Estimating vertebral bone marrow fat unsaturation based on short-TE STEAM MRS

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Purpose: To define a metric for the separability between water and olefinic fat peaks that defines a threshold beyond which the extraction of the olefinic fat peak from vertebral bone marrow short-echo time-stimulated echo acquisition mode MRS at 3T is feasible when using a constrained peak fitting based on the triglyceride fat model.

Methods: The water and olefinic peak height difference was defined as a metric for quantifying the separability of water and olefinic fat peaks. Fat unsaturation was determined using an unconstrained olefinic peak fitting and a constrained fitting of all fat peaks to the triglyceride model. The agreement between the two peak-fitting methods was used to define a threshold on water and olefinic peak height difference separating two groups (A and B), based on L5 short-echo time-stimulated echo acquisition mode (TE = 11 ms) spectra from 252 subjects measured at 3T.

Results: A threshold on water and olefinic peak height difference was defined. Group A with a good agreement of the olefinic fat peak between the two peak-fitting methods showed a mean number of double bounds = 2.95 ± 0.21, a mean number of methylene-interrupted double bounds = 0.94 ± 0.16 and also a significantly lower coefficient of variation for all fatty acid composition parameters compared to group B (p < .001). The water and olefinic peak height difference value showed an inverse association with fat fraction.

Conclusion: A threshold of a metric quantifying the separability of the water peak and the olefinic fat peaks was defined for the estimation of the vertebral bone marrow fat unsaturation from short-echo time-stimulated echo acquisition mode MRS. The proposed methodology shows that the assessment of vertebral bone marrow unsaturation is feasible with a short-echo time-stimulated echo acquisition mode MRS in subjects with a higher fat fraction.

Keywords
bone marrow, fat unsaturation, single-voxel magnetic resonance spectroscopy, triglyceride model
INTRODUCTION

The study of bone marrow adipose tissue is gaining significant attention in understanding the pathophysiology of bone matrix loss in osteoporosis.\textsuperscript{1-3} MRI and MRS techniques have been emerging for noninvasively measuring properties of bone marrow adipose tissue.\textsuperscript{4,5} Most of the recent MRI and MRS work has focused on measuring the bone marrow fat fraction (FF) with a wide range of applications in different diseases.\textsuperscript{6-9} Another property of bone marrow fat, which has been shown to be relevant in relating bone marrow adipose tissue to matrix bone loss has been bone marrow fat unsaturation.\textsuperscript{10,11} Specifically, previous studies have shown a reduction in bone marrow fat unsaturation in osteoporosis\textsuperscript{10,12} and diabetes.\textsuperscript{13,14}

Bone marrow fat unsaturation measurements are typically performed using single-voxel MRS experiments by resolving the olefinic fat peak in the spectral proximity of the water peak.\textsuperscript{15} The visual appearance of MR spectra from yellow bone marrow is similar to MR spectra from subcutaneous fat,\textsuperscript{16} with the exception that in trabecularized bone regions the spectral linewidths are significantly broader because of magnetic susceptibility effects induced by the bone matrix.\textsuperscript{4} The measurement of bone marrow fat unsaturation in red marrow regions is, however, significantly more challenging than in yellow marrow regions.\textsuperscript{11}

In addition to the presence of broad linewidths, red marrow typically encloses a large water peak that might partially overlap with the olefinic fat peak.\textsuperscript{17} A highly clinically relevant red marrow region is the vertebral bone marrow based on the clinical significance and prevalence of vertebral fractures in osteoporotic subjects. Vertebral bone marrow (VBM) contains a large amount of water (with a FF of 50%) and is characterized by broad linewidths with both effects complicating the extraction of the olefinic fat peak.\textsuperscript{17,18}

Many of the existing in vivo MRS studies measuring VBM fat unsaturation metrics have been based on point resolved spectroscopy (PRESS) acquisitions with longer echo times (TEs) in the range between 30 ms and 40 ms at 3T.\textsuperscript{10,12,18} However, J-coupling effects are expected to affect the quantification of olefinic fat peak area in PRESS acquisitions with such long TEs.\textsuperscript{19} Stimulated echo acquisition mode (STEAM) MRS acquisitions with short TEs have been shown to be less sensitive to J-coupling effects compared with PRESS MRS acquisitions.\textsuperscript{19,20} Alternative methods to reduce the effect of J-coupling on olefinic fat peak area determination include long-TE PRESS acquisitions,\textsuperscript{21,22} long-TE STEAM acquisitions,\textsuperscript{23,24} and diffusion-weighted STEAM acquisitions,\textsuperscript{20} but with all three methods being associated with reduced signal-to-noise ratio (SNR). MRS processing of short-TE STEAM MRS employing constrained peak fitting based on the triglyceride fat model has been recently applied in regions of subcutaneous and visceral adipose tissue.\textsuperscript{25,26} However, it is not known for which measured spectra the extraction of the olefinic fat peak is feasible when using short-TE STEAM MRS combined with constrained peak fitting based on the triglyceride fat model.

METHODS

2.1 Subjects

The lumbar spine region of 252 healthy volunteers with an age range between 18 and 77 years (154 women and 98 men; mean age 43.7 ± 15.8 years) was scanned on a 3T whole-body scanner (Ingenia 3.0T, Philips Healthcare, Best, The Netherlands) using the built-in 12-channel posterior coil array. Inclusion criteria were no history of fracture and no pathological bone changes, such as bone metastases or hematological or metabolic bone disorders. The study was approved by the local institutional committee for human research. All subjects gave written informed consent before participation in the study.

2.2 MRS acquisition

MRS was applied to the L5 vertebral body based on appropriate localizing sequences. The L5 vertebral body was selected because it usually has the largest volume of all vertebral bodies. In a small number of subjects, the L5 vertebra was subject to degenerative changes; therefore, L4 was measured. The default MRS voxel size was 15 × 15 × 15 mm\textsuperscript{3}. If necessary, the MRS voxel size was reduced to fit within the vertebral body. Moreover, chemical-shift–displacement effects were considered during the positioning of the volume of interest by visualizing both the water voxel (center frequency selected at the water peak) and the fat voxel (based on the chemical shift of the main fat peak and the STEAM MRS RF pulses bandwidth). A STEAM single-voxel MRS sequence with the following parameters was used: pulse repetition time = 6 seconds (to minimize any T\textsubscript{1}-weighting effects); Mixing Time = 16 ms (set to shortest Mixing Time to minimize J-coupling effects); TE = 11 ms (STEAM with shortest TE to minimize J-coupling effects); eight repetitions with four phase cycles; 4096 sampling points; spectral acquisition bandwidth of 5 kHz; no water suppression; no regional saturation bands. The very asymmetric “spredrex” RF pulses (duration of 7 ms,
bandwidth of 2277 Hz) provided by the vendor were used to achieve a STEAM MRS voxel localization with TE = 11 ms.

2.3 | MRS processing

Frequency-based spectral fitting was performed using in-house written routines in MATLAB (Mathworks, Natick, Massachusetts). Pseudo-Voigt lineshapes\(^{27}\) with a 0.8 × Gauss + 0.2 × Lorentzian lineshape for the fat peaks and a 0.2 × Gauss + 0.8 × Lorentzian lineshape for the water peak were employed. Figure 1A,C show measured VBM fat spectra with fat peaks observed at spectral locations 0.90, 1.30, 1.6, 2.02, 2.24, 2.75, 4.15, 4.30, 5.19, and 5.29 ppm. The letters A and D were assigned to peaks at 0.90 ppm [methyl: \((-\text{CH}_2)_n-\text{CH}_3\)], 2.77 ppm (diallylic methylene: \(-\text{CH}=-\text{CH}_2-\text{CH}=-\text{CH}_2\)), respectively. The letter B was assigned to the superposition of peaks at 1.30 ppm ([methylene: \((-\text{CH}_2)_n\)] and 1.59 ppm \(\beta\)-carboxyl: \(-\text{CO}--\text{CH}_2--\text{CH}_2--\)) and the letter C to the superposition of peaks at 2.00 ppm (\(\alpha\)-olefinic: \(-\text{CH}_2--\text{CH}--\text{CH}_2--\)) and 2.25 ppm (\(\alpha\)-carboxyl: \(-\text{CO}--\text{CH}_2--\text{CH}_2--\)), the letter E to the superposition of peaks at 4.15 ppm and 4.30 ppm (glycerol: \(-\text{CH}_2--\text{O}--\text{CO}--\)) and the letter F to the superposition of peaks at 5.19 ppm (glycerol: \(-\text{CH}_2--\text{O}--\text{CHO}--\text{CH}_2--\)) and 5.31 ppm (olefinic: \(-\text{CH}=-\text{CH}--\)).

Three different peak-fitting methods were employed. First, the method previously described by Dieckmeyer et al.\(^{17}\) was used to estimate the FF. The FF determination method relied on the fitting of the olefinic and glycerol fat peaks constrained to the methylene and methyl peaks based on an a priori

![Diagram of MR spectra with different degrees of separability between olefinic and water peaks.](image)

**Figure 1** Full (A,C) and zoomed (B,D) vertebral bone marrow MR spectra with different degree of separability between the olefinic fat peak and water peak. A,C, MR spectrum with high fat fraction where the olefinic peak is visually separated from the water peak. B,D, MR spectrum with low fat fraction where the olefinic peak is not visually separated from the water peak. At the spectral location of the olefinic fat peak, \(S_{\text{water}}\) is the fitted signal of the water peak, \(S_{\text{olefinic}}\) the fitted signal of the olefinic peak, and \(S_{\text{total}}\) is the fitted signal of all peaks combined. The height difference between the water and olefinic peak is defined based on the above signals. A cut-off value for the WOPHD* is searched to define the range of parameters for which the extraction of the olefinic fat peak is feasible (group A). Groups A and B are defined based on WOPHD* (Figure 2). WOPHD, water olefinic peak height difference.
known average triglyceride model, as previously determined in Dieckmeyer et al.\textsuperscript{17} Second, two methods were used to assess the ability to extract unsaturation parameters with different peak constraints. The unconstrained fitting method used an unconstrained fitting of the olefinic and glycerol fat peaks, independent of the main fat peak. The constrained fitting method constrained all fat peaks to a parameterized triglyceride model. The parameterized triglyceride model consisted of the three main parameters: the mean chain length, the mean number of double bonds (ndb) per triglyceride, and the mean number of methylene-interrupted double bonds (nmidb) per triglyceride.\textsuperscript{25} In both the unconstrained fitting method and the constrained fitting method, a common fat peak linewidth and an independent water peak linewidth were constrained to be below 0.50 ppm and 1.50 ppm, respectively. The spectral locations of the fat peaks were allowed to vary by ±0.03 ppm, and the water peak location was allowed to vary by ±0.10 ppm.

Indicative SNR levels in the in vivo data were calculated in frequency domain, by dividing the highest signal by the standard deviation (SD) of the noise in a spectral region without peaks.

### 2.4 Fat fraction estimation

The previous method proposed by Dieckmeyer et al.\textsuperscript{17} was adopted. After fitting for the peaks A, B, C, and D, as well as for the water peak, the peaks E and F were calculated based on a triglyceride model,\textsuperscript{25} with an assumed value for ndb = 3.13 and nmidb = 0.70.\textsuperscript{17} For the two glycerol-peaks at 4.15 ppm and 4.30 ppm, the total area was calculated as 5.62% of the area of peaks A + B, respectively. Area of the glycerol peak at 5.19 ppm was calculated at 1.41% of the area of peaks A + B and the area of the olefinic peak at 8.79% of the area of peaks A + B. In total, 11 parameters were fitted (fat peak areas of six peaks at A, B, C, and D as describe above; water peak area; spectral location of water peak; common spectral location of all fat peaks; linewidth of water peak; and common linewidth of all fat peaks).

### 2.5 Unconstrained fitting for olefinic fat peak estimation

In the unconstrained fitting method, the spectra were fitted the same way as in the fitting for the fat fraction estimation, but the olefinic peak at 5.29 ppm was not constrained to the area of peak A + B. The glycerol peak at 5.19 ppm was constrained as 1.41% to the area of peaks A + B. The glycerol peaks at 4.15 ppm and 4.30 ppm were calculated as 5.62% of the area of peaks A + B, respectively. In total, 12 parameters were fitted (fat peak areas of six peaks at A, B, C, and D as described above; olefinic fat peak area; water peak area; spectral location of water peak; common spectral location of all fat peaks; linewidth of water peak; and common linewidth of all fat peaks).

### 2.6 Constrained fitting for olefinic fat peak estimation

The constrained fitting method was based on a constrained fitting of all peaks to the triglyceride model by Hamilton et al.\textsuperscript{25} A fixed chain length of 17.33\textsuperscript{28} was assumed. The peaks were fitted for free ndb and nmidb. In total, eight parameters were fitted (ndb; nmidb; triglyceride scaling factor; water peak area; spectral location of water peak; common spectral location of all fat peaks; linewidth of water peak; and common linewidth of all fat peaks).

### 2.7 Metrics definition

In general, the ability to resolve the olefinic fat peak in the spectral proximity of the overlapping water peak depends on both the FF and the water/fat peaks linewidth. A metric of the water and olefinic peak height difference (WOPHD) was thus defined aiming to quantify the ability to resolve the olefinic fat peak in the spectral proximity of the overlapping water peak:

\[ WOPHD = \frac{S_{\text{water}}(\text{ole}) - S_{\text{olefinic}}(\text{ole})}{S_{\text{total}}(\text{ole})} \tag{1} \]

where \( S(\text{ole}) \) is the signal strength of the fitted signal at the spectral location of the olefinic fat peak (Figure 1). \( S_{\text{water}}(\text{ole}) \) is the fitted signal of the water peak at the olefinic fat peak spectral location, \( S_{\text{olefinic}}(\text{ole}) \) the fitted signal of the olefinic peak at the olefinic fat peak spectral location, and \( S_{\text{total}}(\text{ole}) \) is the fitted signal of all peaks combined at the olefinic fat peak spectral location.

To measure the WOPHD metric, the fitting method for FF estimation was used. By definition, WOPHD depends on both the FF and the water/fat peaks linewidth: High WOPHD values are associated with a strong water peak, broader linewidth, and an olefinic fat peak that cannot be separated from the overlapping water peak. Low WOPHD values are associated with a small water peak, narrow linewidth, and an olefinic fat peak that can be separated from the overlapping water peak. The results of the peak-fitting methods were then used to determine the following fat composition metrics, assuming that \( A_{\text{fatpeaks}} \) stands for the area under all fat peaks, \( A_{\text{waterpeak}} \) stands for the area under the water peak and \( A_{\text{olefinicpeak}} \) stands for the area under the olefinic peak at 5.29 ppm.
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The FF was defined as:

\[ FF = \frac{A_{\text{fat peaks}}}{A_{\text{fat peaks}} + A_{\text{water peak}}} \]  

(2)

based on the peak areas determined by the method proposed by Dieckmeyer et al.\textsuperscript{17} (fat fraction estimation method). The olefinic fraction (OF) was then defined as:

\[ OF = \frac{A_{\text{olefinic peak}}}{A_{\text{fat peaks}}} \]  

(3)

and determined based on the peak areas by the two fitting methods (unconstrained/constrained fitting for olefinic fat peak estimation).

A WOPHD cut-off value, labeled as WOPHD*, was defined as the WOPHD value below which the extraction of the olefinic fat peak is feasible. WOPHD* splits the experimentally measured spectra into two groups: Group A was defined as the group of spectra with WOPHD < WOPHD* that allows the separation of water from the olefinic fat peak (Figure 1). Group B was defined as the group of spectra with WOPHD ≥ WOPHD* that does not allow a reliable separation of water from the olefinic fat peak (Figure 1).

To determine WOPHD*, the agreement between the olefinic fraction results between the unconstrained olefinic fat peak-fitting method and the constrained olefinic fat peak-fitting method in the experimentally measured spectra was employed. The slope of the agreement of the OF values between the unconstrained peak-fitting method and the constrained peak-fitting method in group A was plotted as a function of WOPHD* (Figure 2). In addition, the normalized norm of residuals (normalized \( R^2 \)) of the agreement for the OF values between the unconstrained peak-fitting method and the constrained peak-fitting method in group A was plotted as a function of WOPHD* (Figure 2). WOPHD* was defined as the value for which the slope of the agreement of the OF values between the unconstrained peak-fitting method and the constrained peak-fitting method in group A equals one.

2.8 | Numerical simulations

Monte Carlo simulations were performed to verify the WOPHD* extraction based on the experimentally measured spectra. Synthetic MR spectra were generated using the triglyceride model and a fixed chain length of 17.33.\textsuperscript{28} Pseudo-Voigt lineshapes\textsuperscript{27} with a 0.8 × Gauss + 0.2 × Lorentzian lineshape for the fat peaks and a 0.2 × Gauss + 0.8 × Lorentzian lineshape for the water peak were employed and the fat peak locations were defined as for the experimental spectra. Additional parameters were selected in a physiological range: 10% \( \leq \) FF \( \leq \) 100%, 0.6 \( \leq \) ndb \( \leq \) 4, 0 \( \leq \) nmidb \( \leq \) 1.6 with ndb/nmidb \( \geq \) 2, 0.3 ppm \( \leq \) water peak linewidth \( \leq \) 0.8 ppm, and 0.3 ppm \( \leq \) fat peak linewidth \( \leq \) 0.8 ppm. Complex Gaussian noise was added in time domain and the simulations were repeated at different SNR levels: SNR = 60 (low SNR case) and SNR = 170 (average SNR case). At each SNR level and set of parameters, the simulations were repeated 50 times resulting in 841 500 number of simulated spectra per SNR level. The slope of the agreement and the normalized norm of residuals for the OF values between the unconstrained peak-fitting method and the constrained peak-fitting method in group A, now defined based on the simulated spectra, were plotted as function of WOPHD*.

2.9 | Bootstrapping and statistical analysis

To investigate the reproducibility of peak-fitting results, bootstrapping of the experimentally measured spectra was performed. Based on the acquired eight repetitions, employing four phase cycles, 16 different combinations of four repetition were subsampled for bootstrapping. The coefficient of variation (CV) was calculated for ndb, nmidb, and OF for the constrained peak-fitting method.

All statistical analyses were performed with RStudio (Version 1.1.423; RStudio, Inc., Boston, Massachusetts; http://www.rstudio.com/). Tests were performed at a significance level of \(*P < .05, **P < .01, \) and ***\( P < .001\). Two-sided \( t \) tests were performed to test differences in extracted parameters between subject groups A and B. The relationship between FF and WOPHD in the measured spectra was finally investigated to gain additional insights on the main parameters affecting WOPHD.

3 | RESULTS

The spectra from five subjects were excluded because of poor spectral quality. The determination of WOPHD*, which defines the separation between groups A and B, was based on the maximization of the agreement of the OF peak estimation between the unconstrained and the constrained peak-fitting methods (Figure 2A). Figure 2B plots the normalized norm of residuals and Figure 2C plots the slope of the linear agreement between the unconstrained and the constrained peak-fitting methods estimating the OF in group A. The values of WOPHD* that minimize the normalized norm of residuals (blue dot point in Figure 2C) and that result in a slope equal to one (green dot point in Figure 2D) are close and around the WOPHD* value of \(-0.1\). Therefore, WOPHD* was set to \(-0.077\), as for this value WOPHD* results in the slope of the linear agreement between the constrained and unconstrained peak-fitting method in group A equal to 0.997 (and closest to one). Groups A and B were thus defined by spectra with
The following values were extracted based on the constrained peak-fitting method: mean ndb was 2.95 ± 0.21 in group A and 2.62 ± 0.50 in group B. Mean nmidb was 0.94 ± 0.16 in group A and 0.60 ± 0.38 in group B. The mean linewidth of fat was 0.46 ± 0.05 ppm in group A and 0.52 ± 0.07 ppm in group B. The mean linewidth of water was 0.51 ± 0.06 ppm in group A and 0.51 ± 0.07 in group B.

Supporting Information Figures S1-S3 present the dependence of the OF results for both the constrained and unconstrained peak-fitting methods and the dependence of OF on FF, linewidth, and SNR based on the numerical simulations. Figure 3 shows the numerical simulation results at different SNR levels for the dependence of the normalized norm of residuals and the slope agreement between the two peak-fitting methods on WOPHD* (Figure 3A at SNR = 60 and Figure 3B at SNR = 170). The points of the minimum

**FIGURE 2**  A, Agreement of the OF between unconstrained fitting method and constrained fitting method for groups A and B. B,C, The determination of WOPHD* is based on maximizing agreement of the OF between the two fitting methods. Low WOPHD* values are associated with a limited number of samples in group A. High WOPHD* values are associated with spectra where the olefinic fat peak and water peaks cannot be separated. WOPHD* was set to −0.077, resulting in (B) a slope of the correlation line of the OF between the constrained and unconstrained fitting method close to one, and (C) close to the minimum of the normalized norm of residuals in group A. WOPHD, water olefinic peak height difference; OF, olefinic fraction
normalized norm of residuals and slope equal to one (blue
and green points, respectively), have been determined based
on the experimentally measured spectra. The slope of the OF
agreement between the two peak-fitting methods in group A
based on the simulated spectra was equal to 0.9925 at SNR =
60 and equal to 0.9924 at SNR = 170 (Figure 3A,C). The
minimum normalized norm of residuals for the OF
agreement between the two peak-fitting methods in group A
based on the simulated spectra also shifted to higher
WOPHD* values with increasing SNR (Figure 3B,D).

Figure 4 shows the CV resulting from the bootstrapping
analysis. There was a significant difference \( P < .001 \) be-
tween group A and group B in the CV results of the measured
parameters (OF, ndb, and nmidb). The CV of all parameters
was significantly higher in group B compared to group A
\( P < .001 \). The mean ± SD of the CV per parameter for
each group was: OF group A: 2.47% ± 1.22%, OF group B:
5.24% ± 3.21%; ndb group A: 2.34% ± 1.15%, ndb group B:
5.00% ± 3.10%; and nmidb group A: 7.93% ± 4.39%, nmidb
group B: 40.68 ± 64.58, with some values above 100%.
Figure 5 shows a high correlation between the FF and WOPHD in the experimentally measured spectra, including both groups A and B ($R^2 = 0.90$, slope = −49.19, intercept = 50.4).

4 | DISCUSSION

Despite the fact that VBM fat unsaturation has been shown to be linked to bone health, there is currently no consensus on which type of MRS acquisition and peak-fitting method should be employed for extracting the olefinic fat peak in the spectral proximity of the overlapping water peak. The present work employed short-TE STEAM MRS with a constrained peak fitting based on the triglyceride fat model. The short-TE STEAM MRS acquisition was selected because of its known property of reducing J-coupling effects, and its already wide application in studies measuring VBM FF. The constrained peak fitting based on the triglyceride fat model was adopted to reduce the confounding effect of the overlapping
water–fat peaks. A threshold on the separability of water and olefinic fat peaks was determined by comparing the self-consistency of the OF estimation using two different peak-fitting methods. Two groups were defined based on the threshold on the separability of water and olefinic fat peaks in a large pool of experimentally measured spectra. It was demonstrated that there are significant differences between the two groups concerning the mean of OF, ndb, and nmidb. Group A showed higher values for all three parameters compared to group B. The mean ndb and nmidb of group B were distinctly lower than the literature values. Specifically, some results for nmidb with values below 0.2 in group B appeared to be out of the physiological range compared with the literature. In group A, the mean values were close to the values mentioned in the literature, and the within-group SD was smaller compared to group B.

In the employed numerical simulations, the OF results for both the constrained and unconstrained peak-fitting methods showed an increased precision for higher FF (Supporting Information Figure S1), narrower linewidths (Supporting Information Figure S2), and higher SNR (Supporting Information Figure S3). For higher FF and narrowed linewidths, WOPHD decreased, suggesting a better separability of the water and olefinic fat peaks (Supporting Information Figures S1 and S2). The constrained fitting method resulted overall in more precise estimation of OF compared to the unconstrained fitting method (Supporting Information Figures S1-S3).

As no gold-standard measurement for the VBM fat unsaturation was available, the simulation framework was also used to verify that a threshold on the separability of water and olefinic fat peaks can be determined by comparing the self-consistency of the OF estimation using two different peak-fitting methods. The employed simulation framework could simulate noise effects, but no signal model mismatches, in contrast to the experimentally measured data where both noise effects and model mismatches affect the measurements. Similar to the experimentally measured data, the simulation showed that the normalized norm of residuals, a function of WOPHD*, has a local minimum. For lower WOPHD*, fewer spectra are included in group A, and for higher WOPHD*, there is a big overlap of the water and the olefinic peak leading to an increase of the normalized norm of residuals. The shift of the minimum of normalized norm of residuals to higher WOPHD* for higher SNR values can be explained by the fact that at higher SNR and in the absence of model mismatches, the separation of the olefinic and water fat peak can be achieved for higher values of WOPHD. The presented SNR levels of 60 and 170 in the simulations were chosen based on the range of the indicative SNR levels from the experimental data. The simulation verified the definition of WOPHD*, as the WOPHD* value minimizing the normalized norm of residuals based on the simulated data, was close to the WOPHD* value minimizing the normalized norm of residuals based on the experimental data. In addition, the agreement slope for the

**Figure 5** Correlation plot between fat fraction (FF) and WOPHD. Correlation between FF and WOPHD yielded $R^2 = 0.90$ and a slope = $-49.25$ and intercept = 50.47. Dashed line corresponds to WOPHD* and splits the measured spectra in group A (green points) and group B (red points). WOPHD, water olefinic peak height difference.
simulated data at the WOPHD* value determined from the experimental data was close to one.

By performing bootstrapping and calculating the CV for each in vivo spectrum, the reproducibility of the results was studied for groups A and B. For the parameters OF, ndb, and nmidb, group A showed significantly lower CVs than group B, suggesting that the spectra of group A can be measured with a higher reproducibility than the spectra of group B. The CV of nmidb was relatively high in both groups A and B compared with the other parameters, but the CV of nmidb in group A was still significantly lower than the CV of nmidb in group B.

In Figure 5, it can be seen that WOPHD is mainly dependent on the FF, in accordance with the previous work by Xu et al.18 Therefore, the proposed methodology shows that with a short-TE STEAM acquisition, the VBM unsaturation is feasible for WOPHD < −0.077 (primarily spectra with high FF) using either the presently employed unconstrained or constrained peak-fitting methods for the estimation of OF. Attention should be paid when using short-TE MRS for determining VBM unsaturation in subjects with WOPHD > −0.077 (primarily spectra with low FF). However, the present work does not provide a conclusive answer to which acquisition method should be used for short-TE MRS spectra with WOPHD > −0.077.

Instead of short-TE STEAM MRS acquisitions, long-TE PRESS acquisitions,21,22 long-TE STEAM acquisitions,23,24 and diffusion-weighted STEAM acquisitions20 have been previously proposed to reduce the effect of J-coupling on the olefinic fat peak area determination. Long-TE PRESS and long-TE STEAM acquisitions have also been applied for bone marrow fat applications. A major limitation of these methods is the SNR loss caused by increased T2-weighting, which is particularly relevant when measuring VBM fat unsaturation. In addition, multi-TE STEAM MRS with short TEs has been emerging as the method of choice to measure the VBM proton density FF4 and typically includes the shortest-TE scan included in the present study.

Here the work has been based on the agreement of the OF values between two frequency-domain–fitting methods. Both frequency-domain and time-domain peak-fitting methods have been previously employed in the analysis of vertebral bone marrow spectra. Frequency-domain methods are typically limited to fitting the real part of a well-phased spectrum. Instead, time-domain methods fit the complex time-domain data and can more easily incorporate line-shape models and phase parameters, as has been also recently shown in vertebral bone marrow applications.18 The proposed methodology used the frequency representation of the spectra to define WOPHD* based on the agreement of two fitting methods, given that a definition of the metric for the separability of the water and olefinic fat peaks is more intuitive in the frequency domain. However, metrics for assessing the separability of the water and olefinic fat peaks could be also constructed when using time-domain fitting methods.

The present study has several limitations. First, the definition of the threshold based on WOPHD and the determination of the two groups was based on the agreement of the results between two fitting methods, and there was no gold-standard experimental measurement available. In general, it is expected that when the olefinic peak is clearly separable from the water peak, both the constrained and unconstrained peak-fitting methods would give equivalent results for the OF. The application of the above observation on a rich data set of 252 spectra was the basis of the analysis in the determination of the two groups. Despite the lack of gold-standard reference measurements assessing the accuracy of the reported fatty acid composition metrics, the proposed methodology should provide a good estimation for which the VBM unsaturation is feasible and not confounded by overlapping water–fat peaks.

Second, to address the lack of a reference experimental measurement, a Monte Carlo simulation was performed, which has some limitations of its own. The simulation considered only noise effects, which could explain the decrease in the slope for the two fitting methods agreement by increasing WOPHD, whereas the experimental data show an increasing slope with increasing WOPHD. However, including parameters to also simulate a model mismatch would require too many additional degrees of freedom. Third, a measurement of reproducibility would formally require repeated measurements with patient-repositioning. The computation of the CV based on a bootstrapping analysis should in part explain why the OF CV range reported here is significantly lower from the OF CV range reported in previous studies.11 However, the bootstrap analysis was able to characterize the difference between the two groups, given the overall low SNR of the MR spectra in VBM. Fourth, J-coupling effects were minimized, but cannot be totally excluded when using short-TE STEAM MRS acquisition. Fifth, the Voigt lineshape factors in the presently employed frequency domain-based peak-fitting routines were determined empirically and were maintained constant across spectra.

5 | CONCLUSION

A threshold on the separability of the water peak and the olefinic fat peaks was defined for the estimation of the VBM fat unsaturation from short-TE STEAM MRS using a constrained peak fitting based on the triglyceride fat model. The proposed methodology shows that the assessment of VBM unsaturation is feasible with a short-TE STEAM MRS in subjects with a higher FF.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

FIGURE S1 Dependence of A, OF of the constrained fitting method, B, OF of the unconstrained fitting method, C, WOPHD on the fat fraction based on simulated spectra (ndb = 3.0, nmidb = 1.0, linewidth = 0.45 ppm and SNR = 60). The red line indicates the reference OF value

FIGURE S2 Dependence of A, OF of the constrained fitting method, B, OF of the unconstrained fitting method, C, WOPHD on the linewidth based on simulated spectra
(FF = 50%, ndb = 3.0, nmidb = 1.0 and SNR = 60). The red line indicates the reference OF value

**FIGURE S3** Dependence of A, OF of the constrained fitting method, B, OF of the unconstrained fitting method, C, WOPHD on SNR based on simulated spectra (FF = 50%, ndb = 3.0, nmidb = 1.0 and linewidth = 0.45 ppm). The red line indicates the reference OF value

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