right frontal lobe; SCI = structural cerebellar injury; WMD = white matter damage.
[a]Neurodevelopmental tests—ASQ-2 = Ages and Stages Questionnaire-2; BSITD-III = Bayley Scales of Infant and Toddler Development-III; DAS-II = Differential Abilities Scale, Second Edition; SES = socioeconomic status.

and comorbidities in the HDP mothers need to be considered.

Hypoxic–Ischemic Encephalopathy

Hypoxic–ischemic encephalopathy (HIE) at birth is highly associated with long-term neurodevelopmental impairment due to injury to the developing brain; hence, neonatal patients with HIE have been commonly studied using ^{1}H-MRS to monitor the brain biochemistry at birth and the long-term neurodevelopment from birth to early childhood. Among the cross-sectional and serial studies on neonates with HIE within the first week of life, there is a consistent finding of increased Lac, Cho, and Glx, and decreased NAA and Cr compared with healthy controls.[50,82–105] Glycine was occasionally reported in HIE neonates.[86] Furthermore, early measurements of increased Lac/Cho and Lac/NAA ratios and decreased NAA/Cho ratio were associated with neurodevelopmental status including the neuromotor and cognitive outcomes at age 12 months.[83] Reduced NAA/Cho ratios were also reported to be associated with reduced fractional anisotropic in diffusion scans in which abnormalities were frequently observed in white matter (91%) and cortex (70%) in neonates with HIE.[106] It suggested that neonates with HIE suffered brain injuries that adversely affected the brain biochemistry and fiber structure. Persistent Lac detected by ^{1}H-MRS in HIE was associated with alkalosis and an alkaline intracellular pH. The persisting lactic alkalosis was potentially the result of a prolonged effect in the redox state within neuronal cells, presence of phagocytic cells and proliferation of glial cells[107] and thus a potential marker of anaerobic metabolism that elevated Lac in the HIE brain.

Interestingly, brain temperatures were measured in neonates with encephalopathy due to hypoxic–ischemic using the nature of chemical shift differences detected by ^{1}H-MRS. Most scans were performed during the rewarming process (body temperature >35.5°C) following therapeutic hypothermia, as well as approximately one fifth of the scans were acquired during therapeutic hypothermia or early rewarming.[108] The brain temperatures were calculated using a formula derived from a phantom calibration or a formula based on chemical shift differences between water and NAA.[109] Brain temperatures were higher in neonates with more severe HIE than in those with mild HIE. This suggests the potential role of ^{1}H-MRS as a noninvasive method to accurately measure brain temperature during therapeutic hypothermia because maintaining the brain temperature within the therapeutic range for the duration of the therapy is crucial for optimal outcomes of neuroprotection. In a previous randomized clinical trial that studies the efficacy of therapeutic hypothermia, results suggested that a lower temperature and a longer duration of cooling did not change the mortality rate of HIE in NICU.[110] Of note, core body temperatures were measured and monitored using esophageal probes during cooling to estimate a close approximation of brain temperature. In contrast, MRS utilizes the natural properties of water molecules in the brain to estimate brain temperature based on the chemical shift changes between water and a reference metabolite such as NAA.[109]

Congenital Heart Disease

Newborns with congenital heart disease (CHD) displayed significantly lower NAA/Cho and higher Lac/Cho ratios under MRS as compared to healthy-term control infants.[111,112] White matter NAA/Cho ratios were lower in CHD neonates compared to healthy controls, which might reflect a delay in brain maturation.[113] In moderate to severe neonatal CHD cases who underwent cardiac surgeries, white matter NAA increased noticeably in postoperative CHD compared to preoperative cases, and the increased level of NAA was comparable to those in healthy controls. However, no correlation was found between pre- or postoperative white matter NAA/Cho with the 1-year neurodevelopment outcome.[114] The impact of CHD and cardiac surgery on neurodevelopment was uncertain and further longitudinal studies that follow up brain neurochemical on infants with CHD with and without cardiac surgeries may improve our understanding of the clinical impact of altered metabolites.

Other Systemic Illnesses

^{1}H-MRS is also a tool to investigate cerebral metabolism and has been implemented in the setting of generalized systemic illness, including inborn errors of metabolism, transient metabolic derangements, as well as neonatal hyperbilirubinemia and a myriad of other genetic diseases. Brain metabolite alterations were observed in neonates with severe hyperbilirubinemia (bilirubin levels of ≥25 mg/dL). A high level of Lac/NAA and a low level of NAA/Cho ratios are more possible markers, having been observed in one of the five subjects. However, the statistical power of that study was weak given the small sample size.[115] A similar study of six neonates with kernicterus reported elevation of Tau/, Glx/, and mI/Cr ratios and a reduction of Cho/Cr ratio compared with normal neonates.[116] Results from a larger study (N = 31) reported elevated GABA and Cho measurements.[117] The contradiction of Cho could have been the result of the quantification method being applied as the prior study reported Cho as a ratio of Cr and the later reported absolute concentration. Glucose transporter type 1 deficiency syndrome (GLUT1 DS) is a congenital inborn error of metabolism caused by impaired glucose transports across the blood–brain barrier. A case study reported significantly higher Glx/Cr ratio in the thalamus in GLUT1 DS cases compared to healthy controls. Of note, the comparison was based on two cases and two controls, and it was unclear that they were age-matched.[118]

Brain metabolite changes have been investigated in neonates with other rare genetic metabolic disorder including

isolated sulfite oxidase deficiency,[119] mitochondrial diseases including respiratory chain defects[120] and Zellweger Syndrome.[121–123] Common findings included decreased NAA and increased Lac levels, which is similar to the results presented in neonates with brain injuries. In neonates with isolated sulfite oxidase deficiency, ratio of Cho/ and mI/tCr were also increased,[119] whereas in Zellweger Syndrome, mI level was reduced along with increased glutamine compared to healthy infants.[123] Inflammatory cytokines could potentially lead to impaired cerebral oxidative metabolism in neonates with encephalopathy resulting in an abnormal neurodevelopmental outcome at 30 months of age.[124] Results from ^{1}H-MRS suggested that Lac/Cho ratios in the basil ganglia were significantly correlated with various levels of proinflammatory cytokines (IL-1β, IL-6, IL-8, and TNF-α) but NAA/Cho ratios were not associated with any severity of cytokines in both white and gray matters.[124] Collectively, these studies suggest that metabolite changes in the brain may be secondary to generalized or somatic metabolic errors but provide new insight into the neurologic manifestations and may provide additional diagnostic and prognostic information to families/providers. Overall, ^{1}H-MRS opens the gate for noninvasive detection of metabolic disorders. Despite more and stronger evidence is needed to support potential biomarkers from the change of brain biochemistry for diagnosis, invasive procedures such as blood tests and spinal taps that are common for testing metabolic disorders could be possibly replaced in the future.

Besides metabolic diseases, neonates with abnormal body conditions such as Chorioamnionitis and Hydrocephalus have been investigated using ^{1}H-MRS. Chorioamnionitis is an infection of the amniotic fluid during pregnancy with a relatively high prevalence in preterm neonates but is uncommon in term neonates.[125] Researchers studied 31 healthy term neonates with fetal inflammatory response (GA: 39.5 ± 1.3 weeks) and found that decreased NAA/Cho and increased Lac/Cr in basal ganglia were correlated with lower motor and cognitive composite scores, respectively. Developmental outcome at 12-month showed the increase in NAA/Cho ratios postnatally was slower in infants with below average outcomes.[126] Hydrocephalus is a condition rather than a disease and is the result of when too much fluid is accumulated in the brain and spinal cord. Absolute brain metabolite concentrations were reported in a small group of term and preterm infants with hydrocephalus. Lactate, glutamine and alanine were reported to be higher compared to the control group.[127] Another fetal study reported the inositol level to creatine ratio was significantly lower in fetuses with hydrocephalus.[128]

Effects of Medication

The capacity of ^{1}H-MRS can be extended to study the effect of medical interventions on the neonatal brain. Analgesic medications including opioids may be used on neonates with HIE to ease pain and discomfort during clinical care. Opioid treatment was suggested to have neuroprotective effects as those who treated demonstrated significantly less brain injury during the first week of life.[129] To further examine the effect of opioid treatment on brain metabolism, 28 asphyxiated term neonates (8 opioid-treated and 20 non-opioid) and 6 healthy controls were compared. Decreased NAA/Cr ratio and increased Lac measurements in the occipital gray matter were reported in non–opioid-treated group compared with the opioid-treated group.[130] Neuro-metabolite observations using ^{1}H-MRS might support the hypothesis that opioids-treated neonates had less brain injury and received better outcomes.

Interestingly, a study reported Lac/Cho and Lac/NAA ratios were significantly lower in the basal ganglia by 17% and 25% respectively in a group of preterm neonates with pentobarbital sedation compared with the age-matched neonates without sedation during MRS scans.[131] This study highlights the necessity of determining medications and sedation for the accurate interpretation of dynamic imaging sequences, such as MRS.

MRS and Neonatal Prognostic Value

The role of fetal-neonatal ^{1}H-MRS is not limited to studying the dynamic change of brain metabolites in healthy and high-risk neonates or correlation between cognitive neurodevelopmental outcomes to metabolites levels. Its application could be further stretched out to predict the outcome or course of a disease. A study evaluated the prognostic value of metabolite ratios using receiver operating characteristics (ROC) analysis. mI/NAA ratio was found to be the best age-independent predictor for predicting mental developmental outcome of asphyxiated neonates[132] and Lac/NAA, Lac/Cr, Cho/Cr, tNAA, and myo-inositol/NAA ratios also showed good prognostic value to predict abnormal or adverse outcomes with high sensitivity and specificity.[111–113,133–139] NAA/Cho and NAA/Cr ratios in the basal ganglia were found to be significantly correlated with Apgar scores at 5-minute after born and NAA/Cho ratio with Apgar scores at 1-minute in a group of term neonates with possible asphyxia.[140] Lac/NAA ratio was also suggested to be more accurate for early prognosis compared with T_2 relaxation times which positively correlated with developmental outcomes and severity.[137] Besides hypoxic–ischemic encephalopathy, neurometabolite changes due to trauma, seizures, cardiac arrest and surgery and other miscellaneous reasons were reported in a subgroup of 19 term neonates (mean age at the scan: 11 ± 7.5 days). NAA/Cho, Cho/Cr, NAA/Cr, and NAA measurements were significantly different between neonates with good and poor development outcome. Discriminant analysis suggested clinical variables combined with long echo

time data provided up to 95% accuracy on predicting poor outcome on neonates.[141]

MRS in the Living Fetus

In Vivo Brain Metabolite Changes in Healthy Fetuses

Fetal ^{1}H-MRS is particularly useful in monitoring and studying neurodevelopmental changes during pregnancy. It is challenging in terms of acquisition, preprocessing, and quantification due to the inherently small volume of the fetus brain and motion artifact, from both the mothers and fetuses, contributing to the low SNR and poor shimming. One of the earliest MRS studies on fetuses demonstrated the feasibility on measuring metabolites at the early stage of the fetal brain.[142] Longitudinal studies reported the changes of brain metabolite concentrations and results were mostly inline as NAA, Cr, Cho, myo-inositol, and scyllo-inositol were increased as a function of age resulting from the rapid development of the fetal brain between the second and the third trimester[143–145] as reported in Table 5. Results also suggested a faster increase in tNAA and tNAA/tCho ratio during the third trimester compared to the second trimester and a faster growth of Cho and tNAA measurements in female subjects.[145] Some contradicting results were reported as negative correlations were obtained for Cho/Cr ratio in Kok et al[146] and for mI and Cho in Girard et al.[147] The discrepancy might be hinted by the rapid changes of the metabolite concentration in the second trimester of the fetus (between 22 and 27 weeks) neurodevelopment and the measuring method for concentration ratio (i.e., sum of all metabolite signal areas as the denominator) contributing to the inconsistency of the findings. Although contradictory results were reported, brain biochemical profile from healthy developing fetal brain set a benchmark for monitoring neurodevelopment of high-risk fetus during pregnancy. Besides brain development, a recent study demonstrated that elevated maternal depression was associated with decreased Cr and Cho measurements in the fetal brain. This is the first study that has reported on the association between maternal psychological distress and neurobiochemical in fetuses using ^{1}H-MRS.[148]

Brain Metabolite Changes in High-Risk Fetuses

CONGENITAL HEART DISEASE. CHD is one of the most common types of cardiovascular birth defects. It increases the risk of abnormal fetal brain development which begins in utero during pregnancies.[149] Brain metabolites in fetuses with CHD were investigated using ^{1}H-MRS. Data were acquired in a group of fetuses (mean: 31 weeks, range: 25.1–27.1 weeks) with CHD, and those results suggested the rate of increase in NAA/Cho ratio was significantly slower in CHD fetuses when compared to healthy ones.[150] The abnormal development could be a result of hemodynamic deficiency of blood brain circulation. Another recent study that used a larger cohort of fetuses supported these results. The neurochemical profile was compared between CHD fetuses (N = 170) and healthy controls (N = 333).[151] Like prior findings, the results showed a decreased NAA/Cho ratio and an increased Cho level in CHD fetuses. Lac peak was reported in all fetuses, which was associated with increased odds of death before discharge. Altered cerebral metabolites in utero during the third trimester hint at cardiac function in fetuses not being capable enough to supply the oxygen that the brain demanded during its rapid developmental stage resulting from hemodynamic deficiency of CHD. The association between CHD and altered brain metabolite changes indicated the differences in brain growth and maturation in the third trimester between CHD and healthy fetuses.

FETAL GROWTH RESTRICTION. Brain metabolites in fetuses with fetal growth restriction (FGR) were also reviewed as part of our search. NAA/Cr and NAA/Cho ratios were significantly lower in the FGR groups compared with the healthy controls[152–154]; whereas mI/Cho, mI/Cr, and Cho/Cr ratios were not changed between the two groups.[155] Results were mostly in line with the VLBW preterm neonates previously mentioned. As the level of NAA acted as a surrogate marker of neuronal activity, these results explained the association of a NAA decrease to the development of the fetal brain with growth restriction. Lac is a biomarker of fetal metabolic acidemia and was present in the most severe case of FGR which resulted from increased reliance on anaerobic metabolism. Results were consistent with the high lactic acid concentration observed in umbilical blood.[156]

FETAL HYDROCEPHALUS. Decreased levels of the inositol/Cr ratio were observed in fetuses with fetal hydrocephalus compared to the healthy controls. Lac was also presented in spectral of hydrocephalus cases due to the reduced cerebral perfusion from elevated intracranial pressure.[157] Results suggest that inositol played a role in the development of the neurulation process and in the development of the central nervous system.[128]

Gaps in Knowledge

As mentioned in the previous sections, MRS data acquisition in the fetal-neonatal cohorts is more challenging than in adults due to the inherent nature of the subjects and the overall population available for recruitment. For those reasons, questions related to brain metabolites are studied primarily based on adult subjects including (1) the consensus understanding of a brain's biochemistry profile, (2) the physical characteristics, such as T_1/T_2 relaxation times, present in different brain tissues and metabolites, and (3) the influence of macromolecules to LCM. As the easier population to study,

TABLE 5. Brain Metabolites in Healthy and High-Risk Fetuses

	Healthy Fetuses (N)	Sick Fetuses (N)	Conditions	Brain Regions	Field Strength	Vendor	Sequence	Measures	Major Findings
Andescavage et al 2023	221	112	CHD	Mid. brain	1.5 T	GE	PRESS	i.u. /Cho ratio	↑ Cho ↓NAA/Cho in CHD. Presence of Lac in CHD.
Cetin et al 2011	0	5	IUGR	Mid. brain	1.5 T	Philips	PRESS	i.u.	Presence of Lac in severe cases
Evangelou et al 2016	129	0	Healthy fetuses	Mid. brain	1.5 T	GE	PRESS	i.u. /tCho, /tCr	↑ tNAA, tCho and tCr as function of age
Girard et al 2006	N/A	N/A	GA 22–39 weeks	CSO	1.5 T	N/A	PRESS	/sum of all metabolite signal areas ratio	↑ NAA ↓ mI, Cho between GA 22 and 39 weeks
Kok et al 2001	21	0	Healthy fetuses	Parietal and occipital lobes	1.5 T	Siemens	STEAM	i.u.	^{1}H-MRS in fetal brain is feasible
Kok et al 2002	36	0	Healthy fetuses	Mid. brain (include. scalp and extracranial tissue)	1.5 T	Siemens	STEAM, PRESS	i.u. /Cr ratio	↑ NAA, NAA/Cr, NAA/Cho ↓ Cho/Cr Between GA 30 and 41 weeks
Kok et al 2003	36	10	Hydrocephalus	Mid. brain	1.5 T	Siemens	STEAM	/Cr ratio	↓ Ino/Cr
Limperopoulos et al 2010	55	50	CHD	Centrumovale	1.5 T	Siemens	PRESS	/Cho ratio	↑ NAA/Cho between GA 25 and 37 weeks Lower NAA/Cho progress in CHD compared to controls.
Pradhan et al 2020	112	0	Healthy fetuses	Mid. brain	1.5 T	GE	PRESS	i.u.	↑ tNAA, tCr, tCho, sI, tNAA/tCho with GA. tNAA increased faster in the third trimester. tCho and tNAA increased faster in female

TABLE 5. Continued

	Healthy Fetuses (N)	Sick Fetuses (N)	Conditions	Brain Regions	Field Strength	Vendor	Sequence	Measures	Major Findings
Roelants-van Rijn et al 2004a	0	6	Hydrocephalus	BG	1.5 T	Philips	PRESS	i.u.	Lac was presented in two cases.
Sanz-Cortes et al 2015a	55	64	Small for GA	FL	3 T	Siemens	PRESS	i.u. /Cho ratio	↑ Cho/Cr ↓ NAA/Cho in fetuses of small GA
Sanz-Cortes et al 2015b	30/11[a]	31	Late-onset IUGR	FL	3 T	Siemens	PRESS	i.u. /Cho ratio	↓ NAA/Cho in SGA and IUGR compared to AGA group
Story et al 2011	41	28	IUGR	Mid. brain	1.5 T	Philips	PRESS	/Cho ratio /Cr ratio	Lac in five IUGR and three fetuses. ↓ NAA/Cr and NAA/Cho in IUGR groups
Story et al 2013	47	28	IUGR	Mid. brain	1.5 T	Philips	PRESS	/Cho ratio /Cr ratio	↓ Cho/Cr, mI/Cho and mI/Cr with advancing GA in control.
Urbanik et al 2019	32	0	Healthy fetuses	Mid. brain	1.5 T	GE	PRESS	i.u.	↑ NAA, Cr, Cho, and mI with advancing GA
Wu et al 2020	119	0	Healthy fetuses	Mid. brain	1.5 T	GE	PRESS	i.u.	NAA, Cr, and Cho

AGA = appropriate for gestational age; BG = basal ganglia; Cereb. = cerebellum; CHD = congenital heart disease; CSO = centrum semiovale; FL = frontal lobe; Hippo = hippocampus; IUGR = intrauterine growth-restricted; i.u. = institutional unit; SGA = small for gestational age.
[a]Sanz-Cortes et al (2015b) recruited 30 fetuses with AGA and 11 with SGA.

most of the recent research being reported on brain metabolite changes are as functions of age using adult cohorts.[158,159] Metabolite changes as functions of age are anticipated in the fetal-neonatal stage as the brain is in a period of rapid development. However, there is a lack of existing data to support that claim. Despite the measurement of conventional high-concentrated metabolites in the fetal-neonatal brain is possible, it is particularly difficult to study the change of low-concentrated metabolites as artifacts related to motion, poor shimming, and frequency and phase drifts are more sensitive in fetal-neonatal scans due to their involuntary movements, small brain size, and physiological instability. For some low-concentrated metabolites such as GABA and GSH, special spectral editing strategies that utilize known *J*-coupling relationships are required which generally take a longer period of scan time, which is critical in fetal-neonatal acquisitions, to compensate for a better SNR and require sophisticated software for modeling. An ongoing large-scale multicenter study on the developing brain will provide opportunities to study the change of multiple metabolites (including both high and low concentration metabolites) in newborns from the neonatal period to children at the age of 10 using a harmonized and standardized protocol.[160] T_1/T_2 relaxation times for infants cover only for high concentration metabolites including NAA, Cho, Cr, myo-inositol, and Glx. Low concentration metabolites such as Asp, Asc, GABA, GSH, Tau, and Lac are not studied in infants as often, and even less time has been dedicated to fetuses and neonates. Correct measurements of all common metabolites at different ages would allow the proper performance of T_1/T_2 relaxation corrections. Changes to the macromolecules have also been reported in the adult data even with contradictory results due to different field strength, TE, quantification, and localization methods.[161,162] In contrast, macromolecules changes in fetuses and neonates are still unclear and are waiting to be investigated.

Future Direction

As a noninvasive tool to inform the dynamic, metabolic profiles of the developing brain in healthy and high-risk conditions, ^{1}H-MRS has a strong potential for future use and refinement. The MRS research community has published consensus papers dedicated to providing recommendations and standardization guidelines on topics and issues including advanced localization methods,[163] B_0 shimming,[164] motion,[36] and processing and quantification.[10] Along with this emerging technology, standardizing must be addressed to improve MRS as a research tool and transform it into a reliable clinical tool for diagnostic and prognostic purposes. Further refinements to the consensus guidelines are vital for its use in pediatric cases, especially for fetal and neonatal scans, given the unique biologic and technical considerations in these populations. Technical advances, such as real-time tracking tools for motion, localizations that reduce potential artifacts such as chemical displacements and sequences or AI technologies that shortened the scan time that have been used on adults should be tested and implemented into fetal-newborn scans. The development of new MRS editing sequences that allow quantification of low concentration metabolites such as GABA and GSH improves the overall understanding of the neurochemical profile. These new sequences can be applied to study neurodevelopment of healthy fetuses and neonates and correlations between metabolite changes and neurological diseases.

Conclusion

Neuroimaging has a consistent and clear record with ^{1}H-MRS as a tool to document brain metabolite concentrations in health and disease, metabolite profiles as functions of age, physical properties such as T_1/T_2 relaxation times for brain tissues and metabolites, macromolecular concentration, and water concentration in adult cohorts. In neonates and fetuses, ^{1}H-MRS has been used to study the change of brain biochemistry in normal brain maturation and those with adverse conditions or complications such as hypoxic–ischemic encephalopathy and preterm neonates with VLBW. Other applications include long term follow up to study the correlation of neurological diseases or lesions at birth with neurodevelopmental outcomes in childhoods. We have also covered some relatively new and uncommon applications of ^{1}H-MRS in fetal-neonatal scans throughout the review including the use of ^{1}H-MRS to measure brain temperature, to evaluate prognostic values to predict outcome of a disease or disorder and to study the change of metabolites in different sex and brain regional differences. On the technical side, motion artifacts caused by involuntary movements of fetuses and neonates remains one of the biggest challenges. Future application of real-time motion trackers can help to reduce the impact. LCM and segmentation can be further improved with proper data and analysis from the newborn cohort. This review paper brings forward valuable insight on the basic principles, applications, challenges, and future direction of ^{1}H-MRS implementation across healthy individual neonates and fetuses and patients with varied conditions or diseases.

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Conflict of Interest

The authors declare no conflicts of interest.

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