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In vivo Human MR Spectroscopy Using a Clinical Scanner: Development, Applications, and Future Prospects

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MR spectroscopy (MRS) is a unique and useful method for noninvasively evaluating biochemical metabolism in human organs and tissues, but its clinical dissemination has been slow and often limited to specialized institutions or hospitals with experts in MRS technology. The number of 3-T clinical MR scanners is now increasing, representing a major opportunity to promote the use of clinical MRS. In this review, we summarize the theoretical background and basic knowledge required to understand the results obtained with MRS and introduce the general consensus on the clinical utility of proton MRS in routine clinical practice. In addition, we present updates to the consensus guidelines on proton MRS published by the members of a working committee of the Japan Society of Magnetic Resonance in Medicine in 2013. Recent research into multinuclear MRS equipped in clinical MR scanners is explained with an eye toward future development. This article seeks to provide an overview of the current status of clinical MRS and to promote the understanding of when it can be useful. In the coming years, MRS-mediated biochemical evaluation is expected to become available for even routine clinical practice.

Keywords: clinical utility, magnetic resonance spectroscopy, metabolite, multi-nuclei

Introduction

In vivo MR spectroscopy (MRS) was initially performed as phosphorus (³¹P)-MRS, which detects biochemical phosphorus compounds in tissue. These substances, which include adenosine triphosphate, phosphocreatine, and inorganic phosphate, participate in energy metabolism in viable cells, enabling researchers to observe dynamic changes in energy metabolism in vivo in specific settings using this technique. In the late 1980s, in the early stage of clinical MR instrumentation, some high-end clinical MRI machines were equipped with multinuclear systems including specific transmitter and receiver coils, and ³¹P-MRS could be used in the clinical setting. However, owing to several disadvantages of this technique, including low sensitivity and complicated procedures for changing frequency and receivers, its clinical applications were strictly limited and the technique was not used in routine clinical practice.

Meanwhile, proton MRS (¹H-MRS) has been recognized as a useful method for evaluating cerebral metabolism that does not require a change in the frequency and receiver coils after brain MRI studies. Its targeted metabolites are several amino acids, lactate (Lac), creatine, and choline-containing compounds. In particular, n-acetyl aspartate (NAA) exhibits high levels in cerebral neurons. In the early 1990s, the characteristics of NAA were examined, with intense focus on its mechanisms and roles. This amino acid was found to be specific to neurons and to be synthesized through the direct acetylation of aspartate in mitochondria. With NAA now considered a neuronal marker, ¹H-MRS subsequently started to receive more attention in clinical research and diagnosis than ³¹P-MRS. In clinical practice, ¹H-MRS is sometimes performed to investigate the levels of metabolites and provides additional information for the diagnostic process.

In this review, we focus on MRS performed using a clinical MR scanner, which is divided into three categories: (1) MRS with a clinical MR scanner, (2) the clinical utility of ¹H-MRS, and (3) the expected use of other nuclei besides protons in clinical MRS. Although we mainly use single-voxel ¹H-MRS in the brain to describe MRS, its principles...
and methods may be applicable to MRS data of other anatomical regions, as well as those of other nuclei and MR spectroscopic imaging (MRSI).

**MRS with a Clinical MR Scanner**

**Metabolites observed in human brain $^1$H-MRS**

**Major metabolites**

With *in vivo* $^1$H-MRS of the brain, there are more than 30 observable metabolites, each with a variety of chemical shift value combinations. The heights of each peak are proportional to the number of protons but decrease with longitudinal relaxation time ($T_1$), transverse relaxation time ($T_2$), and J-coupling inherent to each peak, with the degree of the decrease depending on the MRS sequence and its parameters, including TE and TR. However, if the TR is long enough, the peak is not affected by the $T_1$ (see 2.2.3 and 2.4 for more details). Furthermore, for *in vivo* human measurements, line broadening due to magnetic field inhomogeneity can cause peaks to overlap those of other metabolites or the metabolite peaks with low concentrations to be embedded in noise, meaning that about 5 or 6 metabolites can be clearly observed in a clinical MR scanner (Fig. 1). The typical brain metabolites observed by MRS with a 1.5-T or 3-T clinical MR scanner are NAA and N-acetylaspartylglutamate (tNAA); glycerophosphocholine (including choline-containing compounds) and phosphocholine; Cr, creatine and phosphocreatine; Glx, glutamate and glutamine; mIns, myo-inositol; PRESS, point-resolved spectroscopy; tNAA, N-acetylaspartylglutamate.

**Other metabolites**

Metabolites other than those mentioned above include taurine, ethanolamine, gamma aminobutyric acid (GABA), and glutathione (GSH), but these are usually at low concentrations ($\leq 2.0$ mM). In healthy individuals, their concentrations are not easy to measure accurately because their metabolite peaks overlap those of metabolites with higher concentrations such as Glu and Gln, but in specific pathological conditions, these metabolites may be identified by an increase in their concentration. In addition, GABA and GSH can be measured using the J-difference editing method (e.g., MEGA-PRESS) with scanners over 2.0 T.

**MR spectral quality in clinical $^1$H MRS**

The spectrum needs to have a good SNR. Improving SNR requires adjustment of the uniformity of the magnetic field, which affects the linewidth of the spectrum, and adjustment of sequence parameters such as TE and TR, voxel size, and the number of signal averages. The spectral quality may also be improved by correcting spectral distortion due to the eddy current effect.
The SNR is defined as the ratio of the largest metabolite peak height to twice the root-mean-square standard deviation of the noise in areas with no peaks and/or artifacts of the spectrum in the frequency domain (Fig. 2). However, it is sometimes defined as the ratio of the largest metabolite peak height to the root-mean-square standard deviation or the free induction decay (FID) signal amplitude at t = 0 divided by the noise at the end of the FID in the time domain.

Linewidth
The full-width at half-maximum (FWHM) of the spectral height is usually used as the definition of linewidth (Fig. 3). The FWHM of a metabolite or water peak is determined by its T2 and the local inhomogeneity of the magnetic field (ΔB0). Furthermore, if the multiplication of any line as a window function is applied to the FID data before the Fourier transform, the related value will also be included in the FWHM of the spectral data.

\[
FWMH = \frac{1}{\pi T_2} + \gamma \Delta B_0 + (WinFunc) \quad [1]
\]

Here, γ is the gyromagnetic ratio and WinFunc is related to the multiplied window function value. The T2 of the major brain metabolites (tNAA, tCr, tCho, mlns, Glx, and Lac) at 3-T is reported to be 134–318 ms, which means that is calculated to be 1.0–2.4 Hz. To achieve local homogeneity, shimming is performed using a vendor-provided shim tool. Shimming methods include 3D shim, which is based on spherical harmonic analysis, and fast
automatic shimming technique by mapping along projections (FASTMAP) shimming, which is based on measuring $B_0$ field plots along projections.\(^42\)

Voxel size, TE and TR, and number of signal averages

Voxel size: This parameter is also known as volume of interest (VOI). The SNR is said to be proportional to the VOI\(^{35}\) but the VOI is preferably 8 mL or less in in vivo brain MRS.

TE: A short TE (< 35 ms) avoids a signal decrease due to $T_2$ decay and signal phase modulation due to J-coupling and detects numerous metabolites (Fig. 4). However, macromolecules and/or lipids with a short $T_2$ and J-couplings are also detected as broad peaks (Fig. 4),\(^{43-45}\) and these broad peaks may interfere with the quantification of metabolite peaks. At a TE of 144 ms, the lactate peak (1.33 ppm) is inverted owing to the phase modulation by J-coupling (Fig. 5), and in addition, the macromolecules and lipid peaks are relaxed and phase-modulated, resulting in a significant decrease in peak height. Therefore, a TE of 144 ms (or 135 ms) is mainly used for Lac peak evaluation.\(^{46}\) A longer TE is used to observe the metabolite peaks of a relatively longer $T_2$.\(^{47,49}\) Moreover, for a metabolite with J-couplings between multiple $^1$Hs, the peak is decreased owing to the phase modulations with a longer TE time, as shown in Fig. 5.\(^{50,51}\) Therefore, a short TE is required to observe mIns, Glu, and/or Gln, and a TE setting of 144 ms or longer may be used to exclude other metabolites for the evaluation of tNAA, Cr, Cho, and/or Lac (Fig. 4).

TR: As for TR, it should be set to 2000 ms or more to avoid signal loss due to insufficient longitudinal relaxation with a too short TR (see Metabolite quantification for more details).

Number of signal averages: This parameter is also known as the number of excitations (NEX) in MRS. Because the SNR is said to be proportional to the square root of the number of signal averages, if the resulting spectrum does not have a sufficient SNR, an increase in the number of signal averages may give a better result.
Eddy current effect correction

Eddy currents induced by rapid gradient switching generate short-time B0 field fluctuations. If this fluctuation remains during the acquisition window, the FID data are affected by the fluctuation, which leads to MRS signal distortion (Fig. 6). Correction is performed by using a water-unsuppressed signal with an identical VOI, gradient strengths, and timings to the water-suppressed data. The same eddy current effects as the water-unsuppressed data are also included in the phases of the water-suppressed data. By subtracting this phase function from both the water-suppressed and water-unsuppressed FID signals, the spectra can be free from the eddy current effects (Fig. 6).

MRS data acquisition methods

MRS data collection methods include single-voxel MRS and MRSI, which is also called chemical shift imaging (CSI). Furthermore, VOI localization methods include localization by sequences such as point-resolved spectroscopy (PRESS) which generate a cube, rectangular, or parallelepiped voxel, and localization by a surface coil with a sequence such as depth-resolved sequence (DRESS), which is often used when the target organ or tissue is in a region of the body other than the head and when the target nucleus is a non-proton nucleus, such as 13C or 31P.

Water signal suppression and outer volume suppression pulse

Prior to the execution of the localization sequence, water signal suppression and outer volume suppression (OVS) are usually performed, as shown in Fig. 7. Water has a concentration of 10000 times or more than that of metabolites and, in the spectrum, distortion due to water peaks may make it difficult to quantify the metabolite peaks. Therefore, a chemical shift selective (CHESS) pulse is used for the water signal suppression. The CHESS sequence comprises a frequency-selective RF pulse followed by a spoiler gradient. In a clinical MR scanner, three repetitions of CHESS pulses are used to enhance the water suppression effect; because the repetition of CHESS pulses generates unwanted echoes, the direction of the spoiler gradient is switched for each CHESS pulse (Fig. 7a1) or its intensity is gradually increased (Fig. 7a2). When spectra of an identical VOI with a water-unsuppressed signal are also acquired to correct eddy current-induced phase shifts and quantify metabolite concentrations (see Eddy current effect correction and Metabolite quantification for more details), the CHESS RF pulse is specifically turned off and the spoiler gradient is used as is. OVS is a method for spatially saturating and reducing the signal outside the VOI. The strength of the slice gradient for the saturation pulse should be higher in order to minimize the chemical shift displacements of metabolites.

Single-voxel MRS

STEAM: The STEAM sequence is installed by default on MRI devices capable of MRS. The STEAM sequence uses three 90° pulses for localization and generates four to five echoes; the echo generated after the third 90° pulse is called the stimulated echo and is used in MRS as the desired signal (Fig. 7c1). Spoiler gradients destroy the echoes and FIDs outside the VOI. The interval between the second and third pulses is called the mixing time (TM). During the
TM period, the magnetization is at the longitudinal (z-) axis, has no transverse components, and does not experience T2 decay. Therefore, the effective TE is the sum of the times between the first and second pulses (TE/2), and the third pulse and the signal acquisition (TE/2). The maximum signal intensity of the stimulated echo can be calculated as follows:

\[ S \propto \frac{M_0}{2} \cdot \sin \alpha_1 \cdot \sin \alpha_2 \cdot \sin \alpha_3 \cdot \exp \left( -\frac{TE}{T_2} \right) \cdot \exp \left( -\frac{TM}{T_1} \right), \]

where \( M_0 \) is the equilibrium magnetization along the z-axis and \( \alpha_i \) is the \( i \)th pulse angle. If each pulse is a perfect 90°, and TE and TM are both short enough compared with the \( T_1 \) and \( T_2 \), the maximum possible signal is 50% of the \( M_0 \).

PRESS: The PRESS sequence\(^{28}\) is also installed by default on MRI devices capable of MRS. The PRESS sequence comprises mainly three slice-selective pulses (90°–180°–180°), each of which has a field gradient for localization, and a single voxel is finally excited. Spoiler gradients on both sides of the field gradient destroy the echoes and FIDs outside the VOI mainly generated by imperfect RF pulses\(^{18,28,41}\) (Fig. 7c2).

PRESS is more widely used because its SNR is twice of that of STEAM.\(^{18,76}\) However, the advantages of STEAM over PRESS are that (i) TE can be shortened in STEAM owing to the difference in the echo generation mechanism described above, and (ii) by using only 90° pulses, it has a sharper slice profile, a wider pulse bandwidth, smaller chemical shift displacements of metabolites, and a smaller specific absorption rate.\(^{18,41,77}\)

**Metabolite quantification**

There are several software packages for in vivo MRS data analysis, including LCModel, jMRUI, and Tarquin, all three of which are free to use.\(^{78-83}\) Here, we will consider LCModel and describe the output metabolite values. LCModel runs on Unix or Linux and quantitatively analyzes peaks of in vivo brain metabolites, muscle, or liver from the MRS data collected.\(^{34,79}\) LCModel uses linear analysis to separate a spectrum into peaks of individual metabolites by comparison with a set of model spectra, called a basis set (see the last paragraph of this section for more details). When FID data are input into the LCModel, preprocessing such as fast Fourier transform and phase correction\(^{43,54,84,85}\) is automatically performed, and the MR spectrum and various items are output, including the quantitative values of each metabolite, Conc., %SD, \%/Cr+PCr, and Metabolite (Fig. 1). The Conc. values of each metabolite are calculated using the following equation:

\[ \text{Conc.} = \frac{\text{Metabolite peak area}}{\text{Metabolite proton numbers}} \times \text{Water concentration}, \]

where the metabolite peak area is that of the spectrum of the water-suppressed signal and the water peak area is that of the spectrum of the water-unsuppressed signal obtained from the identical VOI. The calculation process for Conc. does not consider the relaxation of the water and metabolite peaks. However, by default, only the water peak area is multiplied by 0.7, which takes into
account the reduction in the water peak by $T_2$ at $TE = 30\; ms$.\textsuperscript{34} The correction of the peak relaxation by $T_1$ and $T_2$ for water and metabolites will be discussed later. Water concentration describes the water concentration in the VOI. The default value is 35.88 M, which is that of adult white matter.\textsuperscript{34} In addition, a concentration of 43.30 M is listed in the LCModel manual as that of adult gray matter.\textsuperscript{34} However, the VOI region may contain a mixture of gray matter, white matter, and CSF,\textsuperscript{86,87} and the ratio of these components must be measured to more accurately determine the water concentration. Moreover, water concentrations vary with age\textsuperscript{8,47,88,89} and may be altered in diseases such as edema and some tumors.\textsuperscript{90–92} Therefore, it should be noted that serious errors in the results can occur if metabolite Conc. values are obtained using the same water concentration for different age groups, such as children and adults, or for healthy and disease groups.

Depending on the TE and TR settings, the water and metabolite peaks will decrease with their respective $T_1$ and $T_2$.

\textbf{Fig. 7} An example of a single-voxel MRS scheme (not to scale). \textbf{a)} CHESS pulse for water suppression. \textbf{b)} Outer volume suppression pulse. \textbf{c1)} STEAM sequence. TM = mixing time. \textbf{c2)} PRESS sequence. TE1 = first PRESS echo time; TE2 = second PRESS echo time.
\[ M_{xy} = M_0 \left\{ 1 - \exp\left( -\frac{TR}{T_1} \right) \right\} \cdot \exp\left( -\frac{TE}{T_2} \right) \]  

where \( M_{xy} \) is the magnetization on the xy-transverse plane and \( M_0 \) is the equilibrium magnetization along the Z-axis. The first exponential equation is the \( M_0 \) recovery rate according to \( T_1 \) at \( t = TR \), and the \( M_0 \left\{ 1 - \exp\left( -\frac{TR}{T_1} \right) \right\} \) is equal to the \( M_{xy} \) magnetization just after being inverted to the xy-transverse plane by the 90° excitation pulse. The second exponential equation is the signal decay due to \( T_2 \) relaxation after excitation. Therefore, adding to Equation [3] a consideration of the decrease in metabolite and water peaks due to \( T_1 \) and \( T_2 \), we get the following equation:

\[
\frac{\text{Metabolite peak area}}{\text{Metabolite proton numbers}} \times \frac{\text{Water peak area}}{\text{Water proton numbers}} \times \frac{\left\{ 1 - \exp\left( -\frac{TR}{T_1} \right) \right\} \exp\left( -\frac{TE}{T_2} \right)}{\left\{ 1 - \exp\left( -\frac{TR}{T_1} \right) \right\} \exp\left( -\frac{TE}{T_2} \right)},
\]

Equations [4] and [5] show that the combination of a longer \( TR \) and shorter \( TE \) results in a smaller decrease in magnetization. At 1.5- and 3-T, the \( T_1 \) of adult brain metabolites is up to about 1.6 s,\(^{8,38,93-96}\) and if the \( TR \) is set to 5 s or more, that is, \( \left\{ 1 - \exp\left( -\frac{TR}{T_1} \right) \right\} \approx 1 \), it is not necessary to consider the correction of magnetization reduction by the \( T_1 \). On the other hand, children have a higher brain water concentration and longer \( T_1 \) and \( T_2 \) compared with adults,\(^{8,47,89}\) and even adults may have a higher water concentration in pathological tissues such as tumors,\(^{91}\) and may therefore need a longer \( TR \) setting to ignore the \( T_1 \) effect. To avoid the decrease in magnetization due to \( T_2 \) (and \( J \)-couplings, if present), a shorter \( TE \) is useful,\(^{8,38,43,47,49,89,97-102}\) but this needs to take into account the contribution of the macromolecule peaks (see Voxel size, TE and TR, and number of signal averages for more details). To determine the metabolite concentrations, it may also be necessary to consider the chemical shift displacements of metabolite peaks with different frequencies from the excitation frequency, which is usually set to the water resonance.\(^ {18,103}\)

The value of %SD is equal to the Cramér–Rao lower bound and is used as an index of the uncertainty in the metabolite concentration estimates.\(^ {34}\) The \( \text{Cr}^+\text{PCr} \) for each metabolite represents the value of its metabolite value displayed in Conc. divided by that of \( \text{Cr}^+\text{PCr} \) displayed in Conc.\(^ {34}\) The \( \text{Cr}^+\text{PCr} \) value (i.e., metabolite to \( \text{Cr}^+\text{PCr} \) ratio) can be obtained without a water-unsuppressed spectrum and is a simpler method than that for determining the metabolite concentration.

Clinical utility of \(^1\)H-MRS

Even in the 2010s, the clinical dissemination of \(^1\)H-MRS as a routine diagnostic tool was very slow, and the availability of \(^1\)H-MRS was generally limited to specialized institutions or hospitals with specialists in \(^1\)H-MRS technology. The reasons for the limited clinical use of \(^1\)H-MRS include its technical feasibility, lack of methodological standardization, and difficulties in interpretation. Some MR communities have published several excellent reviews to help establish a methodological and clinical consensus in the 2010s\(^ {105,106}\) because the increased availability of 3-T MRI instruments was considered to be an excellent opportunity to promote clinical MRS. Our MRS community in the Japan Society of Magnetic Resonance in Medicine (JSMRM) published a consensus guideline in Japanese on the clinical utility of \(^1\)H-MRS in 2013 (http://fa.kyorin.co.jp/jsmrm/hattuyo_new_MRS_guideline2013.pdf), which can be downloaded from the website of the society. Here, we have attempted to update this consensus guideline to include recent progress related to the technology and its generally accepted findings.

Physiological changes in metabolites with development and age observed with \(^1\)H-MRS

The signals of major metabolites observed with \(^1\)H-MRS are affected by brain development and aging, and their concentrations also differ among anatomical cerebral regions. The intensities of Cho and NAA signals are generally higher in white matter than in gray matter, whereas those of Cr, mlNs, and Glx are higher in gray matter than in white matter.\(^ {107,108}\) Furthermore, the intensities of Cr and Cho signals are usually highest in the cerebellum.\(^ {108}\)

Many studies have shown that NAA and Cr signals are increased in childhood and that Cho and mlNs signals decrease rapidly within the first year of life as development progresses, with a gradual decline until about 20 years of age.\(^ {107}\)

In a systematic review of the brain metabolite changes measured by \(^1\)H-MRS during healthy aging,\(^ {109,110}\) most data were derived from the frontal region and indicated no change in older individuals in 18 studies. However, a meta-analysis
showed a decrease in frontal NAA and increases in parietal Cho and Cr. This review suggested that NAA may decrease, and Cho and Cr increase with age, and more data are therefore needed from older individuals to better characterize the effect of age. Accordingly, metabolite ratios in older people should be interpreted with caution.

**Clinical utility in the diagnosis of brain tumors and tumor-like disorders**

The clinical utility of $^1$H-MRS can largely be classified into five categories: 1) grading of gliomas, 2) evaluation of therapeutic effects, including differentiation between recurrence and radiation necrosis, 3) differentiation between gliomas and brain metastasis, 4) differentiation between cystic tumors and brain abscess, and 5) prediction of isocitrate dehydrogenase (IDH) mutant gliomas using the detection of 2-hydroxyglutarate (2-HG).

For glioma grading, several studies have reported that automated algorithms can classify high- and low-grade gliomas with sensitivity and specificity exceeding 90%. However, the accuracy of the differentiation between grade III and IV is lower. The ability to differentiate between gliomas and metastasis was found in good responders to tamoxifen. The accuracy of the differentiation between gliomas and metastasis was 60%–80% and lower than that of the grading of glioma (about 90%). The ability to differentiate between gliomas and metastasis was suggested to be related to a greater increase in lipids in metastasis than in gliomas. Cystic tumors and abscesses were successfully differentiated using the signal derived from succinate, acetate, alanine, and glycine, with 96% sensitivity and 100% specificity.

IDH mutant gliomas have recently received more attention due to their better prognosis than IDH wild-type gliomas. IDH overproduces 2-HG, which is a suitable biomarker of IDH mutant glioma. 2-HG has five protons with a complicated J-coupling interaction and shows complicated multiplet signals at about 1.9 ppm, 2.3 ppm, and 4.0 ppm. These signals usually overlap the major peaks of NAA, Glx, and Cr, and accurate detection of 2-HG by $^1$H-MRS is therefore difficult and is still undergoing refinement. Thus far, several $^1$H-MRS methods have been proposed, such as a short TE or long TE conventional $^1$H-MRS sequence using prior-knowledge spectral analysis software (e.g., LCModel), the difference editing technique, and the 2D method. Most of the studies have been conducted using a conventional sequence with specific analysis software and showed higher sensitivity than specificity, with ranges of 77%–100% for sensitivity and 72%–100% for specificity. Although a few studies have been performed with the difference editing technique, a recent prospective study using spectral editing MRS with jMRUI software showed a sensitivity of 80% and specificity of 81%. Preoperative edited MRS also appears to be able to help identify IDH mutant gliomas with high specificity, but the accuracy requires improvement.

**Clinical utility of $^1$H-MRS in neurodegenerative diseases**

Neurodegenerative diseases, which include Alzheimer disease, Parkinson disease, Huntington disease, amyotrophic lateral sclerosis, and multiple system atrophy, have been studied using clinical $^1$H-MRS. Most of the studies concern Alzheimer disease and other types of dementia. In Alzheimer disease, the major findings were a decrease in NAA and Glu and increase in mIns in the affected regions. The decrease in NAA in the affected brain region is consistent with that of other neurodegenerative diseases, but the tendency was not disease-specific. Several studies have monitored the treatment response in Alzheimer disease. A transient increase in NAA concentration was associated with a short-term functional response during donepezil treatment in Alzheimer disease, indicating that NAA also reflects functional integrity and recovery. It is considered that the utility of $^1$H-MRS will be in patient management rather than in disease diagnosis.

**Clinical utility of $^1$H-MRS in pediatric brain disorders**

$^1$H-MRS can be used to detect characteristic metabolic changes, especially in metabolic or degenerative pediatric diseases, such as lysosomal storage disease, peroxisome disease, and mitochondria disease. $^1$H-MRS is sometimes more useful than MRI-mediated morphological diagnosis in certain metabolic or degenerative diseases, and its utility can be classified into the following four categories. Although these categories comprise rare diseases, $^1$H-MRS plays significant roles in their clinical diagnosis.

**Deficiency in Cr signals**

Three metabolite diseases have thus far been reported in this category. Guanidinoacetate methyltransferase (GAMT) deficiency and creatine transporter (CRTR) deficiency show a loss of Cr signal and an increase in guanidinoacetate, which is a precursor of Cr, with signals appearing at about 3.7–3.9 ppm. Arginine:glycine amidinotransferase (AGAT) deficiency also shows a loss of Cr signal, with no increased signal from metabolites.

**Increased NAA signal**

Canavan disease and Salla disease are the two representative diseases in this category. Canavan disease causes decreased activity in aspartoacylase, which induces NAA accumulation. In addition, Cr and Cho are decreased and mIns is increased. Salla disease is an abnormality in sialic acid metabolism that involves the accumulation of n-acetylneuraminic acid (NANA), whose signal is close to that of NAA, which complicates their differentiation. This disease may be recognized as an increase in NAA signal, especially at 1.5-T. Furthermore, Pelizaeus-Merzbacher...
disease is also known by an increase in NAA and Cr, and decrease in Cho.

Appearance of specific peaks that cannot usually be identified
a) Sjögren–Larsson syndrome: This disease is an abnormality in lipid metabolism involving a deficiency in fatty aldehyde dehydrogenase. The peaks derived from long-chain fatty alcohols are found at 0.9 and 1.3 ppm. A peak is observed at 0.9 ppm with a short TE sequence due to the short T2 values of this metabolite.131
b) Succinate dehydrogenase deficiency: This disease is a type of mitochondrial encephalopathy. The peak of succinate is found at 2.4 ppm, in addition to the Lac peak.132
c) Maple syrup urine disease: This disease is an abnormality in branched chain amino acid metabolism. The peaks of valine, leucine, and isoleucine are observed at 0.9–1.0 ppm, in addition to a decrease in NAA and an increase in Lac.133
d) Galactosemia: This disease is caused by an abnormality in an enzyme related to galactose metabolism. The peaks of galactitol are found at 3.67 and 3.74 ppm.134
e) Nonketotic hyperglycinemia: This disease is due to an abnormality in the glycine cleavage enzyme. The peak in glycine is observed at 3.55 ppm, which overlaps with that of mlns.135 However, the T2 value of glycine is much longer than that of mlns, which means that the peak at 3.55 ppm is usually observed in these patients with a long TE sequence. There are no abnormalities in NAA, Cr, and Cho with this disease.
f) Ribose-5-phosphate isomerase deficiency: This disease involves an abnormality in polyol metabolism. 1H-MRS shows peaks of D-arabitol and ribitol at 3.6 and 3.8 ppm, respectively, which reflect the accumulation of polyols.136
g) Sandhoff disease: This lysosomal disease is caused by a deficiency in hexosaminidases A and B. The peak of N-acetylgalactosamine is found at 2.12 ppm, and 1H-MRS also detects a decrease in NAA and increase in mlns.137
h) GABA transaminase deficiency: Glu is metabolized to succinate from GABA through succinic semialdehyde. This pathway is called the GABA shunt. This disease causes an increase in GABA and 1H-MRS can show the increase in GABA signal.27
i) 3-hydroxy-3-methylglutaryl-CoA lyases (HMG-CoA) deficiency: HMG-CoA is transferred to acetyl-CoA and individuals with this deficiency show a peak at 2.42 ppm from 3-hydroxyisovalerate and 3-hydroxy-3-methylglutarate.138 In addition, 1H-MRS also identifies a decrease in NAA and increases in Cho and mlns.

Clinical studies of developmental disorders
Developmental disorders, including autism spectrum disorder, are an important target for clinical pediatric investigation and many studies have reported pathophysiological changes in this disorder using 1H-MRS. However, the difference in metabolites between developmental disorder and normal development has not been characterized, and 1H-MRS is not considered useful for the diagnosis of this disorder. On the other hand, oxytocin has recently been reported to be useful for improving autistic behavior, with its effect revealed in a randomized controlled trial using 1H-MRS and functional MRI.139 1H-MRS may be useful for evaluating the improvement in brain function with some medications.

Clinical utility of 1H-MRS in psychological disorders 
Schizophrenia
Of psychological disorders, schizophrenia is the most popular target for clinical 1H-MRS. The main findings of schizophrenia are decreased NAA or NAA/Cr in the frontal lobe, thalamus, hippocampus, and temporal lobe.140,141 On the other hand, there are no significant differences in Cr and Cho between schizophrenic and healthy individuals. However, these findings are not specific to schizophrenia alone, and the utility of 1H-MRS in clinical diagnosis remains low.

Major depression
The number of published studies concerning major depression is lower than that for schizophrenia, and there is no difference in NAA between individuals with major depression and healthy people in most previous work. Some studies suggested a decrease in Cho/Cr in the basal ganglia and a decrease in Glx in the frontal lobe,142,143 and one study indicated a decrease in GABA in the posterior lobe with a 3-T MRI instrument.144

Bipolar disorders and anxiety disorders
There are, thus far, no consistent findings of 1H-MRS in bipolar disorder and anxious disorder. Several studies showed decreased NAA or NAA/Cr in the basal ganglia and hippocampus in bipolar disorder,145 but others reported no change in NAA. In anxiety disorder, functional MRI suggested abnormalities in the orbitoprefrontal cortex, caudate nucleus, and thalamus, but there is no consensus on the changes in metabolites observed with 1H-MRS. The utility of 1H-MRS in bipolar and anxiety disorders is limited to the clinical diagnosis and evaluation of therapeutic efficacy.

Utility of 1H-MRS in demyelinating diseases (multiple sclerosis, clinically isolated syndrome, and neuromyelitis optica spectrum)
The utility of 1H-MRS in the diagnosis of multiple sclerosis (MS) can be summarized as follows: 1) comprehension of the pathological changes in the plaques and normal-appearing white matter (NAWM); 2) evaluation of the therapeutic efficacy and monitoring of the clinical course; and 3) prediction of the transformation from clinically isolated syndrome (CIS). The typical findings of MS are increases in Cho, Lac,
Lip, mlNs, and Glu and a decrease in NAA in the acute phase; in the chronic phase, the decrease in NAA and the increase in mlNs continue, but the signals of Lac, Cho, and Lip gradually normalize.\(^{146}\) The increase in Glu is limited in the acute phase and may be related to Glu-associated axonal injury and toxicity. In particular, a prominent increase in Glx has been found in tumoractive MS.\(^{147}\) One study determined an increase in mlNs only in MS plaques, and not in NAWM, and mlNs may be used to differentiate between MS plaques and NAWM. Therapy responders showed an increase in NAA/Cr.\(^{148}\) Several studies reported predictors of the transformation from CIS to MS, and useful predictors may be a significant decrease in NAA in NAWM in the early phase and an increase in mlNs.\(^{149,150}\)

\(^{1}\)H-MRS studies in patients with neuromyelitis optica (NMO) spectrum disorder are still limited, but most of the studies have found that the differentiating finding of NMO from MS is the lack of metabolite abnormalities in NAWM in patients with NMO, except for mlNs.\(^{151}\)

**Acute ischemic disease**

Multiple studies have evaluated acute ischemic lesions with \(^1\)H-MRS, with decreased NAA and increased Lac consistent findings in acute ischemia, which are related to mitochondrial function and energy failure. One study reported an increase in Lac in the ischemic lesion before the appearance of a high signal in diffusion-weighted imaging.\(^{154}\) However, no study has determined the level of metabolites on \(^1\)H-MRS at which thrombolysis therapies should be applied, and there is no consensus on a suitable quantification method for application to acute ischemic diseases. To avoid time-consuming processes that delay clinical decision-making, further improvements in the measurement time and simplification of the procedure for accurate quantification are required to enable a multicenter prospective study to determine the threshold for the application of thrombolysis in patients with acute ischemic attacks.

**Nuclei other than protons possibly useful in clinical MRS**

Besides protons, all nuclei with a magnetic moment can theoretically be used for nuclear magnetic resonance (NMR).\(^{155}\) Since the NMR frequency of each nucleus in a static magnetic field is different, it is necessary to install a transmission and reception system in the MR system and to prepare coils that support the frequency. In vivo MRS of nuclei other than protons has been performed for decades, but its application to in vivo humans has been enabled by the recent increase in static magnetic field strength and advances in fast and efficient sampling and reconstruction techniques.\(^{43,61,156,157}\) The nuclei other than protons that may be useful in clinical MRS in the coming years are \(^{13}\)C, \(^{17}\)O, \(^{23}\)Na, and \(^{31}\)P. Their nuclear spin properties are summarized in Table 2. Relative receptivity is obtained with the following equation:\(^{158}\)

\[ R_A(X) = \frac{\gamma(X)}{\gamma(A)} \times \left( \frac{I(X)}{I(A)} \right) \left( \frac{I(A+1)}{I(A)} \right) \times \frac{N_{\text{abd}}(X)}{N_{\text{abd}}(A)} \]  \[ \text{[6]} \]

where \(R_A(X)\) is the relative receptivity of nucleus X, A is a reference nucleus such as \(^1\)H, \(\gamma\) is the gyromagnetic ratio, I is the spin quantum number, and \(N_{\text{abd}}\) is natural abundance.

\(^{13}\)C: Its natural abundance is about 1%. Oral or intravenous administration of labeled \([1–^{13}\text{C}]\) D-glucose and/or \([2–^{13}\text{C}]\) has been used to monitor the cerebral tricarboxylic acid (TCA) cycle, the Glu–Gln cycle, the metabolism from glucose to amino acids in the brain, the cerebral metabolic rate of oxygen consumption (CMRO\(_2\)), and the amount of glucose and glycogen in the liver.\(^{12,67,159–163}\) With the hyperpolarization (HP) technique, \(^{13}\)C-labeled substances increase their MRI signals at a rate of > 10000.\(^{18,164–166}\) \([1–^{13}\text{C}]\) Pyruvate has been approved for use in clinical HP studies by the US Food and Drug Administration and has been used to monitor some metabolic pathways, such as glycolysis, the TCA cycle, and amino acid biosynthesis.\(^{166}\) In vivo human studies have reported metabolic changes in patients with prostate cancer, brain tumor, or breast cancer.\(^{167–169}\) Other \(^{13}\)C HP probes used comprise endogenous biomolecules such as \([2–^{13}\text{C}]\) pyruvate for probing mitochondrial metabolism\(^{170–172}\) and \(^{13}\)C urea for imaging perfusion.\(^{173}\)

\(^{17}\)O: \(^{17}\)O has a spin of 5/2, but the \(^{17}\)O\(_2\)–gas phase and \(^{17}\)O\(_2\) dissolved in water have strong paramagnetism and hence are undetectable in \(^{17}\)O NMR. The \(^{17}\)O signal becomes detectable only when \(^{17}\)O is incorporated into water.\(^{162}\) \(^{17}\)O has been used to measure the CMRO\(_2\) and as a contrast agent.\(^{162,174–182}\) There are two types of \(^{17}\)O-based NMR approaches. One approach involves the direct detection of \(^{17}\)O signal from \(^{2}\)H\(_2\)\(^{17}\)O by intravenous injection of \(^{2}\)H\(_2\)\(^{17}\)O or by inhalation of \(^{17}\)O\(_2\) gas, which is converted to \(^{2}\)H\(_2\)\(^{17}\)O via oxidative phosphorylation in the brain.\(^{162,174,178–181}\) The second approach is to indirectly detect \(^{17}\)O with \(^1\)H-MRI. The protons in \(^2\)H\(_2\)\(^{17}\)O have various Larmor frequencies due to scalar coupling with \(^{17}\)O.\(^{183}\) The chemical exchange of water protons among \(^2\)H\(_2\)\(^{17}\)O and \(^2\)H\(_2\)\(^{16}\)O makes the \(T_2\) of the water protons shorter. As a result, the \(T_2\)-weighted imaging signal intensity of the region including \(^2\)H\(_2\)\(^{17}\)O is lower than that containing only \(^2\)H\(_2\)\(^{16}\)O. Therefore, it is used to observe water flow by dynamic \(T_2\)-weighted \(^1\)H-MRI.\(^{175–177,182,184–187}\)

\(^{23}\)Na: Its natural abundance is 100%. \(^{23}\)Na has a spin of 3/2, which causes electrostatic field sensitivity and fast \(T_2^*\) signal decay.\(^{41,188,189}\) \(^{23}\)Na is endogenous to human tissue and can be imaged without external contrast. The concentration of sodium ions (Na\(^+\)) is reported to be raised in solid tumors, and in vivo human \(^{23}\)Na-MRI studies have been conducted in patients with brain tumor, breast tumors, prostate cancer, and serous ovarian cancer.\(^{190–196}\)
31P: Its natural abundance is 100%. In 1H-MRS, Cho, phosphocholine (PCho), and glycerophosphocholine are observed as total Cho, with 1H from the methyl group (-CH3) having almost the same chemical shift value (3.2 ppm). However, 31P-MRS, PCho, and glycerophosphocholine have a phosphate group, and the chemical shift value of each 31P is different and individual quantification is possible. In vivo 31P-MRS provides information on energy metabolism, cell membrane, and pH by monitoring peaks, such as adenosine triphosphate and phosphocreatine, phosphomonoesterase and phosphodiesterase, and inorganic phosphate, respectively. Especially in cancer, Cho phospholipid metabolism, particularly PCho and tCho, are elevated and can be used in cancer diagnosis and staging by using 31P-MRS.

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### Conflicts of Interest

The authors have no conflicts of Interest.

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