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Fat spectral modeling on triglyceride composition quantification using chemical shift encoded magnetic resonance imaging

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Abstract

Purpose: To explore, at a high field strength of 7T, the performance of various fat spectral models on the quantification of triglyceride composition and proton density fat fraction (*PDFF*) using chemical-shift encoded MRI (CSE-MRI).

Methods: MR data was acquired from CSE-MRI experiments for various fatty materials, including oil and butter samples and in vivo brown and white adipose mouse tissues. Triglyceride composition and *PDFF* were estimated using various a priori 6- or 9-peak fat spectral models. To serve as references, NMR spectroscopy experiments were conducted to obtain material specific fat spectral models and triglyceride composition estimates for the same fatty materials. Results obtained using the spectroscopy derived material specific models were compared to results obtained using various published fat spectral models.

Results: Using a 6-peak fat spectral model to quantify triglyceride composition may lead to large biases at high field strengths. When using a 9-peak model, triglyceride composition estimations vary greatly depending on the relative amplitudes of the chosen a priori spectral model, while *PDFF* estimations show small variations across spectral models. Material specific spectroscopy derived spectral models produce estimations that better correlate with NMR spectroscopy estimations in comparison to those obtained using non-material specific models.

Conclusion: At a high field strength of 7T, a material specific 9-peak fat spectral model, opposed to a widely accepted or generic human liver model, is necessary to accurately quantify triglyceride composition when using CSE-MRI estimation methods that assume the spectral model to be known as a priori information. CSE-MRI allows for the quantification of the spatial distribution of triglyceride composition for certain in vivo applications. Additionally, *PDFF*

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Declarations of interest
None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mri.2018.06.012>.

quantification is shown to be independent of the chosen a priori spectral model, which agrees with previously reported results obtained at lower field strengths (e.g. 3T).

Keywords

Fat quantification; Fatty acid triglyceride composition; Fat spectral model; Magnetic resonance imaging and spectroscopy

1. Introduction

Chemical-shift encoded magnetic resonance imaging (CSE-MRI) water-fat separation has recently emerged as a useful method for the quantification of triglyceride composition in fatty tissues. In comparison to magnetic resonance spectroscopy (MRS), which only allows for the quantification of the average triglyceride composition in a localized volume of interest, CSE-MRI water-fat separation techniques allow for the quantification of triglyceride composition over an entire volume of interest with high spatial resolution [1–6]. Water-fat separation techniques, in general, have also been shown to produce results that are comparable to those obtained using spectroscopy methods [3, 7–14]. The ability to non-invasively quantify the spatial distribution of triglyceride composition may provide valuable clinical information about the development of obesity related diseases and assist in the evaluation of obesity related treatments through the monitoring of brown adipose tissue (BAT) activation and white adipose tissue (WAT) beiging, which have been shown to change triglyceride composition [15–18] and occur heterogeneously throughout the tissue [19–22].

CSE-MRI water-fat separation techniques have primarily been used to only separate the water and fat components of the MR signal to quantify proton density fat fraction (*PDFF*) [23–32] and has focused mostly on the study of nonalcoholic fatty liver disease and hepatic steatosis [9, 10, 12, 13, 33], as well as liver iron quantification [34–40]. These methods generally assume the liver fat spectral model (chemical shifts and relative amplitudes of the fat resonance peaks) to be known a priori, and therefore, the triglyceride composition is also assumed to be known a priori. The triglyceride composition determines the relative amplitudes of the fat peaks in the spectral model based upon three parameters: 1) the number of double bonds per molecule (*ndb*), which is associated with monounsaturated fatty acid composition, 2) the number of methylene-interrupted double bonds per molecule (*nmidb*), which is associated with polyunsaturated fatty acid composition, and 3) the fatty acid chain length (*cl*) [41, 42] (Table 1).

Hamilton et al. [41–43] has shown using MRS that the triglyceride compositions of brown, white, surface and deep subcutaneous, and visceral adipose tissue differ from that of liver tissue, and significant variations in triglyceride composition were observed across subjects [43]. Additionally, Leporq et al. [5, 6] has shown the presence of spatial variability in triglyceride composition within human liver tissue and murine visceral adipose tissue. This raises questions as to whether or not previously published fat spectral models can provide accurate results when used across various biological tissues, and even if using a tissue specific model, how natural spatial variability within the tissue and variability across

subjects, as well as dynamic processes, such as BAT activation and WAT beiging, can affect triglyceride composition estimations.

In modeling the CSE-MR signal for adipose tissues, many different multi-fat peak models have been proposed [14, 30, 41, 42, 44, 45]. NMR spectroscopy performed at higher magnetic fields (e.g. 7T) has shown that the fat spectrum of adipose tissue may consist of as many as 10 different resonance peaks [45], with the chemical shifts being relatively fixed for all types of fatty tissues and materials, while the relative amplitudes of the peaks vary from sample to sample (Fig. 1) based on triglyceride composition (Table 1). Despite availability of this 10-peak model, many studies have used fat spectral models containing less than 10 peaks to minimize errors arising from nonuniform excitation profiles and difficulties differentiating nearby fat resonance peaks due to spectral profile broadening at lower field strengths (e.g. 1.5 T and 3 T) [44, 45]. The two most commonly used fat spectral models are a 6-peak model and a 9-peak model [42], which are provided in the ISMRM fat-water toolbox (<https://www.ismrm.org/workshops/FatWater12/data.htm>). The relative amplitudes provided in this toolbox are specific to human liver tissue, and these models have become widely accepted and are often used generically for other types of fatty tissues.

Recent works have made comparisons between the performances of different fat spectral models when estimating *PDFF* and the effective transverse relaxation rate (R_2^*) using CSE-MRI. Wang et al. [14] has shown that when evaluating *PDFF* in the human liver at 3T using various published fat spectral models containing a range of 3 to 9 peaks, no particular model proved to be superior, and all models produced results that correlated well with MRS-*PDFF*. Using a 6-peak model with fixed chemical shifts, Hong et al. [46] evaluated the variability in the estimations of both *PDFF* and R_2^* in the human liver at 3T using 60 variant relative amplitude models. Increasing variations in the estimates of *PDFF* and R_2^* were reported with increasing *PDFF* and R_2^* ; however, these variations weren't considered to be significant. These studies were both performed at low field strengths (e.g. 3T) and have focused on obtaining estimations solely within the human liver, which typically has a *PDFF* of less than 40%.

The aim of this paper is to further explore, for materials with higher *PDFF*s and at a high field strength (e.g. 7T), the effect and performance of using different 6- and 9-peak fat spectral models when estimating triglyceride composition (*ndb*, *nmdb*, and *cl*) and *PDFF* with a specific focus on differences in the relative amplitudes of the fat peaks. MRI and NMR spectroscopy experiments were conducted using oil and butter samples and an in vivo mouse model to evaluate the importance of using a material specific fat spectral model and to evaluate the variability in the estimations of triglyceride composition and *PDFF* obtained using various fat spectral models.

2. Material and methods

2.1. NMR spectroscopy experiments

Three samples (corn oil, olive oil, and butter) were obtained from local grocery stores and prepared with a chloroform solvent (CDCl_3). Then standard proton (^1H) NMR spectroscopy (rectangular excitation pulse followed by immediate acquisition) was performed on each

sample using a 300 MHz Mercury Spectrometer (Varian Medical Systems, Palo Alto, CA) (flip angle $FA = 45^\circ$, repetition time $TR = 2.7$ s, spectral width $sw = 4800$ Hz, pulse width $pw = 4.75$ μ s, and 8 acquisitions for averaging).

The intercapsular brown adipose tissues (iBAT) from three 2-month-old C57/BL6 mice ($n = 3$) were surgically removed and prepared using a standard NMR procedure [47]. Standard water suppressed 1H NMR spectroscopy was then performed on each sample using a 600 MHz Varian Inova Spectrometer ($FA = 90^\circ$, $TR = 2$ s, $sw = 6600$ Hz, $pw = 6.3$ μ s, and 16 acquisitions for averaging).

Assuming a 9-peak spectral model (Table 1) [41, 42], the relative amplitudes of the fat peaks for all acquired spectra were determined by performing multiplet analysis using MNOVA (Mestrelab Research, S.L., Santiago de Compostela, Spain). Estimates of ndb , $nmidb$, and cl were obtained from the relative amplitudes using the `mldivide` algorithm provided by Matlab (Mathworks Inc., Natick, MA) to solve the linear system of equations associated with the theoretical peak amplitudes given in Table 1 [41, 42]. For the iBAT tissues, the average relative amplitudes of the three mice were used in estimating ndb , $nmidb$, and cl .

2.2. MRI experiments

For phantom experiments, three small 5-ml vials (one filled with each oil or butter sample) were placed into a larger cylindrical tube (28 mm in diameter, 114 mm in length) filled with water. Then using a 7 T Varian Magnex MRI Scanner, the tube containing the three samples was scanned with a 38 mm volume coil and a 2D multi-echo gradient-echo sequence with a unipolar readout gradient ($TR = 200$ ms, $FA = 20^\circ$, initial echo time $TE_i = 1.7$ ms, echo spacing $TE = 0.7$ ms, 12 echoes, matrix size of 64×64 , field of view $FOV = 35 \times 35$ mm, slice thickness $thk = 1$ mm, and 6 acquisitions for averaging).

In vivo experiments were also performed to scan the iBAT and inguinal white adipose tissue (igWAT) of two two-month old C57/BL6 mice ($n = 2$) using a 40 mm surface coil. Again, a 2D multi-echo gradient-echo sequence with a unipolar readout gradient was implemented ($TR = 75$ ms, $FA = 20^\circ$, $TE_i = 3$ ms, $TE = 0.525$ ms, 12 echoes, matrix size of 128×128 , $FOV = 30 \times 30$ mm, $thk = 1$ mm, and 4 acquisitions for averaging). Animals were anaesthetized using isoflurane, and their respiratory rates were monitored throughout experimentation. A water heating pad was used to help maintain normal body temperature. All experimental procedures were approved by the Institutional Animal Use and Care Committee (IAUCC, University of Georgia).

For MRI experiments, TE was selected based upon Monte Carlo simulations designed to find the optimal echo times for reducing the biases and variations in the estimations of triglyceride composition (ndb , $nmidb$, and cl) arising due to inaccuracies in the relative amplitudes of the fat spectral model. TE s in the range of 1.0 – 1.4 π radians (corresponding to 0.5 – 0.7 ms at 7T) were determined to be optimal (see Supplementary material). A minimally available TE_i was selected in order to reduce biases arising from noise, and Gaussian excitation pulses with full width at half-max bandwidths greater than 12 kHz were used to ensure near uniform excitation of the water and all fat peaks.

2.3. CSE-MRI post processing methods

In CSE-MRI, the MR signal of a voxel containing water and fat, acquired using a series of n echoes, is given by

$$S(t_n) = \left(\rho_W e^{-R_2^* t_n} + \rho_F \sum_{m=1}^M \alpha_m e^{i2\pi f_m t_n} e^{-R_2^* t_n} \right) e^{i2\pi f_B t_n} \quad (1)$$

where t_n is the n th echo time, ρ_W and ρ_F are the water and fat signal densities, α_m is the amplitude and f_m is the frequency shift relative to water of the m th fat peak, f_B is the change in the frequency caused by static field inhomogeneities, and R_2^* and R_2^*, m^* are the effective transverse relaxation rates of the water resonance peak and m th fat resonance peak.

It has been shown that accurate measurements of *PDFF* can be achieved when assuming a common R_2^* for all chemical species [10, 11, 33]. Using this assumption, Eq. (1) can be rewritten as

$$S(t_n) = \left(\rho_W + \rho_F \sum_{m=1}^M \alpha_m e^{i2\pi f_m t_n} \right) e^{i2\pi f_B t_n} e^{-R_2^* t_n} \quad (2)$$

For each CSE-MRI dataset, the field map (f_B) and R_2^* map were estimated first. The field map was estimated using a 2D graphcut algorithm [23, 24], and its contribution was removed from the signal prior to R_2^* estimation. R_2^* was then solved on a voxel by voxel basis using variable projection to perform complex non-linear least squares fitting [23, 24], as complex non-linear least squares fitting of the signal has been shown to outperform other methods in the presence of noise [48, 49]. Both the graphcut and variable projection algorithms assume the fat spectral model, frequency shifts (f_m) and relative amplitudes (α_m), of the signal model [Eq. (2)] to be known as a priori information.

Peterson et al. [3] proposed a linear system of equations which ties information regarding the triglyceride composition and the theoretical amplitudes of the fat resonance peaks together based on an 8 fat peak model. Using the previously estimated f_B and R_2^* maps within an adapted 6- or 9-peak linear system of equations, estimates of the decomposition parameters (ρ_W , ρ_F , ndb , $nmdb$, and cl) were determined on a voxel by voxel basis using the `mldivide` algorithm provided by Matlab to find the linear least-squares solution. The frequency shifts of the fat peaks (f_m), but not the relative amplitudes (α_m), of the fat spectral model are assumed to be known as a priori information within this linear system of equations. The chemical shifts and theoretical peak amplitudes for the 6- and 9-peak models are given in Table 1 [41, 42]. *PDFF* was then calculated using $PDFF = |\rho_F| / (|\rho_F| + |\rho_W|)$. A flowchart depicting the above-described CSE-MRI post-processing procedure is presented in Fig. 2.

For all MRI samples, triglyceride composition (*ndb*, *nmidb*, and *cl*) and *PDFF* were estimated using the above described post-processing methods and various a priori fat spectral models listed in Table 2, which includes the widely accepted liver models provided in the ISMRM fat-water toolbox (models 9 and 10). Each model in Table 2 was either calculated using the theoretical peak amplitudes given in Table 1 and previously published values of *ndb*, *nmidb*, and *cl* [1, 41, 42] or were obtained from conducted spectroscopy experiments. For the published BAT and WAT models, values for *cl* were not presented by Hamilton et al. [41]. The *cl* values for these models were determined using the published *ndb*, *nmidb*, and calculated signal percentages given by Hamilton et al. [41] to solve for *cl* based on the theoretical peak amplitudes (Table 1).

For each sample and selected spectral model, average CSE-MRI estimations were determined throughout a region-of-interest that covered the full volume of the sample while avoiding voxels with partial volume effects. The variability of the CSE-MRI estimates across spectral models was evaluated by dividing the ranges of the average estimates by their corresponding medians. To determine which spectral models correlate better with spectroscopy estimations, the relative errors of the average CSE-MRI estimations with respect to the spectroscopy estimations were summed across *ndb*, *nmidb*, and *cl* for each model and then scaled between zero and one for each material. This provides a scaled bias for each material in which the model that correlates best with spectroscopy across the three parameters will be assigned a value of zero, the model that correlates worst with spectroscopy will be assigned a value of one, and all other models will fall between zero and one.

3. Results

3.1. NMR spectroscopy experiments

While the chemical shifts of the fat resonance peaks are relatively fixed, quantifiable differences in the relative amplitudes of the resonance peaks are observed across samples in the acquired NMR spectra (Fig. 1), as well as differences in the estimations of *ndb*, *nmidb*, and *cl* (Table 2, models 2, 4, 5, and 7, and further referred to as the material specific spectroscopy models). For corn oil and olive oil, all estimations are slightly less than previously published results [1], except for *cl* of olive oil (Table 2). For butter, no previously published result was considered due to the large variation in the triglyceride composition of different types of butters. The estimations of *ndb* and *nmidb* for the iBAT are slightly greater than published values [41], while the *cl* estimate is less than the published value (Table 2).

3.2. MRI experiments

Representative spatial maps of *ndb*, *nmidb*, *cl*, and *PDFF* obtained using the material specific spectroscopy models (Fig. 3a–d) and the 9-peak liver model (Fig. 3e–h) for the oil and butter samples are displayed in Fig. 3. The estimations from the multiple material specific spectroscopy models were combined into one map by simply displaying the estimations obtained with each material specific spectroscopy model within the corresponding material's region-of-interest. Clear qualitative differences in triglyceride composition (*ndb*, *nmidb*, and *cl*), but not *PDFF*, are observed between the models, and

spatial variations within each sample are also observed. Similarly, Fig. 4 displays spatial maps of *ndb*, *nmidb*, *cl*, and *PDFF* for in vivo adipose tissues (intercapsular BAT and WAT). Spatial variations within these tissues are also observed.

To quantitatively evaluate the performance of various spectral models in estimating *ndb*, *nmidb*, *cl*, and *PDFF*, seven models (models 1–5 and 9–10 from Table 2) were examined for corn oil, olive oil, and butter, and five models (models 6–10 from Table 2) were examined for the in vivo iBAT and igWAT tissues. Fig. 5a–c shows box plots that represent the difference between the average CSE-MRI estimations and the spectroscopy estimations for *ndb*, *nmidb*, and *cl* across the examined spectral models for each material. The results for three representative spectral models are individually labeled for comparison. It is observed that the majority of the average CSE-MRI estimations are greater than the estimations obtained using spectroscopy. In sixteen out of the twenty-one (76%) of the *ndb*, *nmidb*, and *cl* groups, use of a material specific spectroscopy model (green squares) produces results that correlate better with spectroscopy estimations (or the published values for igWAT) than the estimations obtained using the 6-peak and 9-peak liver models (blue diamonds and pink triangles). For all groups, the estimates obtained using the 6-peak liver model yield either the lower or higher end of the estimates among all spectral models, and in most cases, this model even produces estimations that are considered outliers (estimates not attached to the box by whiskers) from the rest of the results. Outliers were defined as estimates that were more than 1.5 times the interquartile range away from the top or bottom of the box. Even when excluding the 6-peak liver model, variations in the estimations of *ndb*, *nmidb*, and *cl* across spectral models are still relatively large (Fig. 5a–c and Table 3); however, the variations in the estimations of *PDFF* are observed to be relatively small across all examined spectral models (Fig. 5d and Table 3).

To determine whether the material specific spectroscopy models or other models perform better when estimating triglyceride composition (*ndb*, *nmidb*, and *cl*), a scaled bias was determined for each spectral model for each material (Fig. 6 and Table 4). For four out of five of the materials studied in this work, use of a material specific spectroscopy model produces either the best or second best correlation with spectroscopy across the three parameters, and its estimations always correlate better than the estimations obtained using the widely accepted 6- and 9-peak liver models, except for iBAT. The 6-peak liver model produces the worst or second worst correlation for all five materials.

4. Discussion

The poor performance of the 6-peak model in this work suggests that a more accurate (e.g. 9-peak) spectral model is necessary when estimating triglyceride composition (*ndb*, *nmidb*, and *cl*) at a high field strength of 7T. Although the sufficiency of the 6-peak model has not specifically been explored at lower field strengths in relation to triglyceride composition quantification, many studies have used a 6-peak model to minimize errors arising from nonuniform excitation profiles and peak overlap due to spectral profile broadening when estimating *PDFF* at 1.5T and 3T [9, 12, 42, 44, 45, 49]. At higher field strengths, fat resonance peaks become more discernible from each other as the differences in the resonance frequencies of neighboring peaks increase and less peak broadening occurs.

However, even when using a 9-peak model and ensuring near uniform excitation of all peaks, inaccuracies in the relative amplitudes of the spectral model can still lead to significant biases and variations in the estimations of triglyceride composition (Fig. 5a–c and Table 3).

Despite the high SNR of the acquired data, the results for the phantom experiments still show large variations in the estimates of *ndb*, *nmdb*, and *cl* across the 9-peak spectral models examined. This demonstrates a substantial dependence of triglyceride composition quantification on the relative amplitudes of the fat spectral model and suggests that the choice of model needs to be carefully considered. However, the variations in the estimations of *PDFF* across the examined spectral models are relatively small for all materials, indicating that the *PDFF* estimate is relatively independent of the chosen spectral model. This agrees with previous observations by Hong et al. [46] and Wang et al. [14]; whose studies were performed at lower field strengths (e.g. 3T) and examined variability in the estimation of *PDFF* across spectral models in the human liver, which tends to have a *PDFF* of less than 40%. The work presented here further expands upon these observations for a higher field strength of 7T and for materials with higher *PDFF*s.

The importance of using a material specific spectral model, opposed to a widely accepted or generic spectral model, to estimate triglyceride composition is also demonstrated, as the estimates obtained using a material specific spectroscopy model produce results that correlate better with spectroscopy results in comparison to the other spectral models examined, including the 6- and 9-peak liver models provided in the ISMRM fat-water toolbox (Fig. 6 and Table 4).

Within each fatty material, spatial variability in the estimates are still observed when using the material specific spectroscopy models (Figs. 3–4). This is likely due to natural sample variability and biases arising from two factors: noise and spectral model inaccuracies caused by the natural sample variability. For the in vivo adipose tissues, inhomogeneity of the sample is naturally larger than in the oil and butter samples, and other factors, such as respiratory motion and blood flow, may lead to additional biases. However, using a material specific spectral model may be useful in reducing estimation biases and lead to more accurate spatial quantification.

The need for an a priori material specific spectral model makes the triglyceride composition estimation process seem circular. However, spectral models obtained using spectroscopy methods only quantify the average concentrations of the fat peaks throughout a localized volume of interest. These methods do not allow for the spatial quantification of triglyceride composition, while CSE-MRI methods do allow for spatial quantification.

Accurate spatial quantification is important, as certain types of adipose tissue are known to be spatially heterogeneous [22, 50–53], and dynamic processes, such as BAT activation and WAT beiging, have been shown to change the triglyceride composition of the tissue [15–18] and occur heterogeneously throughout the tissue [19–22]. Therefore, the ability to non-invasively quantify the spatial distribution of triglyceride composition may allow for the monitoring of these two processes, which may provide valuable clinical information related

to the development of obesity related diseases and assist in the evaluation of obesity related treatments.

Opposed to methods that require the relative amplitudes of the spectral model to be known as a priori information, iterative techniques to solve for triglyceride composition without a priori knowledge of the relative amplitudes may be more effective [1–6]. The methods used in this work were chosen as they have been shown to outperform iterative decomposition of water and fat with echo asymmetry and least-squares estimation with T_2^* correction (T_2^* -IDEAL) and weighted least squares T_2^* -IDEAL when estimating $PDFF$ and R_2^* in the presence of noise [48, 49].

There are several limitations to this work. In order to accurately quantify triglyceride composition, multiple factors related to the signal model must be accounted for. Eq. (2) accounts for B_0 inhomogeneity, R_2^* decay, and the spectral complexity of fat. However, other factors not accounted for in the signal model, such as eddy currents [8], T1-weighting [25, 54], and noise [10, 25, 33, 48, 49], can lead to biases in the estimations, and therefore, should also be addressed. It is noted that phase errors arising due to eddy currents were not of concern in this work since upon examination of the phase within all samples and within the water background surrounding the phantom samples, no significant deviations from linear behavior were observed.

T1-weighting biases can be avoided by using a small flip angle of typically 5° or less. The bias in the estimation of $PDFF$ arising from T1-weighting has been shown to lead to overestimations that are most severe for materials with moderate $PDFF$ s and minimal at extremely low and high $PDFF$ s [25, 54]. Therefore, bias in the estimation of $PDFF$ due to T1-weighting is expected to be relatively small within the oil, butter, and igWAT samples due to their extremely high $PDFF$ s; however, the iBAT samples are susceptible to this bias due to their moderate $PDFF$ s. For a flip angle of 20° , the biases due to T1-weighting in the estimations of ndb , $nmidb$, and $c/$ have also been shown to lead to overestimations [54]; therefore, T1-biases may contribute to the overestimations observed in this work in comparison to the spectroscopy results (Fig. 5a–c).

Unlike the bias arising due to T1-weighting, the bias in the estimation of $PDFF$ arising due to noise has been shown to lead to underestimations for materials with high $PDFF$ s, and minimal bias is observed for materials with moderate $PDFF$ s [25]. However, it is unclear how noise will affect the estimations of ndb , $nmidb$, and $c/$. The biases in the estimations due to noise are expected to be relatively small within the phantom samples due to the high SNR of the acquired data. However, the estimations within the in vivo samples are more susceptible to noise biases.

Hamilton et al. [43] recently reported that allowing $c/$ to freely vary while estimating ndb and $nmidb$ in various types of adipose tissues from MRS data acquired at 3T led to unstable estimates, but the stability of the estimates greatly improved when fixing $c/$ to a predefined value. The range of $c/$ values deemed biologically plausible by Hong et al. [46] is 17.35 to 17.55, and previously published values of $c/$ by Bydder et al. [1] for three different types of human adipose tissue are in the range of 17.33 to 17.48. These ranges are smaller than the

ranges for cI that were observed in this work. However, since the biologically plausible range of cI is likely very small, fixing cI may produce results that better correlate with spectroscopy estimations and may reduce the observed variability in the estimations across spectral models.

Some of the triglyceride composition (ndb , $nmidb$, and cI) estimations presented in this work are physically unlikely, as they either differ significantly from the USDA National Nutrient Database for Standard Reference [55] or fall outside of the biologically plausible ranges found in mammals. Despite their unlikelihood, the sensitivity of triglyceride composition quantification on the chosen spectral model is still demonstrated.

5. Conclusion

CSE-MRI water-fat separation is a useful method that allows for the spatial quantification of triglyceride composition, while MRS only provides an averaged estimation within a localized volume of interest. At high field strengths, a more accurate fat spectral model (e.g. a 9-peak model opposed to a 6-peak model) is necessary to accurately quantify triglyceride composition. However, the chosen 9-peak spectral model should be carefully selected, as using various relative amplitude models is shown in this work to produce large variations in the estimations of triglyceride composition, and use of a spectroscopy derived material specific 9-peak spectral model, opposed to a widely accepted or generic spectral model, is shown to produce estimates that more closely correlate with spectroscopy results.

Despite the strong dependence of the chosen spectral model on the estimation of triglyceride composition, the estimation of $PDFF$ is shown to be independent of the chosen spectral model at high field strengths of 7 T, which agrees with previously reported results at lower field strengths (e.g. 3T).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

CSE-MRI	Chemical shift encoded magnetic resonance imaging
PDFF	Proton density fat fraction
ndb	Number of double bonds per molecule
nmidb	Number of methylene-interrupted double bonds per molecule

cl	Fatty acid chain length
BAT	Brown adipose tissue
WAT	White adipose tissue
iBAT	Intercapsular brown adipose tissue
iWAT	Intercapsular white adipose tissue
igWAT	Inguinal white adipose tissue

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Fig. 1.

Representative spectra for corn oil (a), olive oil (b), butter (c), and surgically removed iBAT tissue (d) obtained using ^1H NMR. For each spectrum, the locations of the 9 fat resonance peaks (ppm relative to water) listed in Table 1 and their measured relative amplitudes (%) are provided below and above each peak, respectively. The chemical shifts of the 9 peaks are consistent across materials; however, the measured relative amplitudes of the peaks vary across materials. Table 2 gives estimates of triglyceride composition for each material obtained from the measured relative amplitudes (models 2, 4, 5, and 7).

Fig. 2.

A flowchart depicting the MRI post-processing procedure used in this work. Step 1, the field map (f_B) was estimated from the complex multi-echo data using a 2D graphcut algorithm. Step 2, R_2^* was estimated using the previously estimated field map from step 1 and a variable projection algorithm. Step 3, using the field map and R_2^* estimations from steps 1 and 2, the decomposition parameters (ρ_W , ρ_F , ndb , $nmldb$, and cl) were determined by solving the adapted 6- or 9-peak linear system of equations using the mldivide algorithm. Finally, $PDFF$ was estimated using the already estimated ρ_W and ρ_F maps. The necessary a priori spectral model information for each algorithm is given below its name.

Fig. 3. Representative *ndb* (first row), *nmidb* (second row), *cl* (third row), and *PDFF* (fourth row) maps obtained using the corresponding material specific spectroscopy models (models 2, 4, and 5 in Table 2) (first column, a-d) and the 9-peak liver model (model 9 in Table 2) (second column, e-h) for olive oil (bottom left vial), corn oil (bottom right vial), and butter (top vial). Clear differences in the estimations are observed between models, except for *PDFF*. Spatial variability within each of the samples is also observed.

Fig. 4.

Representative anatomical image (a) and *ndb* (b), *nmidb* (c), *cl* (d), and *PDFF* (e) maps displaying the intercapsular region, which includes brown (bottom section as shown in e) and white (top-middle section as shown in e) adipose tissue (iBAT and iWAT), of a mouse from the in vivo experiments. Qualitative differences in the estimations are observed between the iBAT and iWAT, especially for *PDFF*. Natural biological, spatial variability within each tissue is also observed. The estimations displayed here were obtained using the material specific spectroscopy model for the iBAT and the published model for iWAT (models 7 and 8 in Table 2).

Fig. 5.

Box plots representing the difference between the average CSE-MRI estimations and the spectroscopy estimations for *ndb* (a), *nmdb* (b), and *cl* (c) across various spectral models (models 1–5 and 9–10 in Table 2 for the oil and butter samples and models 6–10 for the in vivo tissues). The 6-peak liver (blue diamonds), 9-peak liver (pink triangles), and material specific spectroscopy (green squares) model estimations are shown individually. For the igWAT samples, the published WAT model (model 8 in Table 2) was considered to be the material specific model. Box plots representing the average CSE-MRI estimations of *PDFF* (d) are also presented. All outliers shown here are associated with estimations obtaining when using the 6-peak liver model (blue diamonds). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 6.

A scatter plot displaying the scaled bias of each spectral model for each material. The 6-peak liver (blue diamonds), 9-peak liver (pink triangles), and material specific spectroscopy (green squares) model biases are specifically given. For the igWAT, the published WAT model (model 8 in Table 2) was considered to be the material specific model. Scaled biases were obtained by calculating the relative errors of the average CSE-MRI estimations with respect to the spectroscopy estimations for *ndb*, *nmldb*, and *cl*, summing across the three parameters for each model, and then scaling between zero and one for each material. Models that correlate better with spectroscopy tend towards zero and models that correlate more poorly tend towards one. Numeric scaled biases are provided in Table 4. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Theoretical 9 fat peak model [41, 42].

Water-fat chemical shifts (ppm)	Theoretical peak amplitudes (Number of protons)
0.59	$2 * ndb$
0.49	1
-0.50	4
-1.95	$2 * nmdb$
-2.46	6
-2.68	$4 * (ndb - nmdb)$
-3.10	6
-3.40	$6 * (cl-4) - 8 * ndb + 2 * nmdb$
-3.80	9

A theoretical 9 fat peak model with theoretical amplitudes of each fat resonance peak given in terms of the number of double bonds per molecule (*ndb*), the number of methylene-interrupted double bonds per molecule (*nmdb*), and the fatty acid chain length (*cl*) [41, 42]. The 6-peak model combines the peaks located at 0.49 and 0.59 ppm, -2.46 and -2.68 ppm, and -3.10 and -3.40 ppm into common peaks located at 0.60, -2.60, and -3.40 ppm, respectively.

Table 2

Chemical shifts and relative amplitudes (%) of various spectral models and their corresponding triglyceride composition parameters.

Water-fat chemical shifts (ppm)										Triglyceride parameters			
9 peak fat model	-3.80	-3.40	-3.10	-2.68	-2.46	-1.95	-0.50	0.49	0.59	NDB	NMIDB	CL	
Corn Oil													
1. Published (1)	9.01	51.61	6.00	10.25	6.00	3.50	4.00	1.00	8.63	4.31	1.75	17.76	
2. Spectroscopy	7.74	50.83	7.60	9.97	6.64	3.43	4.57	1.22	8.01	3.99	1.58	17.04	
Olive Oil													
3. Published (1)	8.77	58.46	5.84	9.90	5.84	0.68	3.90	0.97	5.63	2.89	0.35	17.74	
4. Spectroscopy	6.90	59.17	6.92	9.96	6.13	0.43	4.23	1.28	4.99	2.78	0.13	18.23	
Butter													
Published	-	-	-	-	-	-	-	-	-	-	-	-	
5. Spectroscopy	8.37	57.50	6.39	6.89	7.33	2.07	4.68	1.10	5.67	2.68	1.02	16.42	
BAT													
6. Published (41)	8.97	59.32	5.98	7.98	5.98	1.40	3.99	1.00	5.38	2.70	0.70	17.28	
7. Spectroscopy	8.10	56.36	6.99	8.74	6.64	1.80	4.86	0.97	5.55	2.83	0.74	16.65	
WAT													
8. Published (41)	8.95	56.43	5.97	9.15	5.97	1.99	3.98	0.99	6.57	3.30	1.00	17.52	
Spectroscopy	-	-	-	-	-	-	-	-	-	-	-	-	
Liver													
9. Published (42)	8.75	64.15	5.83	6.22	5.83	0.62	3.89	0.97	3.73	1.92	0.32	17.45	
Spectroscopy	-	-	-	-	-	-	-	-	-	-	-	-	
Water-fat chemical shifts (ppm)													
6 peak fat model	-3.80	-3.40	-2.60	-1.95	-0.50	0.60	NDB	NMIDB	CL				
Liver													
10. Published (42)	8.75	69.98	12.06	0.62	3.89	4.71	1.92	0.32	17.45				

Spectral models used when performing CSE-MRI estimations. Models 2, 4, 5, and 7 were obtained from conducted spectroscopy experiments, and the triglyceride parameters (*ndb*, *nmidb*, and *cl*) were estimated from the relative amplitude measurements. The rest of the models were calculated using previously published values for the triglyceride parameters [1, 41, 42] and the theoretical peak amplitudes listed in Table 1.

Table 3

Variability (%) in CSE-MRI estimations across spectral models.

Material	NDB	NMIDB	CL	PDFF
Corn Oil	13.2	16.0	6.57	0.04
Olive Oil	15.2	44.7	5.64	0.09
Butter	22.4	28.1	8.10	0.05
iBAT(1)	10.3	6.76	4.49	1.61
iBAT(2)	27.0	18.6	10.3	2.61
igWAT(1)	5.35	11.5	3.35	2.82
igWAT(2)	6.54	6.14	5.37	0.19

Variability was determined as the range of the average CSE-MRI estimations across the examined spectral models for each parameter and material divided by the corresponding median of the estimates. The estimates obtained using the 6-peak model (model 10 in Table 2) were excluded when calculating the variability; therefore, for phantom experiments, the estimates obtained using models 1–5 and model 10 were used, and for the in vivo experiments, the estimates obtained using models 6–9 were used.

Table 4

Scaled bias of each spectral model for each material.

Material	Model						
	1	2	3	4	5	9	10
Corn Oil	0.41	0.15	0.19	0.00	0.25	0.26	1.00
Olive Oil	0.24	0.04	0.11	0.00	0.18	0.20	1.00
Butter	1.00	0.43	0.47	0.00	0.69	0.73	0.95

Material	Model				
	6	7	8	9	10
iBAT	0.24	0.05	0.35	0.00	1.00
igWAT	0.05	0.16	0.00	0.28	1.00

Scaled biases were determined by calculating the relative errors of the average CSE-MRI estimations with respect to the spectroscopy estimations for *ndb*, *nmdb*, and *cl*, summing across the three parameters for each model, and then scaling between zero and one for each material. Models that correlate better with spectroscopy tend towards zero and models that correlate more poorly tend towards one.