Adipose tissue as well as other depots of fat (triglycerides) are increasingly being recognized as active contributors to the human function and metabolism. In addition to the fat concentration, also the fatty acid chemical composition (FAC) of the triglyceride molecules may play an important part in diseases such as obesity, insulin resistance, hepatic steatosis, osteoporosis, and cancer. MR spectroscopy and chemical-shift-encoded imaging (CSE-MRI) are established methods for non-invasive quantification of fat concentration in tissue. More recently, similar techniques have been developed for assessment also of the FAC in terms of the number of double bonds, the fraction of saturated, monounsaturated, and polyunsaturated fatty acids, or semi-quantitative unsaturation indices. The number of papers focusing on especially CSE-MRI-based techniques has steadily increased during the past few years, introducing a range of acquisition protocols and reconstruction algorithms. However, a number of potential sources of bias have also been identified. Furthermore, the measures used to characterize the FAC using both MRI and MRS differ, making comparisons between different techniques difficult. The aim of this paper is to review MRS- and MRI-based methods for in vivo quantification of the FAC. We describe the chemical composition of triglycerides and discuss various potential FAC measures. Furthermore, we review acquisition and reconstruction methodology and finally, some existing and potential applications are summarized. We conclude that both MRI and MRS provide feasible non-invasive alternatives to the gold standard gas chromatography for in vivo measurements of the FAC. Although both are associated with gas chromatography, future studies are warranted.

KEYWORDS
CSE-MRI, fat unsaturation, fatty acid composition, MRI, MRS

1 INTRODUCTION

In recent years, the traditional view of adipose tissue serving solely as a thermal insulator, mechanical cushion, and energy storage depot has been challenged. Instead, growing evidence has been presented for a more active role of adipose tissue as an endocrine organ and as part of the immune system.1,2 Furthermore, distinct metabolic activities...
and functions have been demonstrated for various adipose tissue depots, and the chemical composition of accumulated fat may be linked to various disease scenarios, in addition to being reflective of the diet. Obesity and metabolic disorders may, for example, be associated with an increased proportion of saturated fatty acids in the liver, and in bone marrow, a lower degree of unsaturation has been linked both to reduced bone quality and type 2 diabetes. Furthermore, the degree of unsaturation in adipose tissue may be affected differently close to malignant compared to benign tumors. Thus, in learning more about the role of adipose tissue in health and disease, methods are needed which cannot only localize, but also characterize human adipose tissue. Specifically, this review concerns quantitative estimation of the chemical composition of fatty acids (FAC) using MRI and spectroscopy.

FAC with imaging, and potential applications are summarized. First, we will describe the chemical composition of triglycerides, the main contributor to adipose tissue signal in MR, and discuss various potential FAC measures. Second, we will review acquisition techniques challenging. In addition, a number of potential sources of bias have been identified for both the MRS and the MRI methods, and the measures used to characterize the FAC differ, making comparisons between different techniques challenging.

We aim to review 1H MRS- and MRI-based methods for in vivo quantification of the FAC, whereas 13C-based methods will not be further discussed. First, we will describe the chemical composition of triglycerides, the main lipid contributor to adipose tissue signal in MR, and discuss various potential FAC measures. Second, we will review acquisition and reconstruction methodology and at last, some existing and potential applications are summarized.

2 BACKGROUND

2.1 Human fat depots

Fat, or triglycerides, is mainly stored in adipocytes in white adipose tissue, but may also be found in other forms in, for example, brown adipose tissue or ectopically in the liver, pancreas, and bone marrow. White adipose tissue may be further divided depending on storage depot as subcutaneous or internal adipose tissue, of which the latter includes visceral adipose tissue, surrounding the internal abdominal organs, or intermuscular adipose tissue. In each depot, fat is typically stored as dominating drops within white adipocytes or as smaller droplets in other parenchymal cells. Although triglycerides are only one example of the many types of lipids in the human body, triglycerides are the ones contributing to the MR signal and will be the only type under discussion in the remainder of this review.

2.2 Fatty acid composition

Triglycerides consist of a glycerol backbone with three fatty acid chains of which each is characterized by the number of carbon atoms making up the chain, and the number of double bonds between them (see Figure 1). The gold standard technique for estimation of the FAC is gas chromatography (GC) determination of the relative abundance of each fatty acid in a sample. Typically, the three most abundant fatty acids in human white adipose tissue are (chain length: number of double bonds) 18:1, 16:0, and 18:2 (see Table 1). Alternatively, fatty acids may be categorized in terms of their degree of saturation, as saturated (no double bonds, SFA), unsaturated (at least one double bond, UFA), monounsaturated (one double bond, MUFA), or polyunsaturated (at least two double bonds, PUFA) fatty acids, and the fractions of each type may be estimated from the relative fatty acid abundances. These fractions will be termed saturation fractions, and be denominated as fSFA, fUFA, and fMUFA, etc. In adipose tissue, fUFA is typically higher than fSFA, and fMUFA higher than fPUFA (see Table 1). Whereas GC allows for estimation of each of these sets of parameters through simple arithmetic from the relative fatty acid abundances, MR techniques are limited to estimation of saturation fractions.

2.3 Fat in MR

Each hydrogen proton of the triglyceride molecule contributes to the MR signal. Protons positioned at different locations within a molecule experience slightly different magnetic fields, since the electronic clouds surrounding the nuclei shield the protons from the external field to different degrees. Consequently, the resonance frequencies in the NMR spectrum reflect different molecular positions of the protons. From the protons in a triglyceride molecule (consisting of three fatty acid chains connected to a glycerol backbone), up to 10 different peaks can be resolved (Figure 1 and Table 2) depending on field strength and T2*. The water protons give rise to a single peak, but its position, unlike the fat peaks, may vary slightly depending on the temperature. Thus, the relative amplitude of each fat peak provides some information on the chemical composition of the triglyceride molecules. The various peaks also have distinct relaxation
times (see Table 2), which may be important to consider in absolute quantification of the FAC.

3  |  MRS-BASED TECHNIQUES

3.1  |  FAC estimation

By comparing the total area of all fat peaks with the area of the water peak, after correcting for differences in $T_1$- and $T_2$-relaxation, it is possible to quantify the relative proton density fat fraction (PDFF). Since MRS, theoretically, can provide information about the relative amounts of protons at all positions within the triglyceride molecule, also the chemical composition of the fatty acids can be assessed.

3.1.1  |  Saturation fractions

Based on the facts that all fatty acids contain two protons in position E and all unsaturated fatty acids contain four protons in position D, $f_{UFA}$ can be calculated as $f_{UFA} = \frac{1}{2} \frac{A_D}{A_E}$, where $A_X$ denotes the peak area of peak $X$ in Table 2. Instead of using the two protons in position E for normalization, Corbin et al suggested using the three methyl protons in position A, because they can be better separated from neighboring peaks, yielding the expression $f_{UFA} = \frac{3}{4} \frac{A_D}{A_A}$. The fraction of saturated fatty acids is calculated as $f_{SFA} = 1 - f_{UFA}$. The corresponding expression for diunsaturated fatty acids is $f_{DUFA} = \frac{A_F}{A_E} - 2 f_{UFA}$ (or $f_{DUFA} = \frac{3}{2} \frac{A_F}{A_A} - 2 f_{UFA}$, if peak A is used for normalization), where $f_{DUFA}$ is the fraction of triunsaturated fatty acids, under the assumption that the fraction of fatty acids with more than three double bonds can be ignored. Similar expressions were derived by Ren et al, but with the assumption that also triunsaturated fatty acids could be ignored (i.e., $f_{DUFA} = 0$), because fatty acids with 0, 1, and 2 double bonds constitute ~97-98% of the total fat in humans on ordinary Western diets.

3.1.2  |  Unsaturation indices

In an MRS study of bone marrow fat at 1.5T, where peaks E and F could not be resolved, Yeung et al introduced an unsaturation index (UI), defined as $UI = A_J / (A_J + A_B + A_D + A_F)$. This UI does, however, not provide an absolute quantification.
of the fatty acid unsaturation, but should be regarded as a relative index to compare lipid compositions. Lundbom et al defined lipid unsaturation as \( \frac{A_J}{A_B + A_J} \) and calibrated this ratio against oils with known composition to obtain the average number of double bonds per fatty acid. Machann et al defined the simple ratios \( \frac{A_J}{A_A} \) and \( \frac{A_F}{A_A} \) as unsaturation and polyunsaturation indices, respectively. In bone marrow, where the presence of a strong water signal and broadening of the spectral peaks caused by a short T\(_2^*\) makes estimation of the olefinic peak J challenging, a surrogate UI was suggested by Johnson et al, defined as \( UIS = \frac{A_D + A_F}{A_A + A_D + A_F} \) and a corresponding saturation index \( SIS = 1 - UIS \).

### 3.1.3 Generic triglyceride model

Motivated by the difficulties to resolve the fat spectrum at clinical field strengths, an elegant model was developed by Hamilton et al, which describes a generic triglyceride by only three parameters: the number of double bonds (\( ndb \)), the number of methylene-interrupted double bonds (\( nmidb \)) and the average chain length (\( cl \)) of the fatty acids. Using this model, it is possible to express the relative amplitude of each fat peak in terms of the \( ndb \), \( nmidb \), and \( cl \) by counting the number of hydrogen protons in each position (see Table 2).

### Table 1 Measures of the FAC in human white adipose tissue, as calculated from Hodson et al\(^{12} \)

| Fatty acid | Relative abundance (mol %) |
|-----------|---------------------------|
| 14:0      | 2.8                       |
| 16:0      | 21.5                      |
| 16:1 n−7  | 7.2                       |
| 18:0      | 3.4                       |
| 18:1 n−9  | 43.5                      |
| 18:2 n−6  | 13.9                      |
| 18:3 n−3  | 0.8                       |
| 20:3 n−6  | 0.2                       |
| 20:4 n−6  | 0.3                       |
| 20:5 n−3  | 0                         |
| 22:4 n−6  | 0.1                       |
| 22:5 n−3  | 0.1                       |
| 22:6 n−3  | 0.1                       |

Unsaturation degree Fraction (%)

f\(_{SFA}\) 29.5
f\(_{MUFA}\) 54.0
f\(_{PUFA}\) 16.5

Descriptive measure # per triglyceride

\( ndb \) 2.8
\( nmidb \) 0.6
\( cl \) 17.3

Note: Whereas all three measures, that is, relative abundances, unsaturation degrees, and descriptive measures, can be assessed using gas chromatography, estimation of relative abundances are not typically available using \( ^1H \) MR techniques.

### Table 2 Peak assignments, frequencies, white adipose tissue relaxation times at 3T,\(^29 \) and theoretical amplitudes expressed in terms of the descriptive measures \( ndb \), \( nmidb \), and \( cl \)^\(^{28} \)

| Peak | Chemical shift (ppm) | Type | Proton position | Theoretical amplitude | T\(_1\) (ms) | T\(_2\) (ms) |
|------|----------------------|------|-----------------|-----------------------|-------------|-------------|
| A    | 0.90                 | Methyl | -CH\(_2\)-CH\(_3\) | 9                      | 543         | 80          |
| B    | 1.30                 | Methylenec | -(CH\(_2\))\(_2\)- | 6(\( cl-4\)-8\( ndb\)+2\( nmidb\)) | 280         | 55\(^a\)    |
| C    | 1.59                 | \( \beta \)-Carboxyl | -CH\(_2\)-CH\(_2\)-COO | 6                      | 240         | 55\(^a\)    |
| D    | 2.03                 | \( \alpha \)-Olefin | -CH\(_2\)-CH=CH- | 4(\( ndb\)-\( nmidb\)) | 249         | 52\(^b\)    |
| E    | 2.25                 | \( \alpha \)-Carboxyl | -CH\(_2\)-CH\(_2\)-COO | 6                      | 202         | 52\(^b\)    |
| F    | 2.77                 | Diacyl | -CH=CH-CH\(_2\)-CH=CH- | 2\( nmidb\) | 284         | 46          |
| G    | 4.10                 | Glycerol | -CH\(_2\)-O-CO | 2                      | 154         | -\(^c\)     |
| H    | 4.30                 | Glycerol | -CH\(_2\)-O-CO | 2                      | 154         | -\(^c\)     |
| I    | 5.21                 | Glycerol | -CH-O-CO- | 1                      | -\(^d\)     | -\(^d\)     |
| J    | 5.31                 | Olefin | -CH=CH- | 2\( ndb\) | 421         | 44          |

Note: The hydrogen atoms of interest are written in bold text.

\(^a\)Estimated as one peak.

\(^b\)Estimated as one peak.

\(^c\)Not available due to \( J \)-coupling effects.

\(^d\)Not available due to overlap with olefinic peak J.
to $f_{\text{SFA}}$, $f_{\text{MUFA}}$, and $f_{\text{PUFA}}$ according to the following set of equations:

$$f_{\text{SFA}} = 1 - \frac{ndb - nmidb}{3}$$
(1)

$$f_{\text{MUFA}} = \frac{ndb - 2nmidb}{3} + f_{\text{TnUFA}}$$
(2)

$$f_{\text{PUFA}} = \frac{nmidb}{3} - f_{\text{TnUFA}}$$
(3)

The fraction of triunsaturated fatty acids, $f_{\text{TnUFA}}$, can either be approximated with a fixed value of 2%$^{18}$ or be ignored by setting $f_{\text{TnUFA}} = 0$.$^{17}$

### 3.2 Acquisition protocol

The MR spectra needed for FAC estimation may be acquired by either single-voxel (SVS) or multi-voxel methods, of which the former, and in some implementations the latter, may be further separated into spin echo (PRESS) or stimulated echo (STEAM) signals depending on whether a (90°-180°-180°) or a (90°-120°-120°) excitation scheme is used for excitation of the volume of interest. A third class of methods, MR spectroscopic imaging (MRSI)$^{31}$ will not be further discussed in this review.

The choice between STEAM or PRESS has an impact on FAC estimation accuracy. Although the STEAM signal is half of that from the PRESS sequence, it is nevertheless preferred for an accurate quantification of the peak areas of a triglyceride spectrum because it is less affected by the J-couplings of the lipid protons.$^{32-34}$ It is, however, possible to design the PRESS sequence in a way that minimizes the effects of J-couplings.$^{33,35}$ A further advantage of STEAM is that the minimum TE is shorter than in PRESS. By optimizing both the radiofrequency (RF)-pulse shapes and the spoiler gradients, Gajdosik et al were able to reduce the minimum echo time (TE) of a STEAM sequence to 6 ms.$^{36}$ Furthermore, the PRESS sequence may be more affected by the chemical shift displacement, causing an underestimation of off-resonance frequency components which are not subjected to all three localization pulses.$^{37}$

Different resonances in the fat spectrum have different $T_1$ and $T_2$ relaxation times (Table 2). For FAC measurements, the different $T_1$ relaxation times do not represent a problem because the TR of an MRS experiment is typically much longer than the longest $T_1$ of fat. However, different $T_2$ relaxation times may be problematic. For example, the unsaturation fraction $\frac{3}{4}A_D/A_A$, as defined by Ye et al$^{25}$ is underestimated by 20% with TE = 30 ms if a $T_2$ correction is not performed, but only by 7% if TE = 10 ms. While the importance of $T_2$ correction for measurements of PDFF has been demonstrated,$^{38}$ no consensus appears to be established for FAC measurements, cf. Table 3. In any case, $T_2$ corrections should not be performed with the PRESS sequence and variable TEs, since J-couplings cause a more rapid signal decay at longer TE and, therefore, leads to an underestimated $T_2$.$^{33}$

The olefinic signal (peak J) needed for estimation of the UI may be difficult to resolve when the water signal is strong or when short $T_2$ causes substantial line broadening. As a remedy, water suppression.$^{39}$ or acquisition with sufficiently long TE (TE = 200 ms) to remove the water signal$^{40,41}$ has been suggested. Drawbacks of these techniques are that the quality of water suppression can vary and result in poor quality of the olefinic peak,$^{7}$ and the use of a long-TE PRESS technique requires calibration against known fatty acid compositions for absolute estimation of unsaturation.$^{27}$ Alternatively, a strong diffusion gradient has been suggested as a superior means of suppressing the water peak.$^{34}$

Typically, the data needed for MRS-based fatty acid composition assessment may be acquired within a few minutes per voxel.

### 3.3 Analysis of MRS data

To compute the various measures of unsaturation described in previous sections, the amplitudes of the individual resonances are calculated by fitting lineshape functions to the data, either in the time domain or in the frequency domain.$^{42}$ Commonly used algorithms are AMARES in the time domain$^{43}$ and LCModel in the frequency domain.$^{44}$ Time-domain fitting has the advantage of not requiring preprocessing of the data (apodization, phase correction, baseline correction, etc.); however, more model parameters are needed and time-domain analysis often fails at low signal-to-noise ratio (SNR).$^{45}$

Typically, Lorentzian or Gaussian functions are fitted to the data, but a Voigt lineshape, which is a convolution of Lorentzian and Gaussian lineshapes, can often improve the fit.$^{46}$ especially in bone marrow where imperfect shim and susceptibility variations are common.$^{47}$

Hamilton et al fitted, in a second step, the general tri-glyceride model in Table 2 to the measured peak areas to obtain values for $ndb$, $nmidb$, and $cl$.$^{25}$ Alternatively, the triglyceride model can be directly fitted to the MRS data without prior calculation of individual peak areas, similar to the MRI-based reconstruction. Nemeth et al made a comprehensive comparison of both approaches and found the direct fit superior in terms of robustness and repeatability.$^{48}$ They also concluded that additional robustness can be gained by constraining the relationship between $ndb$, $nmidb$, and $cl$, however, at the expense of potentially biased results.

### 3.4 Validation and performance evaluation

Several studies have been performed to validate the MRS method for assessment of fatty acid composition against GC reference measurements. At a 1.5T scanner, Lundbom
| Location       | Subjects                                      | Unsaturation index | Saturation index | Polyunsaturation index | Year | Method                          | T₂ correction | Reference |
|---------------|-----------------------------------------------|--------------------|------------------|------------------------|------|---------------------------------|---------------|----------|
| **Bone marrow** |                                              |                    |                  |                        |      |                                 |               |          |
| Femur         | Control, female                               | 0.063 ± 0.02       | –                | –                      | 2014 | PRESS, TE = 30 ms               | No            | 69       |
| Femur         | Adolescent girls with anorexia nervosa        | 0.0041 ± 0.0010 (TE = 30 ms) | –          | –                      | 2017 | PRESS, TE = 30 & 60 ms         | No            | 70       |
| Femur         | Young controls                                | 0.086 ± 0.015      | –                | –                      | 2019 | PRESS, TE = 200 ms             | No            | 41       |
| Tibia         | Healthy volunteers                            | 0.709 ± 0.035      | 0.291 ± 0.035    | 0.245 ± 0.031          | 2008 | STEAM, TE = 20 ms, 7T           | Yes           | 17       |
| Vertebrae, femur | Obese, non-diabetic                          | 0.07 ± 0.02        | –                | –                      | 2017 | PRESS, TE = 30 ms               | No            | 39       |
| Vertebrae     | Control, female                               | 0.127 ± 0.031      | –                | –                      | 2005 | PRESS, TE = 25 ms              | No            | 7        |
| Vertebrae     | Control, female                               | 0.079 ± 0.016      | –                | –                      | 2012 | PRESS, TE = 37 ms              | No            | 10       |
| Vertebrae     | Control, female                               | 0.074 ± 0.005      | 0.753 ± 0.011    |                        | 2013 | PRESS, TE = 37 ms              | No            | 68       |
| Vertebrae     | Elderly, age >80                              | 0.044 ± 0.005      | –                | –                      | 2018 | PRESS, TE = 190 ms             | No            | 71       |
| **Liver**     |                                              |                    |                  |                        |      |                                 |               |          |
|               | Male, lean                                    | –                  | 0.82 ± 0.024     | 0.12 ± 0.022           | 2008 | PRESS, TE = 30 ms               | No            | 9        |
| NAFLD patients| 0.53 ($f_{UFA}$)                              | 0.47 ($f_{SFA}$)   | 0.11 ($f_{PUFA}$) |                        | 2011 | STEAM, TE = 10-30 ms           | Yes           | 28       |
| NAFLD patients| 0.81 double bonds/FA (TE = 200 ms)           | –                  | 0.327 ± 0.098 (TE = 135 ms) |                | 2011 | PRESS, TE = 135 & 200 ms       | No            | 27       |
| NAFLD patients| 0.035 ± 0.004                                 | 0.96 ± 0.004       | 0.005 ± 0.0006   |                        | 2013 | PRESS, TE = 30 ms              | No            | 76       |
| Healthy volunteers |                                           | 0.066 ± 0.040      | –                | –                      | 2015 | STEAM, TE = 6 ms, 7T           | Yes           | 36       |
| Controls      | 0.061 ± 0.037                                 | 0.94 ± 0.04        | 0.0043 ± 0.0055  |                        | 2019 | PRESS, TE = 30 ms              | No            | 74       |
| NASH patients | 0.6 ± 0.3                                     | –                  | –                |                        | 2019 | PRESS, TE = 35 ms              | Yes           | 78       |
| **Adipose tissue** |                                             |                    |                  |                        |      |                                 |               |          |
| Breast        | Healthy young females                         | 0.713 ± 0.084      | 0.287 ± 0.084    | 0.227 ± 0.031          | 2012 | STEAM, TE = 24 ms, 7T          | Yes           | 83       |
| SCAT          | Healthy volunteers                            | 0.729 ± 0.042      | 0.271 ± 0.042    | 0.234 ± 0.039          | 2008 | STEAM, TE = 20 ms, 7T          | Yes           | 17       |
| Location | Subjects | Unsaturation index | Saturation index | Polyunsaturation index | Year | Method | T₂ correction | Reference |
|----------|----------|--------------------|------------------|------------------------|------|--------|--------------|-----------|
| VAT      | Male volunteers | 0.536 ± 0.061 | – | 0.104 ± 0.015 | 2013 | STEAM, TE = 20 ms | No | 80 |
| VAT      | Obese and lean volunteers | 0.083 | – | 0.0006 | 2014 | STEAM, TE = 8.9 ms | No | 81 |
| SCAT and VAT | NAFLD patients | 0.86 double bonds/FA (TE = 200 ms) | – | 0.376 ± 0.043 (TE = 135 ms) | 2011 | PRESS, TE = 135 & 200 ms | No | 27 |
| SCAT and VAT | NAFLD and obese subjects | 0.69 (SCAT), 0.68 (VAT) | 0.31 (SCAT), 0.32 (VAT) | 0.25 (SCAT), 0.23 (VAT) | 2017 | STEAM, TE = 10 ms | Yes | 30 |
| SCAT and VAT | Obese and lean volunteers | 0.59 (SCAT), 0.52 (VAT) | – | 0.11 (SCAT), 0.10 (VAT) | 2017 | STEAM, TE = 20 ms | No | 94 |
| SCAT and VAT† | Male volunteers, sedentary | 0.63 (SCAT), 0.48 (VAT) | 0.37 (SCAT), 0.52 (VAT) | 0.19 (SCAT), 0.11 (VAT) | 2019 | STEAM, TE = 14 ms | Yes | 51 |
| SCAT† | Lymphedema patients (healthy leg) | 0.96 ± 0.05 (f_{UFA}) | 0.04 ± 0.04 (f_{SFA}) | 0.31 ± 0.06 (f_{PUFA}) | 2020 | STEAM, TE = 20 ms | Yes | 58 |

Abbreviations: SCAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.
†Reference includes both MRI and MRS results and appears in both Tables 3 and 4.
et al found a fair agreement of the relative contents of di- allylic and olefinic protons in oil samples using short-TE PRESS but concluded in another in vivo study using long-TE PRESS, that estimations of absolute MUFA, PUFA and SFA content was not possible. In a comprehensive study at 3T, Ruschke et al investigated how the TE and mixing time (TM) of PRESS and STEAM sequences affected the olefinic to methylene ratio ($A_J/A_B$). The conclusions were that a STEAM acquisition with TE and TM lower than 45 ms produced an excellent agreement with GC (correlation close to 1, slope close to 1 and intercept close to 0) and was superior to PRESS (Figure 2). In the presence of a strong water signal, STEAM combined with a strong diffusion gradient ($b$-value 1800 s/mm$^2$) was a superior method for water suppression compared to long-TE PRESS (TE = 200 ms). In a recent study with in vivo MRS and GC of biopsy samples of subcutaneous fat, Nemeth et al calculated $ndb$ and $nmidb$ and found a fair correlation between MRS and GC. MRS overestimated both $ndb$ (bias = 0.24, $y = 0.31x + 1.47$) and $nmidb$ (bias = 0.19, $y = 0.36x + 0.19$).

4 | MRI-BASED TECHNIQUES

4.1 | General signal model

Using Dixon and CSE-MRI-based techniques the signals from fat and water may be sorted into separate images. While the original implementation by Dixon only used two images, one in-phase and the other opposed-phase, modern techniques fit a signal model to chemical shift-encoded data, for example, multiple gradient echo images with different, and in general arbitrary, TEs, that captures the phase oscillations between the fat and water signals. If the signal model includes multiple fat peaks (with amplitudes $\alpha_m$ and frequencies $f_m$ relative to the water frequency), $B_0$ frequency offset $\psi$ and $T_2^*$ dephasing, and relaxation effects are either avoided or corrected for, the separated fat and water signals may be used for quantitative estimation of the PDFF.

$$S(t) = \left( W + F \sum_{m=A,B,C...} \alpha_m E_m'(t) \right) e^{2\pi \psi} e^{-\frac{2\pi}{T_2^*} E_m(t)} = e^{i2\pi f_m} \quad (4)$$
Within a short time period, three similar imaging-based methods were suggested for FAC quantification, by reformulations of the signal equation (Equation 4). Although they differed in exact implementation, they all based their method on chemical-shift encoding through multi-echo gradient echo images and used the generic triglyceride model suggested by Hamilton et al as described for MRS above. Using the theoretical amplitudes (see Table 2) each of \( \text{ndb}, \text{nmidb}, \text{and cl} \), as well as a normalization factor \( f \), may be separated using a similar reconstruction algorithm as for fat/water separation (see Equations 4 and 5), including correction of \( B_0 \) inhomogeneities and \( T^* \) dephasing in a joint step. From the \( \text{ndb} \) and \( \text{nmidb} \), the \( f_{\text{SFA}}, f_{\text{MUFA}}, \) and \( f_{\text{PUFA}} \) may be calculated from Equations (1-3) (see Figure 3).

\[
\sum_{m=A,B,C,} \alpha_m E_m(t) = A_1 \cdot f + A_2 \cdot f \cdot \text{ndb} + A_3 \cdot f \cdot \text{nmidb} + A_4 \cdot f \cdot \text{cl} \\
A_1 = 9E_A(t) - 24E_B(t) + 6E_C(t) + 6E_D(t) + 2E_E(t) + 2E_F(t) + E_I(t) \\
A_2 = -8E_B(t) + 4E_D(t) + 2E_F(t) \\
A_3 = 2E_B(t) - 4E_D(t) + 2E_F(t) \\
A_4 = 6E_B(t)
\]

### 4.2.1 Constrained signal models

To further reduce the number of estimates, Bydder et al suggested that the \( \text{nmidb} \) and \( \text{cl} \) could be expressed in terms of the \( \text{ndb} \) using empirical relations based on standard reference FAC values of vegetable oils and literature values of three human adipose tissue depots. However, as the \( \text{nmidb} \) is expressed as a quadratic function of \( \text{ndb} \), this approach is not compatible with the iterative least-squares method most often used for fat/water separation, but requires an alternative curve-fitting technique. Alternatively, linear expressions may be valid in limited ranges of FAC parameters, as those recently derived from GC data of human subcutaneous adipose tissue by Trinh et al. Using these models to constrain the reconstruction to estimation of \( \text{ndb} \) only, an improved image quality of the resulting \( f_{\text{SFA}}, f_{\text{MUFA}}, \) and \( f_{\text{PUFA}} \) maps was obtained, at the cost of a substantially lower accuracy of the \( f_{\text{MUFA}} \) parameter. The results of this study indicate that a free estimation of \( \text{ndb} \) and \( \text{nmidb} \) may be necessary for a reliable distinction between \( f_{\text{MUFA}} \) and \( f_{\text{PUFA}} \).

### 4.2.2 Joint or sequential algorithm

As opposed to the initially presented joint reconstruction approaches where all parameters are estimated simultaneously, later refinements of imaging-based FAC estimation have also suggested sequential approaches for improved robustness. Using this approach, the off-resonance frequency, \( T^* \), and phase errors associated with a bipolar acquisition are estimated in a separate first step. Estimation of \( \text{ndb} \) in a second step and finally estimation of \( \text{nmidb} \) in a third may then be performed using the real-valued signal, as opposed to the complex signal, to reduce noise effects. Also, denoising algorithms have been suggested for improved precision.

### 4.3 Acquisition protocol and field strength

The design of the echo train regarding the number of echoes, inter-echo time, and total readout time has a crucial impact on
the noise performance of FAC estimation, and the number of echoes and the total read-out time of the echo train has an impact on estimation accuracy. Although the stability of the method has been demonstrated to increase with the number of echoes, inaccuracies of the model have an increasing impact on estimation accuracy with increasing total read-out time.

At 3T, a maximum inter-echo time of 1.8 ms and a minimum total readout time of 15 ms (corresponding to nine echoes) has been suggested by Berglund et al for a joint separation of four free FAC parameters. Note that the method by Berglund et al separates an alternative set of four FAC parameters as opposed to the more commonly used ndb, nmidb, and cl. However, the optimal acquisition parameters are likely similar. In another study using 3T, a maximum inter-echo time of 1.23 ms and a readout time of 14-18 ms (corresponding to 11-15 echoes) was suggested for a sequential reconstruction of ndb and nmidb with cl expressed in terms of ndb and with correction of bipolar phase errors by Schneider et al.

Imaging-based FAC estimation has been compared between 1.5T and 3T, and the feasibility also at 7T has been demonstrated, using a preclinical system. In oil phantoms, a bias was found between the estimates at 1.5T and 3T, and a lower reproducibility was demonstrated between the oil estimates at 1.5T and 3T, a bias was found between the estimates at 1.5T and 3T. At 3T, a maximum inter-echo time of 1.8 ms and a minimum total readout time of 15 ms (corresponding to nine echoes) has been suggested by Berglund et al for a joint separation of four free FAC parameters. Note that the method by Berglund et al separates an alternative set of four FAC parameters as opposed to the more commonly used ndb, nmidb, and cl. However, the optimal acquisition parameters are likely similar. In another study using 3T, a maximum inter-echo time of 1.23 ms and a readout time of 14-18 ms (corresponding to 11-15 echoes) was suggested for a sequential reconstruction of ndb and nmidb with cl expressed in terms of ndb and with correction of bipolar phase errors by Schneider et al.

Imaging-based FAC estimation has been compared between 1.5T and 3T, and the feasibility also at 7T has been demonstrated, using a preclinical system. In oil phantoms, a bias was found between the estimates at 1.5T and 3T, and a lower reproducibility was demonstrated between the oil estimates at 1.5T and 3T. Apart from the lower spectral resolution, an increased impact of J-coupling is also expected at the lower field strength which may motivate the choice of 3T over 1.5T. However, the increased frequency differences also reduces the suitable range of inter-echo times as described above, potentially making high resolution imaging more difficult at a higher field strength and possibly making the use of a bipolar readout necessary. The use of a bipolar readout introduces additional phase errors of the acquired images with a detrimental impact on fat/water separation if not corrected. For FAC estimation, correction of these phase errors has been suggested as the first part of sequential reconstruction approaches.

The data acquisition time for imaging-based FAC assessment is typically a few minutes, but may also be limited to a single breath-hold for liver applications.

### 4.4 Potential sources of bias

There are several potential sources of bias in FAC estimation, and likely any inaccuracy of the used model will impact the results. Thus, relaxation effects, J-coupling, or inaccuracies of the fat model, such as fat shift alterations due to temperature or bulk susceptibility, are all plausible causes of bias. In addition, several papers have demonstrated a positional artifactual gradient of the estimated FAC parameters in the frequency encoding direction, and speculated that it may be caused by anti-aliasing filters, asymmetric frequency response of the coil, or in case of a bipolar acquisition scheme, residual phase errors. It has been suggested that the artifact may be reduced using a constrained signal model, but additional studies are needed. Furthermore, a high sensitivity to motion has been reported.

For FF quantification, differences in relaxation times, especially T1, between fat and water have been demonstrated as causes of bias. As there are differences in relaxation values also between individual fat peaks, the potential relaxation bias on FAC parameters is more difficult to predict. The results of a simulation study indicate that the estimated ndb is largely unaffected by T1-related bias, and may be biased by differences in T2 in fat/water mixtures, but not in pure fat. Using a priori knowledge of the T2 values of individual fat and water peaks, T2 correction is possible in combination with voxel-by-voxel estimation of T2.

### 4.5 Validation and performance evaluation

Several publications have investigated the performance of imaging-based FAC estimation using both phantoms and in vivo experiments. High in vitro reproducibility has been demonstrated, with accuracy comparable to that of MRS. Although the method, in theory, allows for simultaneous estimation of fat content and FAC also in the presence of water signal, the accuracy and robustness of the technique was found to be lower in water/fat mixtures in vitro. Example phantom images of a range of vegetable oils are shown as Figure 4.

While absolute in vivo quantification of the fatty acid composition is challenging also with MRS, it is nevertheless commonly used as a reference for the MRI-based methods, because of the practical difficulties to use GC as a reference. Promising agreement with MRS results has been found in adipose tissue and bone marrow, but one group did note a large intra-subject variability and commented on the need for masking of extreme values. Most recently, Martel et al measured unsaturated and saturated lipid fractions in the proximal femur of osteoporosis patients with MRI and MRS, with a good correlation between the methods (r ~ 0.98) and a small bias (~2 percentage points). Another recent study by Nemeth et al, in which visceral and subcutaneous fat of volunteers were investigated, also found fair agreement between MRS and MRI (ndb: r = 0.82, bias = 0.06, nmidb: r = 0.82, bias = 0.02). However, both methods overestimated ndb and nmidb compared to GC (bias 0.24 and 0.19, respectively), similar to previous in vitro and in vivo validation studies. In a recent investigation of the subcutaneous fat composition in lymphedema patients using both MRI and MRS with GC as a reference, Trinh et al found that both the accuracy and the precision was better with MRI than with MRS. For fMUFA and especially fSFA, the MRI method was in good agreement with GC, while
$f_{PUFA}$ was underestimated. The correlation between the GC and MRI methods was strong for all three parameters.\textsuperscript{58}

Out of the three descriptive parameters, $ndb$ appears to be the most reliant as the estimates of $nmidb$ and especially $cl$ have been reported to be less accurate and robust.\textsuperscript{20,22,59} Similarly, the test-retest reliability of $f_{SFA}$ was found “good” to “excellent”, but only “moderate” to “good” of the $f_{MUFA}$ and $f_{PUFA}$ estimates,\textsuperscript{60} which can be explained by $f_{MUFA}$ and $f_{PUFA}$ being derived from $nmidb$ (cf. Equations 2-3).

5 | FEASIBLE APPLICATIONS

The main targets for reports on the use of both MRI and MRS to quantify the fatty acid composition \textit{in vivo} have been adipose tissue (subcutaneous and visceral), bone marrow, and liver. A survey of studies investigating the FAC in these organs using MRS are shown in Table 3, where a large variation in the reported values is evident. This could be explained by the different definitions of saturation, unsaturation, and polyunsaturation indices used by the cited studies. A corresponding summary of studies using MRI is presented in Table 4.

5.1 | Bone marrow

Bone marrow is a challenging tissue due to its rapid signal decay and an often strong water signal.\textsuperscript{21} Nonetheless, both MRI and MRS has been used to investigate an association between the bone marrow FAC and for example, bone quality. The FAC of the bone marrow in the femoral head has also been
| Location               | Subjects                              | $F_{SFA}$ (%) | $F_{MUFA}$ (%) | $F_{PUFA}$ (%) | ndb    | nmidb   | Year      | Method                  | Reference |
|------------------------|---------------------------------------|---------------|----------------|----------------|--------|---------|-----------|-------------------------|-----------|
| Bone marrow            |                                       |               |                |                |        |         |           |                         |           |
| Femoral head           | Premenopausal women                   | 50.7 ± 7.3*   | 35.2 ± 3.2*    | 14.1 ± 5.5*    | –      | –       | 2018      | 3T, 12 echoes, $TE = 1.2-14.4\text{ ms}$. Restricted cl. | 66        |
| Femoral head           | Osteoporotic                          | 48.8 ± 3.2*   | 37.3 ± 1.8*    | 13.8 ± 2.7*    | –      | –       | 2018      | 3T, 12 echoes, $TE = 1.2-14.4\text{ ms}$. Restricted cl. | 67        |
| Liver                  |                                       |               |                |                |        |         |           |                         |           |
| Liver                  | Male, either obese or with suspected steatosis | 57 ± 9.5      | 26 ± 14.2      | 17 ± 6.8       | 1.80 ± 0.25 | 0.51 ± 0.21 | 2014      | 3T, 8 echoes, $TE = 1.15-9.2\text{ ms}$. Restricted cl. | 59        |
| Simple steatosis       |                                       | 44 ± 4        | 36 ± 1         | 19 ± 3         | –      | –       | 2017      | 3T, 8 echoes, $TE = 1.15-9.2\text{ ms}$. Restricted cl. | 6         |
| Adipose tissue         |                                       |               |                |                |        |         |           |                         |           |
| SCAT and VAT           | Men, either obese or with suspected steatosis | 41 ± 6.9 (SCAT) 43 ± 2.9 (VAT) | 28 ± 6.6 (SCAT) 29 ± 4.1 (VAT) | 31 ± 5.4 (SCAT) 28 ± 4.8 (VAT) | 2.72 ± 0.31 (SCAT) 2.53 ± 0.21 (VAT) | 0.94 ± 0.16 (SCAT) 0.84 ± 0.14 (VAT) | 2014      | 3T, 8 echoes, $TE = 1.15-9.2\text{ ms}$. Restricted cl. | 59        |
| SCAT and VAT           | Male, healthy                         | –             | –              | –              | –      | –       | 2018      | 3T, 8 echoes, $TE = 1.15-9.2\text{ ms}$. Restricted cl. | 48        |
| SCAT and VAT           | Male volunteers, sedentary            | 37 (SCAT) 42 (VAT) | 44 (SCAT) 43 (VAT) | 19 (SCAT) 15 (VAT) | 2.43 ± 0.04 (SCAT) 2.20 ± 0.03 (VAT) | 0.56 ± 0.02 (SCAT) 0.46 ± 0.02 (VAT) | 2019      | 3T, 8 echoes, $TE = 1.15-9.2\text{ ms}$ | 51        |
| VAT                    | Mice, low-fat diet                    | 24.8 ± 2.9    | 52.4 ± 1.8     | 22.8 ± 1.2     | 2.94 ± 0.12 | 0.69 ± 0.04 | 2016      | 7T, 16 echoes, bipolar gradients, $TE = 1.58-12.68\text{ ms}$. Restricted cl. | 62        |
| SCAT                   | Control                               | 30.0 ± 3.8    | 64.1 ± 3.5     | 5.9 ± 2.9      | 3.86 ± 0.24 | 1.016 ± 0.108 | 2020      | 3T, 12 echoes, $TE = 1.31-18.47\text{ ms}$. Restricted cl. | 58        |
| BAT                    | Non-obese subjects                   | –             | –              | –              | 3.05 ± 0.13 | –       | 2018      | 1.5T, 8 echoes, bipolar gradients, $TE = 2.3-18.4\text{ ms}$. Restricted nmidb and cl. | 85        |

Abbreviations: BAT, brown adipose tissue; SCAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

*Estimated from diagram.

†Reference includes both MRI and MRS results and appears in both Tables 3 and 4.
shown to be heterogeneous, thus, making use of the added possibilities of an imaging approach,66,67 (see Figure 5).

Yeung et al studied the vertebral marrow fat of postmenopausal women (mean age 70 y) and found that the UI was lower in osteoporotic (UI = 0.091) and osteopenic (UI = 0.097) subjects, compared with normal subjects (UI = 0.114) and young controls (mean age 28 years, UI = 0.127).7 Another study showed that diabetic patients with fragility fractures had significantly lower UI in vertebral bone marrow than controls.68 The UI may, therefore, be a biomarker of skeletal integrity.

The UI may also be a marker of metabolic risk. The vertebral bone marrow in postmenopausal women had lower UI in subjects with type 2 diabetes than in healthy controls,10 and in a study of morbidly obese patients, the UI of the femoral diaphysis was significantly lower in subjects with type 2 diabetes compared to non-diabetic subjects.39 In a study comparing women with anorexia nervosa against normal-weight controls, the degree of fat saturation in the femur was inversely related to the bone mineral density, suggesting that saturated fat may have a more negative effect on bone.69 However, a later study of girls with anorexia nervosa did not find a statistically significant association between bone mineral density and UI.70 In a study on obese and non-obese premenopausal women, no difference between the groups was found in the UI of vertebral marrow fat. However, an inverse association was found between the UI and the amount of visceral fat.71 Lundbom et al performed a study of red and yellow bone marrow in femur on young (age 20-31 y, female/male = 17/16) volunteers.41 The UI of red marrow was significantly lower for women than for men (7.8% vs. 9.9%), but did not differ in yellow marrow. The water content in red marrow was significantly higher for the women (44% vs. 34%), indicating a higher relative red marrow fraction in women. In both men and women, the fat UI was inversely associated with the red marrow water content. A lower UI in women was also found by Xu et al who investigated the vertebral marrow of an elderly population (age >80 y). The UI was 3.3% for women and 4.2% for men.47

5.2 | Liver

MRS measurements of the fatty acid composition in the liver are more difficult than in adipose tissue or bone marrow, due to motion and a prominent water signal. Especially the latter factor affect the MRI-techniques as well and previous attempts for FAC quantification were not considered sufficiently reliable in cases of <15% fatty infiltration of the liver.6

In an MRS study by Johnson et al, significant increases of the saturation index and significant decreases of the polyUI (defined as $PUI = A_F / (A_A + A_B + A_D + A_F)$), were found in obese men with normal hepatic lipid levels and in obese men with hepatic steatosis, compared to lean controls.9 The PUI was furthermore significantly lower in the men with steatosis compared to the obese men without steatosis. These results were in line with previous studies using biopsy and subsequent gas-liquid chromatography,72,73 and additionally showed that obesity and steatosis are independently associated with alterations of hepatic FAC.

Hamilton et al used MRS to investigate 121 subjects with biopsy-proven non-alcoholic fatty liver disease (NAFLD) or at risk for NAFLD.28 The average lipid composition across all subjects was $ndb = 1.32$, $nmidb = 0.32$, and $el = 17.45$. In another recent study on NAFLD patients, saturation, unsaturation, and polyunsaturation indices according to Johnson et al9 were assessed.74 The saturation index was significantly higher in the NAFLD group compared to the controls, while the UI was significantly lower. The polyunsaturation index did not differ between the groups, but was correlated to the maximal oxygen consumption ($VO_{2max}$). In an MRI study of patients with more severe fatty infiltration of the liver, a slightly higher SFA was found in patients with non-alcoholic steatohepatitis compared with patients with simple steatosis.6

Comparing hepatic lipids to adipose tissue, Lundbom et al used PRESS MRS with long TE to estimate the number of double bonds per fatty acid.27 In subjects with elevated hepatic fat (>5%), the number of double bonds per fatty acid was 0.81 in liver fat, significantly lower than 0.86 in both subcutaneous and visceral fat, possibly due to differences in the de novo lipogenesis.75 In addition, a strong correlation ($r = 0.84$) was found between the unsaturation of liver fat and subcutaneous fat. The number of double bonds reported by Lundbom et al are much larger than the values obtained by Hamilton et al,28 since the latter values count double bonds per triglyceride as opposed to per fatty acid.
MRS has also been used to monitor FAC changes of the liver in several intervention studies, investigating the effect of exercise or changes in diet and lifestyle. In a study on NAFLD patients with hepatic fat >5%, PRESS MRS was used to investigate whether short-term physical exercise would affect hepatic lipid saturation, insulin resistance, and markers of inflammation and oxidative stress. The subjects were examined before and after a 7-d training period and indices of lipid saturation, unsaturation, and polyunsaturation were calculated according to Johnson et al.9 The training improved maximal oxygen consumption (VO2max) but did not affect body weight or adiposity. The saturation and unsaturation indices also did not change, but the polyunsaturation index increased significantly from 0.47% to 0.60%. The polyunsaturation index was positively associated with an increase of plasma high molecular weight adiponectin. Low levels of this protein is a marker of increased risk for metabolic syndrome, hypertonia, and type 2 diabetes.

Deibert et al studied the effect of either meal replacements (one meal per day replaced by a soy-yoghurt preparation) or complete lifestyle change (including nutrition and physical exercise) during 24 wk on a group of NASH patients. The fraction of unsaturated lipids, fUL, was calculated as suggested by Ye et al, by normalizing with the methyl signal. At baseline, the fUL was 0.6 ± 0.3. Both interventions significantly reduced the amount of hepatic fat, but neither intervention significantly reduced the fUL.

5.3 Adipose tissue

Characterization of the lipid composition in adipose tissue is easier than in bone marrow or the liver, because there is no strong water signal interfering with the olefinic peak, which can be resolved in MR spectra without water suppression. Ren et al investigated the fat composition of adipose tissue at 7T and found a saturated fraction of 27% subcutaneously and 29% in tibial marrow, well in agreement with literature values. The monounsaturated fraction was, however, underestimated compared to values obtained in biopsy studies.

Several studies have focused on the role of the FAC of adipose tissue in obesity and metabolic disorders. In a study of volume and composition of visceral fat in volunteers, a strong negative correlation (r = −0.92) was found between the unsaturation and the amount of visceral fat. Another study which investigated visceral and perirenal fat, did, however, not find a significant overall difference in unsaturation ratio between obese and non-obese volunteers, but did find a significantly higher polyunsaturation ratio in the omental fat of the obese subjects. Hamilton et al investigated visceral and subcutaneous fat in 340 subjects with proven or suspected NAFLD, or with obesity. The visceral fat was more saturated (fewer double bonds per triglyceride) than subcutaneous fat, and deep subcutaneous fat was more saturated than superficial. The latter finding was in agreement with a study by Lundbom et al, albeit with different values, potentially due to different acquisition strategies.

Comparing the FAC of various adipose tissue depots, Machann et al measured the composition of fat at six different locations (subcutaneous fat in the neck, visceral, and subcutaneous (deep and superficial) in the abdomen, subcutaneous and marrow fat in the lower leg) in lean and obese subjects. The UI was highest in calf subcutaneous fat, and lowest in tibial bone marrow. There was a trend towards higher UI for male subjects, in agreement with studies of bone marrow, but no association with age or BMI. Furthermore, the UI of bone marrow was found to correlate negatively with insulin sensitivity (Matsuda index), and the UI of visceral fat correlated negatively with the volume of the visceral fat. In breast adipose tissue of healthy volunteers, Dimitrov et al measured a fat composition in good agreement with the composition of subcutaneous fat in the calf, as measured in an earlier study by Ren et al.17

MRI techniques have been used to measure the FAC in adipose tissue in diet intervention studies. In mice, a high-fat diet resulted in significant changes of the visceral adipose tissue fSFA, fMUFA, and fPUFA compared to a fructose diet in mice. Similarly, an overfeeding protocol in non-obese human subjects led to especially fMUFA alterations of the subcutaneous and visceral adipose tissue depots, which could be detected using MRI, but not MRS, due to a larger inter-subject variability of the latter technique. Yet, another interesting MRI application is research on brown adipose tissue, in which the ndb was different between obese and non-obese young men, and decreased as a response to cold.

Furthermore, an MRI approach was used to compare the edematous and healthy subcutaneous adipose tissues in a subject group suffering from lymphedema in one of their lower limbs. Similar to the GC results for the same subject group, the MRI approach was able to detect a significantly higher proportion of SFA and lower fraction of MUFA in the edematous tissue, whereas the results for PUFA were contradictory between the two methods.

5.4 Cancer

MRS has also been used for investigation of relations between body fat composition and risk of cancer, as lipid metabolism often can become dysregulated during tumor development. Ex vivo measurements of excised adipose tissues have indicated elevated levels of monounsaturated lipids near colorectal and prostate tumors. In contrast, Thakur et al investigated the lipid profiles in vivo of 168 women with histopathologically confirmed breast lesions and found significantly lower concentrations of
unsaturated lipids in malignant versus benign tumors.\textsuperscript{8} However, the observations by Thakur et al were in agreement with a study by Freed et al, who found a significantly higher saturated fraction and lower monounsaturated fraction in invasive ductal carcinomas compared to benign lesions, in postmenopausal, but not in premenopausal, women.\textsuperscript{90} A few initial studies have also used imaging techniques to explore the FAC in soft tissue fat tumors in response to radiation therapy.\textsuperscript{91,92}

6 | DISCUSSION

The interest in the FAC in health and various disease scenarios has extended beyond the subcutaneous adipose tissue to applications also deeper within the body. Thus, a non-invasive alternative to the gold standard GC of biopsy samples would simplify and improve the accessibility of future clinical research studies. Although measurement of the relative abundance of individual fatty acids is not feasible using neither MRI nor MRS, both enable assessment of quantitative fractions of various saturation degrees (e.g., $f_{SFA}$, $f_{MUFA}$, and $f_{PUFA}$) fully comparable to independent measures. Measures provided by both MRI and MRS, albeit often biased, have been shown to be associated with GC, and thus, have the potential to replace invasive biopsy exams. Compared with MRI, MRS may be able to provide more detail on the FAC at ultra-high field strength. Furthermore, MRI has the advantage of mapping the various FAC measures, which enables simultaneous measurements in several fat depots as well as detection of regional variations.

Over the past decades, numerous studies have used MRS to investigate the fatty acid composition in vivo, see Table 3. While the results tend to agree when equal acquisition techniques and definitions of saturation and unsaturation have been used, other studies arrive at very different results, which often are difficult to compare because of different FAC definitions. For MRS, one problem is that there does not yet exist a consensus about acquisition techniques (STEAM vs. PRESS, water suppression or not, etc.), spectrum analysis (time domain vs. frequency domain, lineshape to use) and how peak amplitudes should be translated to fatty acid saturation, unsaturation, and polyunsaturation. The lack of consensus in methodology is also true for the MRI techniques, and further method development and validation studies are needed before such a consensus can be reached. In addition to the presence of a strong water signal proving problematic also for the MRI-based methods, an artificial spatial gradient in the readout direction of the resulting FAC maps is an important challenge to overcome. With respect to the analysis of data, however, the MRI methods typically rely on variants of an iterative reconstruction, originally developed by Reeder et al for separation of water and fat,\textsuperscript{53} which do not involve interaction or decisions from the user, except for selection of a proper fat model. This is in contrast to MRS, where decisions regarding which peaks to analyze, lineshapes, apodization, baseline correction etc., must be made individually for each experiment. MRI therefore holds promise to be a more user-independent technique for FAC analysis than MRS.

Quantitative expressions for $f_{UFA}$, $f_{SFA}$, and $f_{PUFA}$ were suggested by Strobel et al, involving the $\alpha$-carbonyl (peak E) and diallylic (peak F) methylene signals.\textsuperscript{18} Since these peaks are often difficult to quantify at clinical field strengths (Strobel et al made their experiments at 7T), a variety of qualitative unsaturation indices have been proposed, many of those involving the olefinic methine signal (peak J), which can be difficult to resolve in case of substantial water signal. Also, qualitative indices are difficult to compare to both other MR measures and to GC.

As an alternative, the approach by Hamilton et al to express the triglyceride composition in terms of the quantitative $n_{db}$, $n_{midb}$, and $cl$ measures, is promising as the information from many peaks can be expressed by only three unknowns.\textsuperscript{28} In addition, the $n_{db}$ and $n_{midb}$ values can easily be converted to $f_{SFA}$, $f_{MUFA}$, and $f_{PUFA}$ fractions, which are directly comparable to GC measures of the FAC. The same set of measures are also commonly used in imaging approaches to map the FAC.\textsuperscript{22,59} However, although both $n_{db}/n_{midb}$ and $f_{SFA} / f_{MUFA} / f_{PUFA}$ are suitable quantities, there is currently no consensus on which set of measures to present in clinical studies, making direct comparisons impractical. As the $n_{db}$ and $n_{midb}$ measures were suggested specifically for MRI and MRS purposes, the saturation fractions may offer the more universally applicable alternative.

Further simplifications of the FAC model are tempting in the interest of improving the robustness of the MRI-based technique as well as its susceptibility to image artifacts. Thus, it has been suggested that $n_{db}$ alone can be used as a measure of the FAC\textsuperscript{21} or to estimate each of the FAC parameters using a sequential approach.\textsuperscript{59} To limit the number of unknowns, vegetable oil data have been used to empirically predict $cl$ and $n_{midb}$ from $n_{db}$,\textsuperscript{21} but the range of FAC parameters of vegetable oils is considerably larger than that expected in human tissue, and the degree of unsaturation is higher. Thus, models based on human subcutaneous adipose tissue may be more suitable.\textsuperscript{58} However, a recent study concludes that a free estimation of the $n_{db}$ and $n_{midb}$ may be necessary for accurate distinction between MUFA and PUFA.\textsuperscript{58} Due to the low inter-subject variability of the $cl$ this parameter is often fixed or restricted,\textsuperscript{21,58,59} likely without any substantial impact on estimation accuracy. In the interest of improving robustness and image quality while maintaining estimation accuracy, the optimal signal model and the appropriate choice of a joint or a sequential reconstruction approach needs to be investigated further. Another important subject of future investigations
is determining the methods’ robustness in terms of repeatability and reproducibility in multi-center trials, similar to those previously performed for PDFF quantification.\textsuperscript{93}

As to data acquisition for imaging-based FAC quantification, some recommendations may be suggested based on the reviewed literature. Using a multi-gradient echo sequence, the first TE should be kept as short as possible while some care should be taken in the choice of inter-echo time and number of acquired echoes, depending on field strength and fat model.\textsuperscript{20,60} In this interest, bipolar readout gradients, and correction of the associated phase errors, may be necessary at higher field strengths.\textsuperscript{59} Furthermore, SNR may be improved by the use of some T\textsubscript{1}-weighting if the PDFF will not be estimated from the same set of images.\textsuperscript{61}

### 7 | CONCLUSIONS

Both MRI and MRS provide a non-invasive alternative to the gold standard gas chromatography for in vivo measurements of the chemical composition of various fat depots in the human body. Although both MRI and MRS measures are associated with gas chromatography, future studies are warranted. For MRS, a consensus is needed on how spectra should be acquired and analyzed. Especially, the use of semi-quantitative indices should be avoided if possible and replaced by quantitative measures more directly comparable to the gold standard. For MRI, further investigations of the optimal signal model and reconstruction approaches are needed for improved robustness of the estimation of f\textsubscript{MUFA} and f\textsubscript{PUFA}, whereas f\textsubscript{SFA} may be reliably estimated using existing techniques. Furthermore, as in vivo experiences of the MRI-based approach are limited so far, more clinical studies using this technique are needed to fully explore its potential.

### ORCID

Pernilla Peterson https://orcid.org/0000-0002-7533-8503

Lena Trinh https://orcid.org/0000-0002-9843-5381

Sven Månsson https://orcid.org/0000-0002-5706-5142

### REFERENCES

1. Pond CM. Adipose tissue and the immune system. *Prostglandins Leukot Essent Fatty Acids*, 2005;73:17-30.

2. Wensveen FM, Valentic S, Sestan M, Wensveen TT, Polic B. Interactions between adipose tissue and the immune system in health and malnutrition. *Semin Immunol*. 2015;27:322-333.

3. Machann J, Stefan N, Wagner R, et al. Intra- and interindividual variability of fatty acid unsaturation in six different human adipose tissue compartments assessed by (1)H-MRS in vivo at 3 T. *NMR Biomed*. 2017;30:e3744.

4. Iggman D, Arnlov J, Vessby B, Cederholm T, Sjogren P, Riserus U. Adipose tissue fatty acids and insulin sensitivity in elderly men. *Diabetologia*. 2010;53:850-857.

5. Velan SS, Said N, Durst C, et al. Distinct patterns of fat metabolism in skeletal muscle of normal-weight, overweight, and obese humans. *Am J Physiol Regul Integr Comp Physiol*. 2008;295:R1060-R1065.

6. Leporq B, Lambert SA, Ronot M, Vilgrain V, Van Beers BE. Simultaneous MR quantification of hepatic fat content, fatty acid composition, transverse relaxation time and magnetic susceptibility for the diagnosis of non-alcoholic steatohepatitis. *NMR Biomed*. 2017;30:e3766.

7. Yeung DK, Griffith JF, Antonio GE, Lee FK, Woo J, Leung PC. Osteoporosis is associated with increased marrow fat content and decreased marrow fat unsaturation: A proton MR spectroscopy study. *J Magn Reson Imaging*. 2005;22:279-285.

8. Thakur SB, Horvat JV, Hancu I, et al. Quantitative in vivo proton MR spectroscopic assessment of lipid metabolism: Value for breast cancer diagnosis and prognosis. *J Magn Reson Imaging*. 2019;50:239-249.

9. Johnson NA, Walton DW, Sachinwalla T, et al. Noninvasive assessment of hepatic lipid composition: Advancing understanding and management of fatty liver disorders. *Hepatology*. 2008;47:1513-1523.

10. Baum T, Yap SP, Karampinos DC, et al. Does vertebral bone marrow fat content correlate with abdominal adipose tissue, lumbar spine bone mineral density, and blood biomarkers in women with type 2 diabetes mellitus? *J Magn Reson Imaging*. 2012;35:117-124.

11. Beynen AC, Hermus RJ, Hautvast JG. A mathematical relationship between the fatty acid composition of the diet and that of the adipose tissue in man. *Am J Clin Nutr*. 1980;33:81-85.

12. Hodson L, Skeaff CM, Fielding BA. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. *Prog Lipid Res*. 2008;47:348-380.

13. Hu HH, Li Y, Nagy TR, Goran MI, Nayak KS. Quantification of absolute fat mass by magnetic resonance imaging: A validation study against chemical analysis. *Int J Body Compos Res*. 2011;9:111-122.

14. Reeder SB, Cruite I, Hamilton G, Sirlin CB. Quantitative assessment of liver fat with magnetic resonance imaging and spectroscopy. *J Magn Reson Imaging*. 2011;34:729-749.

15. Beckmann N, Brocard JJ, Keller U, Seelig J. Relationship between the degree of unsaturation of dietary fatty acids and adipose tissue fatty acids assessed by natural-abundance 13C magnetic resonance spectroscopy in man. *Magn Reson Med*. 1992;27:97-106.

16. Wary C, Bloch G, Jehenson P, Carlier PG. C13 NMR spectroscopy of lipids: A simple method for absolute quantitation. *Anticancer Res*. 1996;16:1479-1484.

17. Ren J, Dimitrov I, Sherry AD, Malloy CR. Composition of adipose tissue and marrow fat in humans by 1H NMR at 7 Tesla. *J Lipid Res*. 2008;49:2055-2062.

18. Strobel K, van den Hoff J, Pietzsch J. Localized proton magnetic resonance spectroscopy of lipids in adipose tissue at high spatial resolution in mice in vivo. *J Lipid Res*. 2008;49:473-480.

19. Zancanaro C, Nano R, Marchioro C, Sbarbati A, Boicelli A, Osculati F. Magnetic resonance spectroscopy investigations of brown adipose tissue and isolated brown adipocytes. *J Lipid Res*. 1994;35:2191-2199.

20. Berglund J, Ahlstrom H, Kullberg J. Model-based mapping of fat unsaturation and chain length by chemical shift imaging–phantom validation and in vivo feasibility. *Magn Reson Med*. 2012;68:1815-1827.

21. Bydder M, Girard O, Hamilton G. Mapping the double bonds in triglycerides. *Magn Reson Imaging*. 2011;29:1041-1046.
22. Peterson P, Mansson S. Simultaneous quantification of fat content and fatty acid composition using MR imaging. Magn Reson Med. 2013;69:688-697.

23. Hakumaki JM, Kauppinen RA. 1H NMR visible lipids in the life and death of cells. Trends Biochem Sci. 2000;25:357-362.

24. Hernando D, Sharma SD, Kramer H, Reeder SB. On the confounding effect of temperature on chemical shift-encoded fat quantification. Magn Reson Med. 2014;72:464-470.

25. Ye Q, Danzer CF, Fuchs A, Wolfrum C, Rudin M. Hepatic lipid composition differs between ob/ob and ob/+ control mice as determined by using in vivo localized proton magnetic resonance spectroscopy. MAGMA. 2012;25:381-389.

26. Corbin IR, Furth EE, Pickup S, Siegelman ES, Delikatny EJ. In vivo assessment of hepatic triglycerides in murine non-alcoholic fatty liver disease using magnetic resonance spectroscopy. Biochim Biophys Acta. 2009;1791:757-763.

27. Lundbom J, Hakkarainen A, Soderlund S, Westerbacka J, Lundbom N, Taskinen MR. Long-TE 1H MRS suggests that liver fat is more saturated than subcutaneous and visceral fat. NMR Biomed. 2011;24:238-245.

28. Hamilton G, Yokoo T, Bydder M, et al. In vivo characterization of the liver fat (1)H MR spectrum. NMR Biomed. 2011;24:784-790.

29. Hamilton G, Smith DL Jr, Bydder M, Nayak KS, Hu HH. MR properties of brown and white adipose tissues. J Magn Reson Imaging. 2011;34:468-473.

30. Hamilton G, Schlein AN, Middleton MS, et al. In vivo triglyceride composition of abdominal adipose tissue measured by (1)H MRS at 3T. J Magn Reson Imaging. 2017;45:1455-1463.

31. Bao S, Guttmann CR, Mugler JP 3rd, et al. Spin-Echo planar spectroscopic imaging for fast lipid characterization in bone marrow. Magn Reson Imaging. 1999;17:1203-1210.

32. Hamilton G, Middleton MS, Bydder M, et al. Effect of PRESS and STEAM sequences on magnetic resonance spectroscopic liver fat quantification. J Magn Reson Imaging. 2009;30:145-152.

33. Yahya A, Tressier AG, Fallone BG. Effect of J-coupling on lipid composition determination with localized proton magnetic resonance spectroscopy at 9.4 T. J Magn Reson Imaging. 2011;34:1388-1396.

34. Ruschke S, Kienberger H, Baum T, et al. Diffusion-weighted stimulated echo acquisition mode (DW-STEAM) MR spectroscopy to measure fat unsaturation in regions with low proton-density fat fraction. Magn Reson Med. 2016;75:32-41.

35. Yablonskiy DA, Neil JJ, Raichle ME, Ackerman JJ. Homonuclear J coupling effects in volume localized NMR spectroscopy: Pitfalls and solutions. Magn Reson Med. 1998;39:169-178.

36. Gajdosik M, Chadjynski GL, Hangel G, et al. Ultrashort-TE stimulated echo acquisition mode (STEAM) improves the quantification of lipids and fatty acid chain unsaturation in the human liver at 7 T. NMR Biomed. 2015;28:1283-1293.

37. Wilson M, Andiones O, Barker PB, et al. Methodological consensus on clinical proton MRS of the brain: Review and recommendations. Magn Reson Med. 2019;82:527-550.

38. Dieckmeyer M, Ruschke S, Cordes C, et al. The need for T(2) correction on MRS-based vertebral bone marrow fat quantification: Implications for bone marrow fat fraction age dependence. NMR Biomed. 2015;28:432-439.

39. Yu EW, Greenblatt L, Eajazi A, Torriani M, Bredella MA. Marrow adipose tissue composition in adults with morbid obesity. Bone. 2017;97:38-42.

40. Yahya A, Troitskaya A, Fallone BG. Sci-Sat AM: Brachy - 12: Resolving the olefinic lipid resonance from water in proton magnetic resonance spectra of vertebral bone marrow at 3 T. Med Phys. 2012;39:4647.

41. Lundbom J, Bierwagen A, Bodis K, et al. (1)H-MRS of femoral red and yellow bone marrow fat composition and water content in healthy young men and women at 3 T. MAGMA. 2019;32:591-597.

42. Poulet JB, Sima DM, Van Huffel S. MRS signal quantitation: A review of time- and frequency-domain methods. J Magn Reson. 2008;195:134-144.

43. Vanhamme L, van den Boogaart A, Van Huffel S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. J Magn Reson. 1997;129:35-43.

44. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magn Reson Med. 1993;30:672-679.

45. Sanctuary BC. Structure determination by NMR spectroscopy. In: Clark DE, ed. Evolutionary Algorithms in Molecular Design. Volume 8, Methods and Principles in Medicinal Chemistry. Weinheim: Wiley-VCH; 2000:214.

46. Marshall I, Higinbotham J, Bruce S, Freise A. Use of Voigt line-shape for quantification of in vivo 1H spectra. Magn Reson Med. 1997;37:651-657.

47. Xu K, Sigurdsson S, Gudnason V, Hue T, Schwartz A, Li X. Reliable quantification of marrow fat content and unsaturation level using in vivo MR spectroscopy. Magn Reson Med. 2018;79:1722-1729.

48. Nemeth A, Segrestin B, Leporq B, et al. Comparison of MRI-derived vs. traditional estimations of fatty acid composition from MR spectroscopy signals. NMR Biomed. 2018;31:e3991.

49. Lundbom J, Heikkinen S, Fielding B, Hakkarainen A, Taskinen MR, Lundbom N. PRESS echo time behavior of triglyceride resonances at 1.5T: Detecting omega-3 fatty acids in adipose tissue in vivo. J Magn Reson. 2009;201:39-47.

50. Lundbom J, Hakkarainen A, Fielding B, et al. Characterizing human adipose tissue lipids by long echo time 1H-MRS in vivo at 1.5 Tesla: Validation by gas chromatography. NMR Biomed. 2010;23:466-472.
adipose tissue fatty acid composition against gas chromatography. *Magn Reson Med.* 2020;84:2484–2494.

59. Leporq B, Lambert SA, Ronot M, Vilgrain V, Van Beers BE. Quantification of the triglyceride fatty acid composition with 3.0 T MRI. *NMR Biomed.* 2014;27:1211-1221.

60. Schneider M, Janas G, Lugauer F, et al. Accurate fatty acid composition estimation of adipose tissue in the abdomen based on bipolar multi-echo MRI. *Magn Reson Med.* 2019;81:2330-2346.

61. Peterson P, Svensson J, Mansson S. Relaxation effects in MRI-based quantification of fat content and fatty acid composition. *Magn Reson Med.* 2014;72:1320-1329.

62. Leporq B, Lambert SA, Ronot M, et al. Hepatic fat fraction and visceral adipose tissue fatty acid composition in mice: Quantification with 7.0T MRI. *Magn Reson Med.* 2016;76:510-518.

63. Peterson P, Mansson S. Fat quantification using multiecho sequences with bipolar gradients: Investigation of accuracy and noise performance. *Magn Reson Med.* 2014;71:219-229.

64. Yu H, Shimakawa A, McKenzie CA, et al. Phase and amplitude correction for multi-echo water-fat separation with bipolar acquisitions. *J Magn Reson Imaging.* 2010;31:1264-1271.

65. Mansson S, Peterson P, Johansson E. Quantification of low fat contents: A comparison of MR imaging and spectroscopy methods at 1.5 and 3 T. *Magn Reson Imaging.* 2012;30:1461-1467.

66. Martel D, Leporq B, Bruno M, Regatte RR, Honig S, Chang G. Chemical shift-encoded MRI for assessment of bone marrow adipose tissue fat composition: Pilot study in premenopausal versus postmenopausal women. *Magn Reson Imaging.* 2018;53:148-155.

67. Martel D, Leporq B, Saxena A, et al. 3T chemical shift-encoded MRI: Detection of altered proximal femur marrow adipose tissue composition in glucocorticoid users and validation with magnetic resonance spectroscopy. *J Magn Reson Imaging.* 2019;50:490-496.

68. Patsch JM, Li X, Baum T, et al. Bone marrow fat composition as a novel imaging biomarker in postmenopausal women with prevalent fragility fractures. *J Bone Miner Res.* 2013;28:1721-1728.

69. Bredella MA, Fazeli PK, Daley SM, et al. Marrow fat composition in anorexia nervosa. *Bone.* 2014;66:199-204.

70. Ecklund K, Vajapeyam S, Mulkern RV, et al. Bone marrow fat content in 70 adolescent girls with anorexia nervosa: Magnetic resonance imaging and magnetic resonance spectroscopy assessment. *Pediatr Radiol.* 2017;47:952-962.

71. Ermetici F, Brignati S, Delneo A, et al. Bone marrow fat contributes to insulin sensitivity and adiponectin secretion in premenopausal women. *Endocrine.* 2018;59:410-418.

72. Ayar J, Rodrigo R, Videla LA, et al. Increase in long-chain polyunsaturated fatty acid n-6/n-3 ratio in relation to hepatic steatosis in patients with non-alcoholic fatty liver disease. *Clin Sci.* 2004;106:635-643.

73. Elizondo A, Ayar J, Rodrigo R, et al. Polyunsaturated fatty acid pattern in liver and erythrocyte phospholipids from obese patients. *Obesity.* 2007;15:24-31.

74. Erickson ML, Haus JM, Malin SK, Flask CA, McCullough AJ, Kirwan JP. Non-invasive assessment of hepatic lipid subspecies matched with non-alcoholic fatty liver disease phenotype. *Nutr Metab Cardiovasc Dis.* 2019;29:1197-1204.

75. Diraison F, Dusserre E, Vidal H, Sothier M, Beylot M. Increased hepatic lipogenesis but decreased expression of lipogenic gene in adipose tissue in human obesity. *Am J Physiol Endocrinol Metab.* 2002;282:E46-E51.

76. Haus JM, Solomon TP, Kelly KR, et al. Improved hepatic lipid composition following short-term exercise in nonalcoholic fatty liver disease. *J Clin Endocrinol Metab.* 2013;98:E1181-E1188.

77. Kishida K, Funahashi T, Shimomura I. Adiponectin as a routine clinical biomarker. *Best Pract Res Clin Endocrinol Metab.* 2014;28:119-130.

78. Deibert P, Lazzaro A, Schaffner D, et al. Comprehensive lifestyle intervention vs soy protein-based meal regimen in non-alcoholic steatohepatitis. *World J Gastroenterol.* 2019;25:1116-1131.

79. Field CJ, Angel A, Clandinin MT. Relationship of diet to the fatty acid composition of human adipose tissue structural and stored lipids. *Am J Clin Nutr.* 1985;42:1206-1220.

80. Machann J, Stefan N, Schabel C, et al. Fraction of unsaturated fatty acids in visceral adipose tissue (VAT) is lower in subjects with high total VAT volume—A combined 1 H MRS and volumetric MRI study in male subjects. *NMR Biomed.* 2013;26:232-236.

81. Schrover IM, Leiner T, Klomp DW, et al. Feasibility and reproducibility of free fatty acid profiling in abdominal adipose tissue with 1H-magnetic resonance spectroscopy at 3 T: Differences between lean and obese individuals. *J Magn Reson Imaging.* 2014;40:423-431.

82. Lundhomb J, Hakkarainen A, Lundhomb N, Taskinen MR. Deep subcutaneous adipose tissue is more saturated than superficial subcutaneous adipose tissue. *Int J Obes.* 2013;37:620-622.

83. Dimitrov IE, Douglas D, Ren J, et al. In vivo determination of human breast fat composition by (1)H magnetic resonance spectroscopy at 7 T. *Magn Reson Med.* 2012;67:20-26.

84. Viallon M, Leporq B, Drinda S, et al. Chemical-shift-encoded magnetic resonance imaging and spectroscopy to reveal immediate and long-term multi-organs composition changes of a 14-days periodic fasting intervention: A technological and case report. *Front Nutr.* 2019;6:5.

85. Deng J, Neff LM, Rubert NC, et al. MRI characterization of brown adipose tissue under thermal challenges in normal weight, overweight, and obese young men. *J Magn Reson Imaging.* 2018;47:936-947.

86. Arluckkas SP, Browning EA, Poptani H, Delikatny EJ. Imaging of cancer lipid metabolism in response to therapy. *NMR Biomed.* 2019;32:e4070.

87. Mosconi E, Minicozzi A, Marzola P, Cordiano C, Sbarbati A. (1) H-MR spectroscopy characterization of the adipose tissue associated with colorectal tumor. *J Magn Reson Imaging.* 2014;39:469-474.

88. Iordanescu G, Brendler C, Crawford SE, Wyrwicz AM, Venkatasubramanian PN, Doll JA. MRS measured fatty acid composition of periprosthetic adipose tissue correlates with pathological measures of prostate cancer aggressiveness. *J Magn Reson Imaging.* 2015;42:651-657.

89. Venkatasubramanian PN, Brendler CB, Plunkett BA, et al. Periprosthetic adipose tissue from obese prostate cancer patients promotes tumor and endothelial cell proliferation: A functional and MR imaging pilot study. *Prostate.* 2014;74:326-335.

90. Freed M, Storey P, Lewin AA, et al. Evaluation of breast lipid composition in patients with benign tissue and cancer by using multiple gradient-echo MR imaging. *Radiology.* 2016;281:43-53.

91. Skorpil M, Ryden H, Berglund J, Brynolfsson P, Tsagozis P. Soft-tissue fat tumours: Differentiating malignant from benign versus breast MR imaging pilot study. *Prostate.* 2016;76:510-518.
92. Skorpil M, Ryden H, Wejde J, Lidbrink E, Brosjo O, Berglund J. The effect of radiotherapy on fat content and fatty acids in myxoid liposarcomas quantified by MRI. *Magn Reson Imaging*. 2017;43:37-41.

93. Hernando D, Sharma SD, Aliyari Ghasabeh M, et al. Multisite, multivendor validation of the accuracy and reproducibility of proton-density fat-fraction quantification at 1.5T and 3T using a fat-water phantom. *Magn Reson Med*. 2017;77:1516-1524.

94. Machann J, Stefan N, Wagner R, et al. Intra- and interindividual variability of fatty acid unsaturation in six different human adipose tissue compartments assessed by (1) H-MRS in vivo at 3 T. *NMR Biomed*. 2017;30:e3744

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