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HEPATIC FAT CONTENT ASSESSMENT USING MR-BASED METHODS

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ABSTRACT

Magnetic resonance based methods are increasingly used for liver fat quantification as a non-invasive alternative to liver biopsy in diagnostic studies, observational studies and clinical trials. Many studies have addressed the diagnostic accuracy of MR-based methods and of other non-invasive imaging methods (CT and Ultrasound). Important advantages of MR based methods over CT and Ultrasound are their quantitative nature and lack of ionizing radiation exposure. In this review we give an overview of the most commonly available MR-based techniques (MR-imaging and MR-spectroscopy) for liver fat detection and quantification. We discuss technical aspects, advantages, disadvantages and diagnostic accuracies.
INTRODUCTION

According to various population studies, at least 30% of the general Western adult population has a fatty liver [1-4]. A liver is considered ‘fat’ or ‘steatotic’ when fat-containing vacuoles accumulate in the hepatocytes and the total fat content exceeds 5% of the wet weight of the liver [5,6]. The majority of fatty livers are related to non-alcoholic fatty liver disease (NAFLD). NAFLD is currently one of the most common causes of chronic liver disease in both children and adults because of the strong association with diabetes and obesity [4,5]. The prevalence of NAFLD increases to 40-69% when diabetes is present and up to 91% in obese patients [4]. Moreover, diabetes, insulin resistance and obesity are independent predictors of mortality in patients with chronic liver disease [7]. Other conditions that are associated with hepatic steatosis include excessive alcohol consumption, chronic viral infection (hepatitis C) and metabolic or storage disorders. Certain drugs and toxins can also induce hepatic steatosis [8,9].

Detection and quantification of hepatic steatosis is clinically important in several situations: in NAFLD, steatosis is recognized as the earliest biomarker and necessary feature for the development of non-alcoholic steatohepatitis (NASH). NASH is a condition in which hepatic steatosis coexists with liver cell injury and inflammation [10]. While the presence of steatosis alone (“simple steatosis”) in itself is considered benign, patients with NASH have an increased risk of liver-related complications and mortality [5]. Recently, a follow-up study showed that simple steatosis can progress to NASH: of 13 patients with simple steatosis at baseline, five developed borderline NASH and three developed NASH after three years [11]. Early diagnosis and treatment of NASH and monitoring of patients with simple steatosis is therefore important.

In hepatitis C, steatosis is associated with more severe fibrosis and rapid disease progression while an adequate response to antiviral treatment results in a decrease of steatosis [12]. In addition, the presence of hepatic steatosis impairs the regenerative capacity of the liver in both donor and recipient in liver transplantation surgery and is associated with primary non-function of the liver graft [6, 13-15]. The maximum amount of fatty infiltration for liver grafts accepted by most transplantation centers varies between 10- 30% [13,16]. Therefore, an accurate tool with which to determine the exact amount of fat in the liver is essential.

Liver biopsy is the reference standard for the assessment of hepatic steatosis. Liver biopsy has a number of disadvantages including patient discomfort (20% of patients experience moderate pain, 3% experience severe pain [17], complication- and mortality risk (0.31 and 0.03% respectively) [18]. Moreover, the histological examination of the liver biopsy by pathologists is subject to inter and intra observer variability and the small volume of the liver biopsy sample (30 µl) can cause sampling errors [17-19]. For these reasons, liver biopsy is unsuitable for monitoring patients or for large-scale clinical trials.

MR-based methods (MR-imaging and proton MR-Spectroscopy) can detect and quantify hepatic steatosis non-invasively. Other available imaging techniques are Ultrasound (US) and Computed Tomography (CT). MR methods have a higher diagnostic accuracy than US and CT for evaluating hepatic steatosis and are capable of detecting and quantifying even small amounts of hepatic fat [20-22]. Although not always readily available and relatively expensive, MR–based methods have the advantage of being accurate and quantitative. Moreover they do not involve radiation exposure and can easily be combined with other MR protocols. Proton MR spectroscopy
(1H-MRS) is considered the most accurate technique and is increasingly used as reference standard instead of liver biopsy in clinical trials, diagnostic studies and observational studies [23-35].

In this review we give an overview of the most common available MR-based techniques (MR-imaging and proton MR-spectroscopy) for liver fat detection and quantification.

**CHEMICAL SHIFT TECHNIQUES**

Chemical shift imaging utilizes the difference in resonance frequency of protons in water and protons in fat* to detect and measure hepatic fat content [36,37]. After excitation by a radiofrequency pulse, protons in water will resonate slightly faster than protons in fat, due to the difference in chemical environments. This difference in resonance frequency of protons is called chemical shift. The difference in resonance frequency is linearly related to the magnetic field strength $B_0$. The resonance frequency $\omega_0$ (MHz) is defined by the Larmor equation:

$$\text{Resonance frequency } \omega_0 = \frac{\gamma}{2\pi \cdot B_0} \quad \text{(eq. 1)}$$

In this equation, the resonance frequency $\omega_0$ is expressed in megahertz; $\gamma$ represents the gyromagnetic ratio ($\gamma / 2\pi = 42.58 \text{ MHz/T}$ for protons in water) and $B_0$ represents the magnetic field strength in Tesla (T). The chemical shift frequency difference ($\Delta\omega_{cs}$) between protons in water and fat is proportional to the magnetic field strength: at body temperature, this difference is approximately 145 Hz at 1T, 217 Hz at 1.5T and 434 Hz at 3T. Chemical shifts are usually expressed in parts per million (ppm), which are independent of $B_0$. The chemical shift difference between water and fat is approximately 3.4 ppm:

$$\text{Fat - water chemical shift} = \frac{\Delta\omega_{cs}}{\omega_0 \cdot 10^{-6}} = \frac{145 \cdot B_0}{42.58 \cdot B_0} = 3.4 \text{ ppm} \quad \text{[26]} \quad \text{(eq. 2)}$$

How can this principle be used to quantify hepatic steatosis in MR imaging? At the exact moment the excitation radiofrequency pulse is sent into the liver tissue, all protons are exactly in-phase, meaning that all magnetisation vectors point in the same direction. Immediately after the radiofrequency pulse is turned off, the protons will start to dephase. The fat-protons will dephase slightly faster than the water-protons. As a result, at

$$t = \frac{1}{2} \cdot T_{E_{1p}}$$

fat protons will be 180° out of phase, meaning they point in exactly the opposite direction as the water protons (Figure 1):

$${\text{Out-of-phase signal}} \ S_{OP} = S_{\text{water}} - S_{\text{fat}} \quad \text{(eq. 3)}$$

At $t = T_{E_{1p}}$, all protons will be in phase again, meaning that water and fat signals add up (Figure 1).

* Methylene is the most abundant chemical structure within a triglyceride molecule and therefore the most dominant signal that arises from the triglyceride molecule. Unless specified otherwise, the term “fat” in this paper refers to methylene.
In-phase signal $S_{IP} = S_{water} + S_{fat}$  \hspace{1cm} (eq. 4)

The echo times (TE) at which fat and water signals are in-phase and out of phase can be calculated for different MR field strengths $B_0$ (Table 1):

$$TE_{IP}(ms) = \frac{1000}{\text{chemical shift} \cdot \gamma \cdot B_0} = \frac{1000}{3.4 \cdot 42.58 \cdot B_0}$$  \hspace{1cm} (eq. 5)

$$TE_{OP}(ms) = \frac{1}{2} \cdot TE_{IP}$$  \hspace{1cm} (eq. 6)

**Dual-echo chemical shift MRI (dual-echo IP/OP imaging)**

For this technique, the hepatic fat fraction is calculated by comparing the signal intensities on in-phase (IP) and opposed-phase (OP) images:

$$\text{Fat signal fraction} = \frac{S_{IP} - S_{OP}}{2 \cdot S_{IP}}$$  \hspace{1cm} (eq. 7)

The fat fraction can be calculated when applying equation 7 to signal intensities measured in corresponding ROIs on IP and OP images (Figure 2a,b) [38]. Also, instead of selecting ROIs, a fat signal fraction map can be generated for the complete MR slice, showing the spatial distribution of the fat signal intensity values throughout the liver (Figure 2c). IP and OP echoes must however be acquired after a single RF excitation pulse, with identical calibration for both echoes. Otherwise, if the IP and OP echoes are acquired separately, an internal reference (spleen) should be used to correct the hepatic signal intensity values [39].

**Advantages**

The dual echo IP/OP imaging technique is fast, can easily be performed in routine examinations and is widely available. Moreover, it allows for fat quantification of the entire liver. It can be performed at different magnetic field strengths and is relatively insensitive to magnetic field heterogeneity [38].

**Disadvantages**

There are several important limitations to dual-echo IP/OP imaging: the fat-water signal dominance ambiguity and confounders such as $T2^*$ effects, $T1$ effects and fat spectral complexity effects [38,40].

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**Figure 1.** Signal vector diagram for in-phase and opposed-phase imaging. The signals of water and fat are combined and the resultant signal is observed.
Fat-water signal dominance ambiguity limits fat quantification in case of severe fatty liver. As explained in the previous section and in Figure 1, the signal intensity on an IP or OP MR image reflects the sum of the magnetisation vectors of water and fat. If the magnetisation vectors of water and fat are equal, their signals will cancel each other out in the OP image. If however the magnetisation vectors are unequal, which will normally be the case, then it is not possible to determine whether the resultant signal intensity on the OP image originates from either fat or water. Correct liver fat quantification will not be possible unless additional information is acquired from for instance multiple flip angles or repetition times, fat suppression or field mapping [41-43]. Fat fractions greater than 50% however are very uncommon [3], meaning that the resultant signal on OP images will normally originate from water.

T2* effects: dual-echo IP/OP imaging does not correct for T2* effects. The IP and OP images are acquired at different echo times. During the TE interval, T2* decay occurs, resulting in signal loss. The signal loss between IP and OP images is used to calculate fat content, so additional information is required to correct for T2* effects.
signal loss due to T2* effects will interfere with fat detection and lead to errors of interpretation. If the OP image is acquired first, additional signal loss on the IP images due to T2* effects will cause the hepatic fat content to be underestimated. If on the other hand the IP image is acquired first, then signal loss on the OP image due to T2* effects will lead to overestimation of hepatic fat content. This T2* bias is even stronger in case of iron overload, which can coexist with fat in chronic liver diseases and is associated with liver cirrhosis [44]. T2* effects can be minimized by choosing the first consecutive OP and IP echoes. T2* effects can also be corrected for by measuring T2* separately. More advanced imaging techniques based on IP/OP imaging such as triple-echo and multiecho techniques have been developed to take these T2* effects into account and will be discussed later in this paper [26,35,38,41,45,46].

T1-effects: T1-weighting in dual-echo IP/OP imaging causes a bias since water and fat have different T1 values. The shorter T1 time of fat causes the fat signal to be artificially amplified in a T1-weighted image. This T1 bias can be avoided by using a low (10º) flip angle [38,40,47].

Fat spectral complexity effects: in dual-echo IP/OP imaging, the ratio between the signal from water (4.7 ppm) and the signal from the methylene fat peak (1.3 ppm) is calculated. As shown in the MR spectrum in Figure 3a, fat has other (smaller) spectral peaks that also contribute to the total fat content but that are ignored in dual-echo IP/OP imaging (e.g. diacyl at 2.75 ppm; a-carboxyl at 2.24 ppm; a-olefinic at 2.02 ppm and methyl CH₃ at 0.9 ppm). These peaks cause complex phase interferences (fat-fat interference effects), leading to inaccuracies in fat and water signal measurements [48]. A correction model for these effects has been described by Yokoo et al [35]. Moreover, fat peaks at 5.29 ppm (olefinic) and at 4.20 ppm (glycerol) account for 8.6 - 15% of the total fat content [49,50]. These peaks however lie so close to the water peak at 4.7 ppm that their signal will add to the water signal, leading to further quantification errors. Because there may only be limited variation in the fat spectrum of the liver, the magnitude of these peaks can be corrected for when the magnitude of the other fat peaks is known [49].

Accuracy
Studies that compared the diagnostic accuracy of dual-echo IP/OP imaging with liver biopsy as the reference standard were analysed in a meta-analysis [20]. Summary estimates of sensitivity and specificity for detecting liver fat with a threshold of 0-5% fat on liver biopsy were 82% (95% CI: 64-92%) and 90% (95% CI: 81-95%), respectively. With a threshold of 10-20% liver fat, sensitivity and specificity summary estimates were 90% (95% CI: 73-97%) and 95% (95% CI: 83-99%), respectively. With a threshold of 30-33% liver fat on biopsy, sensitivity was 97% (95% CI: 84-100%) and specificity was 76% (95% CI: 50-91%). No separate analyses were performed for the presence of iron, fibrosis stages or underlying liver disease. All studies were performed at a magnetic field strength of 1.5T.

Two recently published papers that compared the accuracy of US, CT, dual-echo IP/OP MR-imaging and ¹H-MRS with liver histopathology were not included in this meta-analysis. Both studies used a 3T MR system for MR-imaging and MR-spectroscopy. For dual-echo IP/OP MR-imaging with a threshold of >5% liver fat on histopathology, Van Werven et al found a sensitivity 90% and a specificity of 91% for steatosis detection in 46 patients who underwent liver resection [22]. The area under the curve (AUC) was 0.93. They did not correct for T1 or T2* effects. The performance of dual-echo IP/OP
imaging was better than the performance of US and CT (AUC: 0.77 and 0.76 respectively). $^1$H-MRS performed slightly better than dual-echo IP/OP imaging with an AUC of 0.97.

Lee et al assessed the accuracy of US, CT, dual-echo IP/OP imaging and $^1$H-MRS in 161 potential living liver donors compared with liver biopsy [21]. For dual-echo IP/OP imaging with corrections for T2* effects, the sensitivity, specificity and AUC with a threshold of >5% fat on liver biopsy were 77%, 87% and 0.883 respectively. With a threshold of >30% fat on liver biopsy, sensitivity, specificity and AUC were 91%, 94% and 0.995 respectively. In this study, dual-echo IP/OP imaging performed significantly better than US and CT. There was no significant difference in performance between dual-echo IP/OP imaging and $^1$H-MRS. The results from both Lee et al and Van Werven et al are in close agreement with the results from the meta-analysis [21,22].

**Multiecho chemical shift MRI**

New techniques have been described that address the discussed confounding influences of T1, T2 and fat spectral complexity effects [25-27,30,32,35,41,45,47,51-54]. In summary, T1 is accounted for by using a long TR and a low (10º) flip angle; T2* relaxation effects are estimated and corrected for by triple or multiecho acquisition and the fat-fat interference effect is corrected for by incorporating this component into the model (fat spectral modelling). Images can be acquired in one or two breath holds. An additional post-processing step is required that includes a reconstruction algorithm to generate the fat signal fraction (FSF) map [39].

**Accuracy**

All studies that investigate the diagnostic accuracy of multiecho techniques for liver fat quantification have used $^1$H-MRS as the reference standard. To our knowledge, no studies have been published with liver biopsy as the reference standard.

Yokoo et al compared low flip angle (10º) dual-echo IP/OP imaging, triple-echo, multi-echo and multi-interference techniques with $^1$H-MRS as the reference standard at 1.5T MRI [47]. All

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**Figure 3.** Left: MR spectrum of a severe fatty liver at 3T MRI. The most dominant signals are the resonance signal from water at 4.7 ppm and the main fat peak (methylene - CH$_2$) at 1.3 ppm. The chemical shift difference between water and fat is 3.4 ppm. The other peaks represent the resonances from other protons along the fatty acid chain: (a) olefinic at 5.29 ppm; (b) diacyl at 2.75 ppm; (c) a-carboxyl at 2.24 ppm; (d) a-olefinic at 2.02 ppm and (e) methyl CH$_3$ at 0.9 ppm. Right: MR spectrum of a non-fatty liver. Only the water peak at 4.7 ppm is visible, no fat peaks are observed.
four techniques suppressed T1 effects by low 10° flip angle. Dual-echo IP/OP imaging did not include T2* correction or fat spectral modelling. Triple and multi-echo techniques included T2* correction but did not correct for fat-fat interference effects. The multi-interference technique covered all three confounding influences. With a diagnostic threshold of 6.25% for the presence of a fatty liver with 1H-MRS, the sensitivity of dual-echo IP/OP imaging was 82%. This was significantly lower than the sensitivities of triple-echo (97%), multi-echo (95%) and of multi-interference imaging (98%). Specificities were 100%, 88%, 100% and 88%, respectively. A systematic underestimation of the liver fat fraction with dual-echo IP/OP imaging of 2.9% resulted in the low sensitivity and high specificity.

Guiu et al assessed the systematic errors in liver methylene fraction resulting from fat-fat interference effects from non methylene peaks with dual-echo IP/OP gradient recalled echo (GRE) imaging and triple-echo GRE imaging at 3T MRI [27]. They found that non-methylene peaks produced a ~10% systematic relative underestimation of the liver methylene fraction in both techniques. T2* decay was responsible for an absolute systematic error of 1.9 – 4.2% in liver methylene fraction measurement.

Most recently, Yokoo et al compared the accuracy of dual- three- and six- echo MRI methods in 163 subjects with 1H-MRS as the reference standard at 3T MRI [35]. For each of the three MRI methods the fat fraction was calculated with single-frequency (methylene, 1.3 ppm) and with multifrequency (all measurable fat peaks) fat signal modeling. The multiecho methods were corrected for T2*-effects, the dual-echo methods were not. The classification accuracies of T2* corrected multi frequency three- and six-echo imaging methods were highest (accuracies of 95-96% for both methods depending on the fat fraction threshold). The accuracy of single frequency fat fraction measurement was highest for three-echo MRI with an accuracy of 94-96%.

FREQUENCY SELECTIVE FAT SATURATION

Frequency selective imaging enables suppression of a signal of interest, such as that of water or fat. This is different from dual-echo IP/OP imaging, where both fat and water protons are excited to produce the MR signal. When the signal from the main fat peak (methylene) in the liver is suppressed, the resultant signal will approximate the signal from the water peak. To suppress the methylene peak, a frequency selective pre-saturation RF pulse with the same resonance frequency as that of the methylene fat peak is applied. The bandwidth of the pulse needs to be selected in such a way that it does not affect the water frequency (4.7 ppm). The flip angle of this radio-frequency pulse needs to be exactly 90° so that all longitudinal magnetization in the fat peak will be tipped into the transverse plane and will thus be saturated. The pulse is followed by a crusher gradient to spoil all transverse magnetization of fat. Immediately after this pre-saturation pulse, a standard imaging sequence is started. The zero net magnetization that results from the pre-saturation pulse has no time to recover, resulting in suppression of signal from the main fat peak and the remaining signal thus originates from water [55].

To calculate the fat percentage with frequency selective fat saturation (FS) imaging, two spin-echo or in-phase T2-weighted MR images need to be acquired: one with a presaturation FS pulse and one without a presaturation FS pulse. Both images should be acquired with the same imaging parameters so that confounding influences such as T2* effects are balanced.
If the FS and non-FS images are not obtained with identical imaging parameters, an internal reference (e.g. spleen) is necessary to normalize the signal intensity values of the liver [38]. Non-FS images show the signal intensity of water and fat protons together, FS images that of water only (Figure 4). The fraction of relative signal intensity loss on images without FS and with FS is calculated as follows:

\[
\text{Fat signal fraction} = \frac{\text{non FS} - \text{FS}}{\text{non FS}} \quad \text{(eq. 8)}
\]

As with IP/OP imaging, manually selected co-localized ROIs can be placed in the liver or a separate fat signal fraction map can be created.

**Advantages**

The frequency selective fat saturation pulse can precede any MR-imaging sequence. Signal in non-fat tissue is unaffected as long as the bandwidth and saturation pulse frequency are accurately selected. Fat saturation pulses are very effective when the main magnetic field \(B_0\) as well as the transmit RF field \(B_1\) are homogeneous in the selected field of view. Although few clinical studies have been published on the diagnostic accuracy of frequency selective fat saturation imaging for liver steatosis quantification, FS imaging could have benefits over dual-echo IP/OP imaging as FS imaging is less susceptible to T2* effects than dual-echo IP/OP imaging [42,56-59,42].

**Disadvantages**

\(B_0\) heterogeneities: Fat saturation pulses are sensitive to \(B_0\) heterogeneities that shift the position of the water and fat peaks with respect to the pre-saturation pulse. Such \(B_0\) heterogeneities can lead to incomplete or failed saturation of the targeted signal. The saturation pulse can even be so far off that it saturates the water peak instead of the fat peak. Therefore, successful fat suppression with pre-saturation pulses requires a homogeneous magnetic field.

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**Figure 4.** Fifty-three year old female patient with focal liver steatosis. A: T2 weighted image without fat saturation; B: T2 weighted image with fat saturation. The T2 weighted fat saturated image shows substantial signal loss in the right liver lobe due to focal liver steatosis, whereas this was not visible on the T2 weighted image without fat saturation.
B1 heterogeneities: The perfect fat saturation pulse requires an exact 90-degree flip angle in order to saturate the longitudinal magnetization of fat. A smaller or larger flip angle results in incomplete suppression of the fat signal. Flip angles however are typically accurate only within a 5-10 degree range as they are sensitive to heterogeneities in the radiofrequency field B1. The use of surface coils can also distort the transmitter field [60].

Accuracy
Qayyum et al retrospectively compared the relative accuracy of fat-saturated fast spin-echo MRI and that of dual-echo IP/OP imaging and with liver biopsy in patients with and without cirrhosis [42]. In patients without cirrhosis, FS/ non-FS imaging correlated better with liver biopsy than dual-echo IP/OP imaging: r = 0.92 vs. 0.69, p<0.01. In patients with cirrhosis, FS/ non-FS imaging correlated with liver biopsy: r= 0.76, p< 0.01 whereas dual-echo IP/OP imaging did not correlate with liver biopsy (r= 0.25, p = 0.36). Increased liver iron levels in patients with cirrhosis could have caused T2* effects and confounded dual-echo IP/OP measurements. Liver iron however was not measured.

Cotler et al validated the FS technique in phantoms and in 10 patients with biopsy proven NAFLD [56]. MRI measured fat content of patients correlated strongly with histopathology (r= 0.96, p< 0.001).

Cowin et al compared FS/ non-FS imaging, dual-echo IP/OP imaging and ¹H-MRS with liver biopsy in twelve patients with hepatic steatosis [57]. Correlations were 0.935 (p<0.0001) for FS/ non- FS imaging, 0.942 (p<0.0001) for dual-echo IP/OP imaging and 0.928 (p <0.0001) for ¹H-MRS. In two patients, mild liver iron was present.

FS/ non-FS imaging and dual-echo IP/OP imaging were compared with liver biopsy in fifty-two patients with liver disease by Bahl et al [59]. No liver iron was present. Correlations were 0.75 for FS/ non-FS and 0.78 for dual-echo IP/OP imaging (p<0.01).

Mc Pherson et al studied the accuracy of FS/non-FS, dual-echo IP/OP imaging and ¹H-MRS in 94 patients with a wide spectrum of liver diseases with histopathology as the reference standard [58]. Correlations were 0.88 (p< 0.001) for all three techniques. Severe (grade 4) hepatic iron overload was present in two patients. For these patients, dual-echo IP/OP measurements were aberrant, one ¹H-MRS was uninterpretable and the other did not have ¹H-MRS performed. Steatosis estimates observed with FS/ non-FS imaging however were consistent with histology for both patients.

MAGNETIC RESONANCE SPECTROSCOPY
Magnetic resonance spectroscopy (MRS) characterizes the molecular composition of tissue. The signals that originate from protons in different molecular structures within a selected voxel are recorded and plotted in a spectrum (Figure 3). The molecular structures are separated from each other and characterized based upon their differences in precession frequency. The signal intensity and line width of individual peaks of the spectrum give additional information regarding the relative quantity of the chemical moieties. Different nuclei can be used for liver fat quantification with MRS, e.g. protons (¹H) [61], phosphor (³¹P) [62,63] or carbon (¹³C) [64]. ¹H-MRS has the advantage over other metabolites that it is easier to perform, more widely available and that it provides a higher signal-to-noise ratio (SNR). The difference between ¹H-MRS and MR-imaging is that ¹H-MRS does not contain information on the spatial origin of
the signal. Therefore, a $^1$H-MRS voxel needs to be placed in the location of interest on a separately acquired anatomical MR-image (Figure 5). Clinical $^1$H-MR spectra of the liver are measured in voxels with sizes ranging from 1 to 36 cm$^3$ [46,50,58,65-68]. When placing the voxel, large vessels need to be avoided as well as the liver edges (with a margin of 1-2 cm). Especially when spectra are acquired during free breathing, the chest and diaphragm can move substantially as a result of which subcutaneous fat or lung tissue can cause artificial signal contributions. Clinical $^1$H-MRS may be performed by using a torso coil and the following acquisition parameters: TR $>$3000 msec, TE 20-35 msec, spectral width 1000-2500 Hz and 1000-2000 data points for 16-32 acquisitions [69]. Acquisition can be performed with breath holds, with respiratory gating or during free breathing [70,71]. A single voxel is placed in the liver and the acquisition takes approximately 1-15 minutes, depending on the imaging sequence.

The clinical application of $^1$H-MRS to quantify fatty infiltration of the liver was first published by Longo et al in 1993 [61]. Since the results from the Dallas Heart Study were published by Szczepaniak et al in 2005 [3], $^1$H-MRS has often been used as the reference standard for liver fat quantification in diagnostic studies [23,25-28,30,32,47,48] and as a clinical endpoint in observational studies and clinical trials [24,29,34]. Arguments in favour of using $^1$H-MRS as the reference standard are the fact that $^1$H-MRS measures fat content volumetrically and thus is directly comparable with results obtained from MR-imaging techniques. With liver biopsy in contrast, the number of fat-containing hepatocytes are examined, which does not reflect volumetric fat content. Moreover, MR-spectroscopy and MR-imaging can be performed during a single MR examination and results from similar sampling volumes in the same liver region can be obtained, which is not achievable for liver biopsy [35].

For single voxel spectroscopy there are two approaches: point resolved spectroscopy (PRESS) and stimulated-echo acquisition mode (STEAM) [37,50,61,72,73]. PRESS acquisition uses a 90º-180º-180º pulse sequence with a TE larger than 35 ms. STEAM uses a 90º-90º-90º pulse sequence and allows the use of shorter echo times (typically around 10 ms). STEAM sequences are attractive for measuring peaks with short T2 values. The maximum amplitude for a STEAM echo however is one half of that for a PRESS echo at the same TE, therefore yielding lower signal compared with PRESS [74].

Figure 5. Correct placement of MR-spectroscopy voxel in the liver. Large vessels and liver edges are avoided.
Confounders

T1 and T2 effects: Each peak in a MR spectrum has its own longitudinal (T1) and transverse (T2) relaxation time, and the relative signal amplitudes of the peaks vary with the chosen TR and TE. T2 values vary across individuals, mainly due to the presence of variable liver iron content. Accurate fat quantification with 1H-MRS therefore requires individual correction for these effects [27]. T1 effects are minimized by choosing a long TR (> 3000 msec). Correction for T2 effects requires acquisition of MRS at multiple echo times. The T2 value of each peak is then calculated separately, assuming mono-exponential signal decay. By extrapolating the T2 decay curve to an echo time of 0, the relative proton density of each peak is estimated [27]. Some studies correct for T2 effects with fixed T2 times for water and fat [25,71]. This however can cause inaccuracies as T2 times between subjects may vary.

J Coupling: All liver fat resonances exhibit J coupling [75]. J coupling or spin-spin coupling occurs when spins within a molecule interact with each other and affect the local magnetic field around their nuclei. Spin coupling differs from chemical shift: it is independent of the magnetic field strength B₀ and there is always another spin involved in the coupling. Resonances that exhibit J coupling are divided into multiple peaks with different resonance frequencies. In the liver J coupling occurs between adjacent protons along the carbon chain. J couplings give rise to changes in peak amplitude with increasing echo time, thus modulating the apparent peak T2 value, which can result in erroneous estimation of the water/fat ratio in the liver [76]. Hamilton et al have shown that J coupling effects are more prominent in PRESS than in STEAM in the liver. STEAM sequences will therefore give more accurate estimation of liver fat [49].

Fat content measurement

The area under the resonance signal of a specific metabolite is directly related to the concentration of this metabolite. Absolute quantification of peaks however is difficult due to differing conditions between measurements, e.g. B₀ and B₁ heterogeneities. Therefore, for liver fat quantification the ratio between the fat peaks between 0.5 and 3 ppm and the sum of fat and water peaks is calculated:

\[ \text{Liver fat fraction (LFF)} = \frac{\text{fat signal peak area (0.5 – 3 ppm)}}{\text{fat signal peak area (0.5 – 3 ppm)} + \text{water peak area}} \]  

(eq. 9)

Because the fat peaks that resonate under the water peak (at 5.3 and 4.2 ppm) are not considered in this equation, an additional correction needs to be performed: [77]

\[ \text{Liver fat fraction corrected} = \frac{\text{LFF}}{1.138 – 0.339 \cdot \text{LFF}} \]  

(eq. 10)

Dedicated software is available for post-processing and analysis of 1H-MRS data such as jMRUI with the AMARES algorithm [78], LC Model or SAGE-Spectral Analysis [69].

Advantages

1H-MRS is considered a very accurate non-invasive technique to quantify liver fat [20,38,69]. With 1H-MRS, the absolute liver fat concentration can directly be measured and very small amounts of liver fat (as low as 0.5%) can be detected and quantified [38].
The between weeks reproducibility of $^1$H-MRS is high with a coefficient of variation of 9.5%, repeatability coefficient of 1.3% and intraclass coefficient of 0.998 [71].

**Disadvantages**

The complexity of data analysis is the major limitation of $^1$H-MRS. A skilled operator and dedicated post-processing software is needed to analyse and interpret the data.

Clinical MRS is typically performed in a single voxel as measurements of multiple voxels is too time consuming. Additional MR images are mandatory for anatomical orientation. Although a voxel is much larger in size than a liver biopsy, unequal distribution of liver fat can still cause sampling error [71]. The quality of the MR spectrum depends on the homogeneity of the magnetic field, which can be influenced by magnetic field susceptibility effects near organ edges or foreign bodies. Time consuming shimming is usually required to ensure high quality $^1$H-MRS data, lengthening the total examination time. Individual correction for T2 effects requires extra data acquisition at multiple echo times to measure T2 times. The spectral resolution of an MR spectrum depends on the magnetic field strength. Therefore, at clinical field strengths lower than 3T, the spectral resolution of the MR spectrum is not high enough for complete detection of the individual smaller fat peaks.

**Accuracy**

In 1993 and 1995, Longo et al reported correlations of 0.68 and 0.70 between $^1$H-MRS and liver biopsy in two studies of 26 and 29 patients with fatty liver respectively [61,50]. Thomsen et al in 1994 reported a correlation of 0.9 (p < 0.001) between $^1$H-MRS and chemical triglyceride measurement of liver biopsy tissue in 14 patients with alcohol abuse [73]. Szczepaniak et al demonstrated that in vivo $^1$H-MRS measurement of liver fat in dogs and rabbits correlated well with biochemical analysis of liver tissue ($r = 0.93; p<0.0001$) [79]. In 2005 they published the results from the Dallas Heart Study (a multiethnic, probability-based population sample) where the distribution of liver fat was analysed with $^1$H-MRS in 2,349 participants [3]. In this study, the upper limit of normal (95th percentile) for hepatic triglyceride content in 345 healthy subjects without identifiable risks for hepatic steatosis was 5.56%. Since then, $^1$H-MRS has gradually gained acceptance as reference standard instead of liver biopsy.

Meta-analysis of $^1$H-MRS diagnostic accuracy studies showed a sensitivity and specificity of 89% (95% CI: 77-95%) and 92% (95% CI: 81-97%) for detecting liver fat with a threshold of 0-5% fat on liver biopsy [20]. With a threshold of 10% liver fat, sensitivity was 83% (95% CI: 62-93%) and specificity was 94% (95% CI: 80-99%). For detecting moderate amounts of liver

**Table 1. In-phase and opposed-phase echo times for water and fat**

<table>
<thead>
<tr>
<th>$B_0$ (T)</th>
<th>$TE_{op}$ (ms)</th>
<th>$TE_{ip}$ (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>3.46</td>
<td>6.91</td>
</tr>
<tr>
<td>1.5</td>
<td>2.30</td>
<td>4.60</td>
</tr>
<tr>
<td>3.0</td>
<td>1.15</td>
<td>2.30</td>
</tr>
</tbody>
</table>

$B_0$ = magnetic field strength; T = Tesla; TE = echo time; OP = opposed-phase; IP = in-phase.
Hepatic fat content assessment with MR-imaging

Fat (>30%), sensitivity and specificity were 73% (95% CI: 41-91%) and 96% (95% CI: 85-99%). The lower sensitivity of 1H-MRS at higher liver fat levels might be explained from the fact that with 1H-MRS, the absolute volume of liver fat is measured whereas on liver biopsy, the percentage of hepatocytes that contain fat droplets is visually estimated by the pathologist. The latter method overestimates the liver fat volume by a factor of more than two [56,66,77,80]. If not corrected for, this discrepancy becomes more apparent at higher grades of liver fat. Krssak and d’Assignies compared 1H-MRS with visual evaluation of liver fat content on liver biopsy and with biochemical analysis [66] and semi-automatic fat vacuole segmentation [46]. In both studies, the correlation for 1H-MRS was significantly higher with biochemical analysis or semi-automatic fat vacuole segmentation than with visual evaluation liver fat content.

In 46 patients undergoing liver resection, Van Werven et al observed a sensitivity of 91% (95% CI: 70-98%) and a specificity of 87% (95% CI: 65-97%) for 1H-MRS at 3T MRI compared with liver biopsy when applying a threshold of 5% liver fat [22].

Lee et al observed sensitivities of 80% (95% CI: 68-88%) and 73% (95% CI: 43-91%) and specificities of 80% (95% CI: 71-87%) and 79% (95% CI: 72-85%) for 1H-MRS at 3T MRI compared with liver biopsy with respective thresholds of 5% and 30% liver fat [21]. In this study, 161 consecutive potential living liver donors underwent liver biopsy, US, CT, dual-echo IP/OP MRI and 1H-MRS.

Other Imaging Techniques

Ultrasound

US is widely used in clinical practise for the evaluation of the presence of hepatic steatosis as it is a safe and inexpensive examination that is widely available. Criteria for steatosis assessment with US include: liver echogenicity, echotexture, visibility of diaphragm and large vessels and beam attenuation [40]. The positive predictive value for detecting hepatic steatosis has a broad range in literature, ranging from 59-100% [81-98]. The diagnostic accuracy for detecting moderate and severe degrees of hepatic steatosis (>33%) of US is good with a sensitivity of 86% (95% CI: 78-91%) and a specificity of 85% (95% CI: 77-91%). For detection of liver fat with a lower threshold (>0-5% liver fat), the accuracy is lower with a sensitivity of 73% (95% CI 62-82%) and a specificity of 84% (95% CI: 76-90%) [20].

Evaluation with US however is qualitative, operator dependent and has a poor reproducibility. Therefore, US is not the preferred imaging technique for the follow-up of hepatic steatosis or when exact quantification of the amount of hepatic steatosis is required.

Computed Tomography

CT attenuation is related to liver fat content and therefore CT allows for quantitative and qualitative evaluation of hepatic steatosis. The accuracy of CT for assessing hepatic steatosis has been investigated for unenhanced CT [13,14,16,94,99-106], contrast enhanced CT [14,99,100] and dual-energy CT [107]. Kodama et al compared unenhanced with enhanced CT and found that unenhanced CT performed better than enhanced CT scans for prediction of pathologic fat content [100]. For examination of the degree of hepatic steatosis the Hounsfield Units (HU) are measured in the liver. Preferably, the attenuation value of the spleen is used as an internal reference by calculating liver minus spleen HU values or the liver-to-spleen ratio. When
liver HU only is used, the calibration of the CT scanner or the amount of subcutaneous fat can influence the result and thus hamper comparison between different patients and scanners. The specificity of CT for detecting liver fat with liver biopsy as the reference standard in diagnostic accuracy studies is high, ranging from 88 to 95%. The sensitivity for detecting different degrees of hepatic steatosis however is much lower, ranging from 46 to 72% [20]. Other drawbacks are the radiation exposure and susceptibility to confounding effects due to cirrhosis or depositional diseases (e.g. glycogen, iron, amyloid accumulation) [69].

**SUMMARY**

Several MR-based techniques have the ability to detect and quantify fat in the liver. Each technique has important advantages and disadvantages.

*Dual-echo IP/OP imaging* is a fast, easy to perform and widely available technique without complicated post-processing that allows for fat quantification of the entire liver. Limitations however are the fat-water signal dominance ambiguity, T2* effects and fat-fat interference effects. Accuracy of dual-echo imaging is high in the absence of liver iron accumulation.

*Multiecho techniques* correct for T2* and fat-fat interference effects and have a higher accuracy than dual-echo IP/OP imaging, but require more complex post-processing. All studies that investigate the diagnostic accuracy of multiecho techniques for liver fat quantification have used 1H-MRS as the reference standard.

*Frequency selective fat saturation* imaging enables selective suppression of fat. Although few clinical studies on the diagnostic accuracy of frequency selective fat saturation imaging for liver steatosis quantification have been published, FS imaging could have benefits over dual-echo IP/OP imaging because it has less susceptibility to T2* effects. FS sequences however are very sensitive to B₀ and B₁ heterogeneities.

*Magnetic resonance spectroscopy (MRS)* characterizes the molecular composition of liver tissue. 1H-MRS has a high diagnostic accuracy for liver fat quantification and is increasingly being used as the reference standard instead of liver biopsy. With 1H-MRS, a spatially localized MRS sequence such as PRESS or STEAM is used to obtain data within a manually placed voxel. The accuracy of 1H-MRS is improved by correcting for T2 decay of individual frequency peaks by obtaining multiecho spectra. Analysis of MR spectra is complex and requires dedicated post-processing software.

*Ultrasound* has a high accuracy for detecting moderate and severe grades of liver steatosis. For lower amounts of liver fat, US is less accurate. US is widely available and is relatively inexpensive. Evaluation of liver fat content with US however is qualitative of nature and operator dependent.

For CT, the sensitivity to detect liver steatosis is moderate, the specificity is good. Radiation exposure and susceptibility to confounding effects makes CT a less attractive imaging modality for the evaluation of liver steatosis.
CONCLUSIONS AND FUTURE PERSPECTIVE

Liver fat quantification has become important clinically due to the rapidly increasing prevalence of non-alcoholic fatty liver disease. There is a need for an accurate non-invasive test that can detect, quantify and monitor liver steatosis. Over the last two decades a major step has been made in the development and evaluation of MR-based methods for liver fat quantification. MR-based methods have been demonstrated to outweigh CT and US with respect to their ability to detect and quantify hepatic steatosis. $^1$H-MRS is now increasingly being used as the reference standard in clinical trials, observational studies and in diagnostic accuracy studies. Surprisingly, until recently only a small number of human studies were published that investigated the diagnostic accuracy of $^1$H-MRS compared with liver histopathology. The latest publications on this topic show that MR-imaging methods, especially multiecho chemical shift imaging, have a comparable accuracy as $^1$H-MRS. Until now, MR-based techniques for liver fat quantification are still mainly used as a research tool in tertiary centers and are not yet ready for widespread implementation in routine clinical practice. Future research should therefore focus on standardization of the techniques to improve reproducibility and comparability of results from different centers. The role of MR-based techniques as reference standard for liver fat detection and quantification needs to be further established and agreed upon. Finally, the biggest challenge in the near future will be to convince clinicians of the benefits of MR-based techniques for liver fat quantification over liver biopsy, US and CT and to implement the techniques in routine clinical practice.

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HEPATIC FAT CONTENT ASSESSMENT WITH MR-IMAGING


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