MRS in Hepatic Fat

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Magnetic resonance spectroscopy (MRS) is a powerful tool that allows direct quantification of metabolites in tissue or areas of interest. MRS has been applied in both research and clinical studies to assess liver fat noninvasively in vivo. MRS has also demonstrated excellent performance in liver fat assessment with high sensitivity and specificity compared to biopsy and other imaging modalities. Because of these qualities, MRS has been generally accepted as the reference standard for the noninvasive measurement of liver steatosis. MRS is an evolving technique with high potential as a diagnostic tool in the clinical setting.

Keywords: magnetic resonance spectroscopy ; liver fat ; hepatic steatosis ; liver fat fraction ; 1H-MRS

1. Introduction

Several imaging modalities have been used in liver fat assessment, including ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI), and magnetic resonance spectroscopy (MRS). Among these imaging modalities, MRS has shown high accuracy in liver fat quantification with safe, noninvasive, and reproducible results [1]. MRS provides a direct measurement of liver fat from the signal peak of fat and is also commonly accepted as a noninvasive reference standard for liver fat assessment [2][3].

2. The Importance of a Sensitive Method for Liver Fat Assessment Important

Hepatic steatosis or the accumulation of liver fat is the pathological hallmark of NAFLD and has many other clinical implications.

The criterion for the diagnosis of NAFLD is excess fat accumulation in the liver that affects more than 5% of hepatocytes [4]. The degree of liver steatosis is stratified by the percentage of hepatocytes affected by steatosis, as follows: S0 (<5%), S1 (5–33%), S2 (>33–66%), and S3 (>66%) [5].

Currently, the gold standard for identifying more aggressive NASH is a liver biopsy in staging fibrosis and liver steatosis [6]. In addition to providing information for staging liver steatosis, a liver biopsy also assists with identification of the manifestation of other liver diseases that might coexist or have similar characteristics to fatty liver, such as chronic hepatitis C infection [7]. However, the percutaneous liver biopsy is limited by its invasive nature and is not suitable for clinical applications that require real-time monitoring of liver fat levels throughout therapeutic intervention.

Notably, an early stage of NAFLD can easily be reversed with lifestyle modification [8]. While the identification of this early stage may help prevent disease progression, a sensitive and noninvasive method for liver steatosis will prove to be more useful in later stages of NAFLD. Considering the prevalence and severe consequences of advanced NAFLD, sensitive and real-time monitoring tools would help with the evaluation of the therapeutic response that might lead to small changes in liver fat in the early stage of intervention. At present, serum biomarkers and imaging techniques have been proposed as two main approaches for noninvasive liver steatosis assessment [9], and MRS is one of those techniques.

3. Basic Principle for MRS

MRS utilizes the MR principle to identify and quantify the metabolite from the tissue of interest. The signal in MRS is obtained in the same way as MR imaging, that is, a radiofrequency (RF) at specific resonance is applied to nuclei (e.g., ^{1}H, ^{13}C, ^{31}P, etc.) in a static magnetic field to generate a signal [10]. The pulse sequence and MR signal acquisition are shown in **Figure 1**. This signal comes from a specific area of interest or the voxel that is then Fourier transformed from the MR signal to the MR spectrum. Unique chemical properties and environments lead to the unique proton resonance frequency and peak shape of each metabolite. This slight shift of the resonance position along the x-axis of the spectrum

is termed the chemical shift, which is measured in ppm [11]. The calculation of ppm is obtained from the distance in Hertz (Hz) relative to a reference peak such as water, divided by the operating frequency of the MR system [11][12]. The proton resonance frequency is proportional to the MR field strength at 63.9 MHz at 1.5 Tesla, 127.8 MHz at 3 Tesla, and 298.2 MHz at 7 Tesla. Therefore, the chemical shift in ppm can be compared across studies irrespective of MR field strength. The MR field strength is also proportional to the improved signal-to-noise ratio (SNR). Thus, the increased field strength of MR machines improves spectral resolution and the separation of metabolite peaks [13].

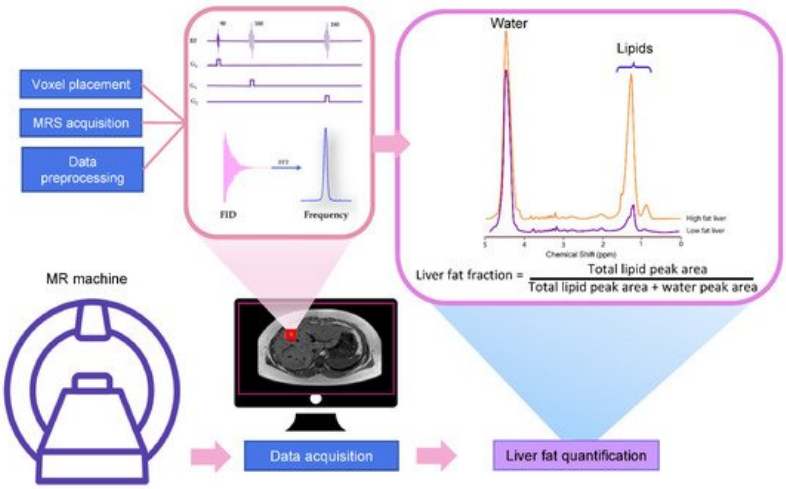

Figure 1. Schematic explanation of how to acquire the MR spectrum. MR spectra were obtained from a region of interest. Free induction decay (FID) is then acquired and converted to resonance frequency spectrum by fast Fourier transformation (FFT). The liver fat fraction can be calculated from the peak area corresponding to fat and water.

3.1. Liver MRS Spectrum

Most of the visible peaks in the MRS liver spectrum obtained from the clinical MR scanner (1.5-3 Tesla) are fat and water. While water shows a single peak at approximately 4.7 ppm, fat shows multiple peaks due to its complex chemical components (**Table 1**) [14][15][16]. Six resonances of fat are usually detected with the main lipid peak at approximately 0.9 to 2.75 ppm. There are also unresolved fat resonances at 4.2 and 5.3 ppm from glycerol and olefinic acid, respectively (**Figure 2**) [15][16][17]. These two peaks overlap with the water peak signal at 4.7 ppm. While the correct identification of the liver fat peak is possible in MR systems with a high field, it is less feasible in a lower field with lower spectral resolution and broader linewidth. The misidentification of lipid peaks leads to quantification errors in liver fat; therefore, these unresolvable peaks are not qualified for diagnostic purposes [11].

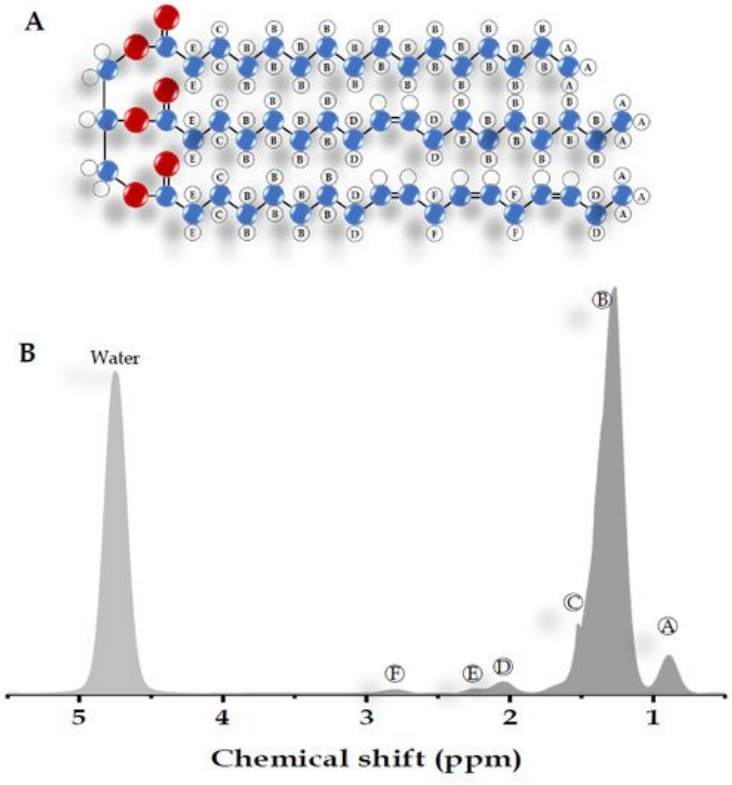

Figure 2. Schematic illustration of a triglyceride molecule and MR spectrum from the liver. (**A**) The molecular structure of the triglyceride. Hydrogen atoms are shown in white (○), carbon in blue (●), and oxygen in red (●). (**B**) The spectrum of all molecules obtained from the liver corresponding to the hydrogen atom position on the molecular structure of a triglyceride.

Table 1. Detectable metabolite peaks from the liver MR spectrum.

Peak	Chemical Shift (ppm)	Type	Hydrogen Atom Position (Bold)
A	0.9	Methyl	$-CH_2-CH_3$
B	1.3	Methylene	$-(CH_2)n-$
C	1.59	β-Carboxyl	$-CH_2-CH_2-COO$
D	2.1	α-olefinic	$-CH_2-CH=CH-$
E	2.25	α-Carboxyl	$-CH_2-CH_2-COO$
F	2.75	Diacyl	$-CH=CH-CH_2-CH=CH-$
-	4.7	Water	H_2O

3.2. The Acquisition of Liver MRS Spectrum

MRS liver spectra are often obtained using a single-voxel technique [18][14]. The advantage of the single-voxel technique is that it provides a high SNR from a large volume of liver sampled. While multivoxel spectroscopy allows larger coverage of the liver than other techniques, the distance from the coil to the organ, longer acquisition time, and reduced shim quality limit its application [14][12][19].

A coil with a multichannel coil array receiver is recommended over a body coil for MRS acquisition to maximize the SNR [11][20]. The quality of the MRS spectrum is sensitive to inhomogeneous magnetic fields. Good magnetic field homogeneity is required for good spectral resolution or small line width to distinguish peaks from each other. The use of shimming of the magnetic field is therefore necessary to minimize field inhomogeneity across the voxel. While most commercially available MR machines have automated shimming prior to MRS acquisition, manual shimming can also be performed to improve field homogeneity.

The most common pulse sequences for MRS spectral acquisition are stimulated-echo acquisition mode (STEAM) and point-resolved spectroscopy (PRESS). STEAM is a stimulated echo-based technique that utilizes three 90° angles to create well-defined voxels and reduce contaminating signals outside the voxel [18]. PRESS is a spin echo-based technique that uses a 90° pulse followed by 180°–180° (**Figure 1**).

3.3. MRS Spectrum Analysis and Liver Fat Quantification

Several commercial and noncommercial software programs are available for the analysis of the MRS spectrum [21][22][23][24]. These specialized software packages provide more flexibility for MRS spectra analysis from the preprocessing process through metabolite quantification.

The liver fat fraction calculation can be obtained from the ratio of lipid peak area to the water peak area. After the visible lipid peaks (0.9–2.1 ppm) and water (4.7 ppm) are identified in the spectrum analysis process, the area under the peak is calculated through peak modeling, such as the Gaussian or Lorentzian model [19]. As previously discussed, only disguisable lipid peaks (0.9, 1.3, and 2.1 ppm) or main lipid peaks (1.3 ppm) have been used for the calculation of lipid signals [7]. A total fat signal is obtained from the summation of individual lipid peak areas from water-suppressed liver MRS spectra. The total water signal is obtained from unsuppressed spectra. The fat fraction (FF) can then be calculated by dividing the total fat signal by the sum of the water and fat signals ($FF = Signal_{fat}/Signal_{fat} + Signal_{water}$) [25].

MRS-derived FF is generally accepted as a reference method for noninvasive liver fat assessment [26][27][28][29]. MRS-derived FF is used as a standard reference in the validation of the method for proton-density FF from MR imaging [27] and other imaging modalities, such as CT and US [26]. While biopsy is the gold standard for liver steatosis, several studies have demonstrated that MRS has excellent correlation with the liver fat content from the histopathologic assessment [1][30][31][32].

4. Application of MRS for Liver Fat Quantification

4.1. Evaluation of Diffuse Liver Fat Disposition

MRS has been used in clinical trials to investigate liver steatosis grading. Previous studies have applied MRS to investigate liver steatosis grading in a large group of subjects [33][34]. This illustrates the feasibility of MRS for hepatic steatosis grading in the general population.

Moreover, MRS can estimate the subspecies of liver fat from MRS from the lipid subspecies index using an equation based on the oil spectra model [35][36][37]. It has been suggested that the degree of saturation of liver lipids may be associated with liver fat accumulation on hepatocellular damage and disease progression [36][38]. Each peak of lipid spectra reflects the different chemical positions within the triglyceride molecule, including unsaturated, saturated, monounsaturated, and polyunsaturated fatty acids [39]. The fatty acid composition quantification obtained from MRS has also shown good agreement with other MRI-based methods [40][41].

Several studies have demonstrated that MRS can evaluate the efficiency of therapeutic intervention [42]. MRS was previously used in a clinical trial of drugs for NAFLD and was able to evaluate the reduction in liver fat content in a dose-dependent manner [43][44]. In a double-blind study of NAFLD patients, MRS-assessed liver fat showed no alteration from symbiotic treatment while reducing fecal dysbiosis [45]. Additionally, MRS can also be used to assess the effect of dietary and lifestyle changes on liver fat content. A previous MRS study demonstrated that short-term exercise improved liver lipid saturation, insulin sensitivity, and oxidative stress in individuals with known NAFLD [37]. Additionally, MRS is regarded as an accurate noninvasive tool for liver fat quantification in NASH, the more severe form of NALD [46][47].

MRS has also been used to study liver-related diseases that have fat accumulation within hepatocytes, such as human immunodeficiency virus (HIV), hepatitis C virus (HCV), excessive alcohol consumption, and hepatotoxic effects from chemotherapeutic agents or antiretroviral therapy [7][48][49].

Due to the high accuracy and sensitivity of MRS, it has also been used in liver fat assessment for liver transplants. Hepatic steatosis not only influences the outcome of translation but also increases the complication risk of both participants and donors [50].

4.2. Cirrhosis

MRS is a promising tool for the evaluation of liver cirrhosis. Chronic hepatitis can be classified into stages based on the level of fibrosis and necroinflammatory activity insults [51]. Elevated cirrhosis is a risk factor for developing hepatocellular carcinoma (HCC) [52]. Therefore, a correct diagnosis and monitoring of cirrhosis is clinically valuable. MRS has previously been used to investigate alterations in lipid and choline levels in liver cirrhosis patients [53].

4.3. Evaluation of Focal Liver Fat Disposition

In addition to diffuse liver fat assessment, MRS has also been used to investigate metabolite alterations in focal hepatic lesions such as benign lesions and malignancies. In addition to lipids, liver spectra can also be used to investigate choline-containing compounds (i.e., choline, phosphoethanolamine, and phosphocholine). These choline-containing compound peaks cannot be resolved in the low-field MR system and appear as a single peak at 3.2 ppm. The alteration of choline-containing compounds has been hypothesized to be associated with elevated cell membrane turnover and cell proliferation [54]. Abnormal choline-containing compounds are suggested to be associated with carcinogenesis [55]. Previous work on in vitro MRS in liver biopsies demonstrated elevated phosphomonoesters (i.e., phosphothanolamine and phosphocholine) and reduced phosphodiesters (i.e., glycerophosphocholine and glycerophosphoetganoalamine) compared to healthy liver tissue [56].

MRS has also been used in other studies, such as MRS of the gallbladder, to study the bile component and metastasis of adenocarcinoma of the liver [57][58]. The improvement of higher-field MR systems and acquisition techniques leads to many possible clinical applications of MRS. With increased SNR, MRS might be used to study the liver in a smaller area and with high spectral resolution, thus improving the ability to resolve metabolite features within the tissue of interest. The evidence suggests that MRS is a powerful tool for the noninvasive assessment of liver fat content that can be used in both research and clinical settings. However, performing MRS requires extensive resources, and thus, MRS remains mainly a research modality despite its potential for accurate liver fat quantification in clinical applications.

5. Possible Confounders and Limitation of Liver MRS

Several confounders could affect the accuracy of liver fat measurement from MRS. However, this can be avoided by careful design of MRS acquisition and correction of these confounding factors. While MRS spectroscopy is the most direct measurement of liver fat signals, it is affected by T1 bias and T2 relaxation effects, which lead to errors in liver fat fraction measurements [59].

One of the confounders is motion artifacts. Motion artifacts in liver MRS can arise from several sources, including gross movement, respiration, and cardiac pulsation. Rhythmic motion, such as respiratory and cardiac motion, often leads to phase and frequency shifts, spectrum line broadening, and a reduced degree of water suppression [12].

Another limitation is that the MRS technique for liver fat quantification is complex, expensive, and not widely available. MRS of liver fat requires an MR machine with a homophonous magnetic field with MRS capability, a complex analysis method, a specialized software package, and user expertise to carry out the measurement. Therefore, MRS is mostly used in research and clinical trials rather than clinical settings. In addition, MRS is sampled from only a small area of the liver parenchyma. Therefore, MRS liver FF does not reflect the lipid content of the whole liver [60].

Despite these limitations of MRS, continuous effort is being made towards technical refinement and standardized MRS for translation into the clinical setting. The developments in the MR instrument and software have made the MRS sequence a rather standard addition with a clinical scanner. The improvements of the MR system have led to the reduction of MRS scan time, and the possibility of data acquisition and analysis remote controlled by skilled specialists improves its feasibility in a clinical setting.

6. Conclusions

MRS has been shown to be one of the most precise and accurate techniques for liver fat assessment, and it provides a straightforward quantitative measurement of liver steatosis. Moreover, MRS has the potential to become an alternative substitution for liver biopsy and thus avoid complications such as bleeding and infection. Given the excellent sensitivity of MRS, it also plays an important role in the initial detection of liver fat content and monitoring the response to treatment. While the application of MRS was previously limited to clinical trials and research settings due to its requirement of user expertise, the continued refinement and validation of instrument and acquisition technique now lead to the possibility to incorporate its robust application into clinical routine.

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