In vivo comparison of MRI-based and MRS-based quantification of adipose tissue fatty acid composition against gas chromatography

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Purpose: To compare MR-based fatty acid composition (FAC) quantification methods against the gold standard technique, gas chromatography (GC), with comparison of a free and a constrained signal model. The FAC was measured in the healthy and edematous legs of lymphedema patients.

Methods: In vivo MRS and MRI data were acquired from 19 patients at 3 T. Biopsies were collected from subcutaneous adipose tissue of both thighs during liposuction. The saturated, monounsaturated, and polyunsaturated fatty acid fractions ($f_{SFA}$, $f_{MUFA}$ and $f_{PUFA}$, respectively) were estimated with the MR-based methods using two signal models: free and constrained (number of methylene-interrupted double bonds expressed in number of double bonds, based on GC data). Linear regression, Bland–Altman plots, and correlation coefficients were used to evaluate the MR methods against the GC of the biopsies. Paired t-test was used to compare the FAC difference between edematous and healthy legs.

Results: The estimated parameters correlated well with the GC data ($r_{SFA}$, $r_{MUFA}$, and $r_{PUFA}$ = 0.82, 0.81 and 0.89, respectively) using the free model MRI-based approach. In comparison, the MRS-based method resulted in weaker correlations and larger biases compared with MRI. In both cases, correct estimation of $f_{MUFA}$ and $f_{PUFA}$ fractions were not possible using the constrained model. The difference in FAC of healthy and edematous legs were estimated to 0.008 ($P = .01$), −0.009 ($P = .005$), and 0.002 ($P = .03$) for $f_{SFA}$, $f_{MUFA}$, and $f_{PUFA}$.

Conclusion: In this study, MRI-based FAC quantification was highly correlated, although slightly biased, compared with GC, whereas the MRS-based approach...
INTRODUCTION

Methods for quantification of fat fraction using MR have become well-established and are used widely for in vivo investigation of fat accumulation. Recently, both MRS and MRI methods for estimating also the chemical composition of fat have emerged. With these methods, potential applications in obesity, metabolic disorders, inflammatory conditions, and cancer have been suggested.

Although both MRS-based and MRI-based methods offer a noninvasive alternative to biopsies, MRI has the additional advantage of providing highly resolved spatial information over a large volume. Previous studies have indicated that the fatty acid composition (FAC) of visceral, deep subcutaneous, and superficial subcutaneous adipose tissue differ from each other and have different metabolic relevance. Using imaging-based techniques, these fat depots can be investigated simultaneously, thus avoiding the repeated measures when using biopsies or MRS. However, the various MRI approaches to measure FAC have been validated primarily against MRS. Further comparison to an independent, gold standard technique is still needed.

Using the MR-based techniques, the fat signal can be described in terms of the number of double bonds (ndb), the number of methylene-interrupted double bonds (nmidb), and the chain length (cl) of the triglyceride molecule. From the estimated ndb and nmidb, the fraction of saturated fatty acids (SFAs), unsaturated fatty acids, monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) can easily be calculated and compared with independent techniques. Since the MR-based methods were first introduced, additional effort has been made to increase robustness by setting cl to a fixed value or expressing cl and/or nmidb as functions of ndb, thus reducing the number of free parameters. However, the suggested models are either based primarily on vegetable oils or on a small sample of in vivo data. Before a more general model can be found, models based on various human tissues are needed, and the impact on estimation accuracy when using a restricted signal model needs to be investigated further.

This study was carried out on a group of lymphedema patients in whom biopsies of subcutaneous adipose tissue for gas chromatography were easily accessible during surgical liposuction treatment of their condition. Lymphedema is a common and disabling complication after cancer treatment, in which insufficient lymph drainage leads to excess accumulation of both fluid and fat, primarily in the subcutaneous compartment. The condition is associated with inflammation of the affected limb but the mechanisms behind the fat accumulation and the chemical composition of the fat are unknown.

This study therefore has three aims: (1) validate MRI and MRS against the gold-standard method, gas chromatography (GC); (2) compare the free and constrained estimation methods for both MRI and MRS, using GC data to design signal models for human adipose tissue; and (3) compare the ability of the various techniques in detecting a difference between the healthy and edematous legs.

METHODS

2.1 Subjects

Twenty subjects with leg lymphedema were recruited to the study. One patient participated only in the MRI and MRS part, and one patient was excluded after MRI due to the presence of bilateral lymphedema, leaving a total of 19 patients with usable MRI and MRS data, of whom 18 contributed with GC samples. Participation was voluntary, and all participating patients signed a written informed consent. The study was approved by the regional ethical review board.

The included subjects underwent MRI examination in which MRI and MRS data were acquired. Two weeks after the MRI scans, the patients underwent liposuction. In connection to the liposuction, biopsies from both healthy and edematous legs were collected and sent for GC analysis.

2.2 Data acquisition

A 3T MRI scanner (TIM Trio; Siemens Healthineers, Erlangen, Germany) was used to acquire MRI and MRS data from both thighs (edematous and healthy sides). Three axial 2D multi-echo gradient-echo images were collected using
flyback gradients, a 12-channel body matrix coil, and with the following parameters: number of echoes = 12, TE1/ΔTE = 1.31/1.56 ms, TR = 250 ms, flip angle = 30°, matrix size = 128 × 128, FOV = 285 × 480 × 5 mm³, and bandwidth = 1953 Hz/pixel. The frequency direction was from right to left, and the total MRI scan time was 1 minute and 56 seconds. The interecho spacing was chosen to be as short as possible while avoiding suboptimal noise performance based on analysis of number of signal averages.4

In fat/water imaging, care must be taken to minimize T1 relaxation bias, such as by choosing a small flip angle. In FAC quantification, however, T1 relaxation effects are of less concern, as it has been shown that ndb and nmidb are not as sensitive to T1 weighting.23

Six STEAM spectra were collected from each thigh, at the same level as the MRI data, with TE = 20, 30, 40, 50, 60, and 100 ms, TR = 2500 ms, mixing time = 10 ms, voxel size = 12 × 12 × 20 mm³, number of data points = 1024, and bandwidth = 1500 Hz. Total spectroscopy scan time was 12 minutes.

All images and spectra were acquired 20 cm above the femoral condyles. Spectroscopy voxels were placed in each thigh, in the medial subcutaneous fat, avoiding large vessels and lymphatic fluid (Figure 1). During liposuctions, 20-mL biopsies were taken from the subcutaneous fat of both healthy and edematous legs by local liposuction medially on the thigh 20 cm above the femoral condyles. The samples were collected approximately in the same location as the MRS voxels and sent for GC analysis at Eurofins Food and Organic Materials.24

2.3 | Data analysis

From the GC analysis, the abundances of each fatty acid were reported as fractions of the total fatty acid weight. The fatty acid fractions were corrected for differences in mass to make the GC results comparable to the MR results. Then, the mean ndb, nmidb, and cl were calculated by

$$\text{ndb} = \frac{\sum_n R_n X_{nn, ndb}}{n}$$

$$\text{nmidb} = \frac{\sum_n R_n X_{nn, nmidb}}{n}$$

$$\text{cl} = \frac{\sum_n R_n X_{nn, cl}}{n}$$

where $R_n$ is the relative mass-corrected abundance of fatty acid $n$, and $X_n$ is the corresponding value of fatty acid ndb/nmidb/cl. The SFA, MUFA, and PUFA fractions ($fSFA$, $fMUFA$ and $fPUFA$, respectively) were calculated as the accumulated relative abundances of each fatty acid with no double bonds, with one double bond, and with more than one double bond, respectively. The relationships between ndb and nmidb, and ndb and cl, were used to create models for the MR-based methods.

For the MR-based methods, FAC was estimated using two models, free and constrained, in which the GC results were used to define relationships between ndb and nmidb, and ndb and cl (Figure 2). The free model estimated ndb and nmidb as free parameters, with cl expressed in ndb. The constrained model freely estimated ndb, with nmidb and cl expressed in ndb.

An iterative least-squares reconstruction algorithm with an eight-peak fat model was used in the MRI-based method.4 Briefly, the algorithm used a signal model as follows:

$$S(t) = \left( W + F_f \sum_{m=1}^8 \alpha_m E_m(t) \right) e^{\imath \psi t}$$

(1)

where $E_m(t) = e^{\imath \omega_m t}$ describes the phase evolution of peak $m$ at time $t$; $\psi$ is a complex field map24; and $f = 1/\sum \alpha_m$ is a normalization constant. The peak amplitudes $\alpha_m$ (Table 1) are functions of ndb and nmidb (free model) or of ndb only (constrained model) according to Table 1. By rearranging the terms of Equation 1, the signal is expressed as

$$S(t) = \left( W + F_f \left( P_0(t) + P_1(t) ndb + P_2(t) nmidb \right) \right) e^{\imath \psi t}$$

(2)

where

$$P_0(t) = E_1(t) + 4E_2(t) + 6E_4(t) + 6E_6 + 73.9E_7(t) + 9E_8(t)$$

$$P_1(t) = 2E_1(t) + 4E_5(t) - 5.7E_7(t)$$

$$P_2(t) = 2E_3(t) - 4E_5(t) + 2E_7(t)$$

for the free model and

$$P_0(t) = E_1(t) + 4E_2(t) - 1.8E_3 + 6E_4$$

$$+ 3.6E_5 + 6E_6 + 73.9E_7(t) + 9E_8(t)$$

$$P_1(t) = 2E_1(t) + E_3 + 4E_5(t) - 5.2E_7(t)$$

for the constrained model.
for the constrained model. To obtain \( ndb \) (and \( nmidb \)), Equation 2 was converted to matrix form and solved in an iterative process described previously.4,25

The regions of interest were defined in the medial area of both legs, approximately where the MRS data and the GC biopsies were retrieved. To avoid veins and lymph fluid, and to minimize partial volume effects as much as possible, only voxels with a fat fraction above 0.9 within the regions of interest were included for further analysis.

The MRS spectra were analyzed using the jMRUI/AMARES software.26-28 Peaks A, B, and G (Table 1 and Figure 1) were modeled using two Gaussian shapes for each peak: Peaks C-E were modeled using one Gaussian each, and peak F was modeled using four Gaussians. The water peak was modeled using one Gaussian. A Levenberg-Marquardt algorithm was used to obtain \( T_2 \) relaxation–corrected peak amplitudes. Due to spectral overlaps, joint \( T_2 \) relaxation times for water and fat peaks A and B, and fat peaks D and E, were calculated. From the \( T_2 \)-corrected amplitudes of peaks A-G, \( ndb \) (and \( nmidb \)) were estimated based on the theoretical amplitude expressions. The water amplitude was also estimated and as a free parameter.

In both the MRI and MRS cases, \( f_{SFA}, f_{MUFA}, \) and \( f_{PUFA} \) were calculated from the estimated \( ndb \) and \( nmidb \). With an assumption that the triglycerides are at most di-unsaturated, the value of \( f_{PUFA} \) can be calculated as 
\[
f_{PUFA} = \frac{nmidb}{3}.
\]
The values of \( f_{MUFA} \) and \( f_{SFA} \) are estimated as 
\[
\begin{align*}
f_{MUFA} &= -(ndb - 2nmidb)/3, \\
f_{SFA} &= 1 - (ndb + nmidb)/3.
\end{align*}
\]

For the constrained model, the following equations were used:
\[
\begin{align*}
f_{PUFA} &= 0.15ndb - 0.24, \\
f_{MUFA} &= -0.035ndb + 0.48, \\
f_{SFA} &= 1.2 - 0.55ndb.
\end{align*}
\]

The constrained model, the amount of \( nmidb \) is assumed to depend only on \( ndb \). However, this is not completely accurate and might affect the estimation of \( f_{SFA}, f_{MUFA}, \) and \( f_{PUFA} \).

2.4 | Statistics

Linear regressions and Bland–Altman analysis were performed to study the agreement between the MRI-estimated and MRS-estimated \( f_{SFA}, f_{MUFA}, \) and \( f_{PUFA} \) using both free and constrained models, to the results from the GC analysis. The obtained slope and intercept of the regression line will be denoted as \( k \) and \( m \), respectively.
respectively, throughout this paper. Correlation coefficients \( r \) for each method, model, and parameter were calculated as well as the confidence intervals for all statistical parameters.

To compare the FAC of the healthy and edematous legs, the differences for all parameters were calculated as (edematous-healthy) leg for all three methods, as well as for both free and constrained models in the MR cases. A paired \( t \)-test was used to test whether the relative differences were significantly \((P \leq .05)\) different from zero.

3 | RESULTS

The relationships between \( ndb \) and \( nmidb \), and \( ndb \) and \( cl \), based on the results from the GC analysis are shown in Figure 2. The following expressions for \( nmidb \) and \( cl \) were obtained: \( nmidb = 0.448ndb - 0.714 \) and \( cl = 0.378ndb + 16.3 \). The corresponding correlation coefficients are 0.86 (confidence interval 0.74-0.93) and 0.89 (confidence interval 0.78-0.94), respectively. The mean \( cl \) of both legs was calculated as 17.31 ± 0.07 from the GC data.

The mean and SDs of the estimated \( ndb \) in the healthy and edematous legs using GC, \( MRS_{\text{free}} \), \( MRS_{\text{constr}} \), \( MRI_{\text{free}} \), and \( MRI_{\text{constr}} \) were 2.63 ± 0.18, 3.81 ± 0.27, 3.86 ± 0.24, 2.28 ± 0.17 and 2.36 ± 0.19, and 2.62 ± 0.17, 3.88 ± 0.21, 3.88 ± 0.19, 2.19 ± 0.20 and 2.27 ± 0.21, respectively. The corresponding values for the estimated \( nmidb \) were 0.457 ± 0.093, 0.946 ± 0.170, 1.016 ± 0.108, 0.170 ± 0.081 and 0.345 ± 0.084, and 0.465 ± 0.091, 1.017 ± 0.149, 1.024 ± 0.087, 0.149 ± 0.099 and 0.301 ± 0.096, respectively.

Examples of the MRI-estimated \( f_{\text{SFA}} \), \( f_{\text{MUFA}} \), and \( f_{\text{PUFA}} \) images of 1 patient, obtained using both models, are presented in Figure 3. Compared with the estimation of \( f_{\text{SFA}} \), a larger variability as well as a gradient of increasing values in the frequency-encoding direction were seen in the \( f_{\text{MUFA}} \) and \( f_{\text{PUFA}} \) images estimated with the free model. In contrast, the \( f_{\text{MUFA}} \) and \( f_{\text{PUFA}} \) images estimated with the constrained model are very homogeneous over the images.

The corresponding linear fits and Bland-Altman plots of the MRS data are presented in Figures 6 and 7, respectively. Lower correlation coefficients were found for the MRS-based method compared with the MRI-based method, especially for \( f_{\text{MUFA}} \) and \( f_{\text{PUFA}} \) estimated using the free model. Similar to the MRI case, the constrained model resulted in worse correlation compared with the free model, except for \( f_{\text{PUFA}} \), in which a higher correlation coefficient was found. Using both the free and constrained models, \( f_{\text{SFA}} \) was underestimated while \( f_{\text{PUFA}} \) was overestimated. In the same manner as the MRI-based method, the constrained model failed to accurately separate \( f_{\text{MUFA}} \) and \( f_{\text{PUFA}} \).

The mean and SD of the estimated \( f_{\text{SFA}} \), \( f_{\text{MUFA}} \), and \( f_{\text{PUFA}} \) of both healthy and edematous legs are summarized in Table 2. The pair-wise differences between the subcutaneous fat of healthy and edematous legs are presented in Table 2 and Figure 8.

The GC analysis revealed significantly higher \( f_{\text{SFA}} \) and \( f_{\text{PUFA}} \), and a lower \( f_{\text{MUFA}} \), in the edematous leg compared with the healthy leg. In addition, the MRI-based methods, using both the free and constrained model, resulted in significant differences. However, the differences between healthy and edematous legs were larger for all parameters and of opposed sign.
Figure 4: Estimated $f_{\text{SFA}}$, $f_{\text{MUFA}}$, and $f_{\text{PUFA}}$ of both healthy (○) and edematous (x) legs using an MRI-based method with the free (A) and the constrained (B) model, compared with GC. The red lines are the linear regression fit, and the black lines are the identity lines. Using the constrained model, the correlations of $f_{\text{MUFA}}$ and $f_{\text{PUFA}}$ are significantly lower compared with the free model case, whereas the estimation of $f_{\text{SFA}}$ appears to be relatively unaffected. The estimation of $f_{\text{MUFA}}$ using the constrained model also results in a constant value with no association to the GC values. CI, confidence interval.

Figure 5: Bland–Altman plots of $f_{\text{SFA}}$, $f_{\text{MUFA}}$, and $f_{\text{PUFA}}$ comparing the results from the MRI-based methods (the free [A] and the constrained [B] models) and GC. (○) represents the healthy legs, and (x) represents the edematous legs. The black solid lines indicate the mean difference between the MRI-based method and GC with the corresponding CIs (black dotted lines). The red dashed lines indicate the limits of agreement. Biases can be seen in all estimated parameters, using both models. Although a similar estimation of $f_{\text{SFA}}$ is obtained, it is obvious that the constrained model has issues with the estimation of especially $f_{\text{MUFA}}$ compared with the free model.
FIGURE 6  Estimated $f_{SFA,MRS}$, $f_{MUFA}$, and $f_{PUFA}$ of both healthy (○) and edematous (x) legs using an MRS-based method with the free (A) and the constrained (B) model, compared with GC. The red lines indicate the linear regression fit, and the black lines indicate the identity lines. Similar to the MRI results, the estimation of $f_{MUFA}$ using the constrained model also results in a constant value with no association to the GC values. However, a larger impact of using the constrained model can be seen on the estimation of $f_{SFA}$, and a stronger correlation to GC was found in the estimation of $f_{PUFA}$ using the constrained model compared with the free model.

FIGURE 7  Bland–Altman plots of $f_{SFA,MRS}$, $f_{MUFA}$, and $f_{PUFA}$ comparing the results from the MRS-based methods (the free [A] and the constrained [B] models) and GC. (o) represents the healthy legs, and (x) represents the edematous legs. The black solid lines indicate the mean difference between the MRI-based method and GC with the corresponding CIs (black dotted lines). The red dashed lines indicate the limits of agreement. Larger biases were found using the MRS-based method compared with the MRI-based methods in the estimation of all parameters except for $f_{MUFA}$. In contrast to MRI, a more robust estimation of $f_{PUFA}$ was obtained when using the constrained model.
for \( f_{PUFA} \) compared with the GC results. In contrast, no significant differences were found with the MRS-based approaches, which resulted in substantially larger SDs than MRI. Using the free model MRS-based approach, the differences follow the same tendency as the GC results, although with large SDs. The results from the constrained model also reveal large SDs but with smaller mean differences compared with GC.

### TABLE 2

Mean ± SD of the estimated \( f_{SFA}, f_{MUFA}, \) and \( f_{PUFA} \) obtained from MRI, MRS, and GC as well as the pair-wise differences (edematous - healthy) ± SD with corresponding \( P \)-values

|          | Healthy     | Edematous   | Difference | \( P \)-value |
|----------|-------------|-------------|------------|--------------|
| GC       |             |             |            |              |
| \( f_{SFA} \) | 0.275 ± 0.038 | 0.283 ± 0.034 | 0.008 ± 0.011 | .01          |
| \( f_{MUFA} \) | 0.602 ± 0.034 | 0.593 ± 0.031 | −0.009 ± 0.012 | .005         |
| \( f_{PUFA} \) | 0.123 ± 0.029 | 0.124 ± 0.028 | 0.002 ± 0.003 | .03          |
| MRI, free|             |             |            |              |
| \( f_{SFA} \) | 0.300 ± 0.038 | 0.328 ± 0.049 | 0.028 ± 0.019 | <.0001       |
| \( f_{MUFA} \) | 0.641 ± 0.035 | 0.622 ± 0.040 | −0.019 ± 0.019 | .0003        |
| \( f_{PUFA} \) | 0.059 ± 0.029 | 0.051 ± 0.035 | −0.008 ± 0.016 | .04          |
| MRI, constrained|       |             |            |              |
| \( f_{SFA} \) | 0.325 ± 0.034 | 0.347 ± 0.042 | 0.022 ± 0.015 | <.0001       |
| \( f_{MUFA} \) | 0.559 ± 0.007 | 0.555 ± 0.008 | −0.004 ± 0.003 | <.0001       |
| \( f_{PUFA} \) | 0.116 ± 0.028 | 0.099 ± 0.034 | −0.018 ± 0.013 | <.0001       |
| MRS, free|             |             |            |              |
| \( f_{SFA} \) | 0.043 ± 0.043 | 0.055 ± 0.059 | 0.005 ± 0.033 | .6           |
| \( f_{MUFA} \) | 0.644 ± 0.049 | 0.668 ± 0.068 | −0.024 ± 0.052 | .06          |
| \( f_{PUFA} \) | 0.313 ± 0.056 | 0.337 ± 0.046 | 0.019 ± 0.045 | .08          |
| MRS, constrained|       |             |            |              |
| \( f_{SFA} \) | 0.051 ± 0.043 | 0.055 ± 0.051 | −0.001 ± 0.030 | .9           |
| \( f_{MUFA} \) | 0.6105 ± 0.0082 | 0.6097 ± 0.0096 | −0.0002 ± 0.0057 | .9          |
| \( f_{PUFA} \) | 0.339 ± 0.035 | 0.336 ± 0.041 | 0.001 ± 0.024 | .9           |

#### FIGURE 8

The mean differences (edematous-healthy) and SDs of \( f_{SFA}, f_{MUFA}, \) and \( f_{PUFA} \) between edematous legs and healthy legs calculated from GC, MRI with the free model, MRI with the constrained model, MRS with the free model, and MRS with the constrained model (*\( P < .05 \); **\( P < .01 \); ***\( P < .001 \)). The SD of \( f_{MUFA} \) and \( f_{PUFA} \) extending beyond the y-axis are −0.076 and 0.064, respectively. Small but significant differences between healthy and edematous legs were found using both GC and MRI; however, in general, larger mean differences were found using MRI compared with GC, which is considered the true difference in this study.

#### DISCUSSION

In this study, the characterization of the chemical composition of fat using MRI and MRS has been compared to GC, the gold-standard method for FAC quantification. Using a model with free estimation of \( ndb \) and \( nmidb \) and constrained cl, a strong correlation was found between the MRI-based method and GC. In comparison, the MRS-based approach with free estimation of \( ndb \) and \( nmidb \) resulted in weaker correlations with GC and larger biases than the MRI method. Especially \( f_{SFA} \) was robustly estimated using MRI with both free and constrained estimation models, and demonstrated similar tendencies in a comparison between healthy and edematous tissue as GC. However, no correct distinction between MUFA and PUFA could be made using the constrained model.
A larger bias was found in the estimation of \(f_{\text{PUFA}}\) compared with \(f_{\text{SFA}}\) and \(f_{\text{MUFA}}\) when using the free model. This is consistent with previous studies demonstrating a lower test-retest reliability of the \(f_{\text{MUFA}}\) and \(f_{\text{PUFA}}\) estimations compared with the \(f_{\text{SFA}}\) parameter,\(^{16}\) and a lower accuracy of the associated \(nmidb\) parameter.\(^1\) In addition to the smaller peak amplitudes linked to the \(nmidb\) parameter, a discrepancy between the MR-based methods and GC is also expected, as the exact definition of \(f_{\text{PUFA}}\) differs between the methods. In the MR-based methods, it is assumed that the triglycerides are at most di-unsaturated, whereas no such assumption is made in the GC analysis. However, based on the GC data of this study, only about 2% of the fatty acids have an unsaturation degree higher than 2. A similar value (2%-3%) has been reported previously.\(^{20}\) Compared with the MRI-based method, weaker correlations and worse agreement were found with the MRS-based approach. This may be due to the impact of J-coupling on the estimated T2 relaxation times.\(^{30}\)

Although the most likely artificial, spatial variation was improved for \(f_{\text{MUFA}}\) and \(f_{\text{PUFA}}\) with the constrained approach, the correlation and accuracy to GC results were weaker, and for \(f_{\text{MUFA}}\), a close to constant value was obtained with no detectable association with GC. In the case of \(f_{\text{PUFA}}\), the correlation between MRI and GC decreased from 0.89 to 0.65, with a 2-fold increase of the confidence interval. In other words, by using a constrained estimation of \(nmidb\), \(f_{\text{MUFA}}\) and \(f_{\text{PUFA}}\) cannot be correctly separated. This is likely due to the assumption of a simple relationship between \(nmidb\) and \(ndb\). First, insertion of \(nmidb\) as a linear function of \(ndb\) in the \(f_{\text{MUFA}}\) expression leads to an almost full cancellation of the \(ndb\) dependency of \(f_{\text{MUFA}}\). Second, as the \(nmidb\) is not only dependent on \(ndb\), but also on the degree of unsaturation, using an expression of \(nmidb\) as a function of only \(ndb\) leads to a partial loss of the information needed for accurate distinction between MUFA and PUFA. In contrast, little effect was found on the estimation of \(f_{\text{SFA}}\), indicating that the separation of SFAs and unsaturated fatty acids is largely unaffected by a constrained \(nmidb\). Other models of \(nmidb\) and \(cl\) have been suggested previously, based on a large number of various, primarily vegetable oils and fats.\(^2\) However, these models involve a wider range of \(ndb\), \(nmidb\), and \(cl\) than what is found in human adipose tissue.

In contrast to \(nmidb\), \(cl\) is not directly linked to the calculation of \(f_{\text{SFA}}, f_{\text{MUFA}}, \) or \(f_{\text{PUFA}},\) and shows a small interpersonal variation based on the present GC results as well as previous reported values.\(^{14,29,31,32}\) Motivated by this and the interest in minimizing the number of free parameters, \(cl\) was therefore constrained in all estimations using MR-based methods in this study.

A recent study compared MR-based FAC quantification methods to GC, and strong correlations were found between the methods in general.\(^{15}\) In contrast to our results, higher correlations were found between MRS and GC compared with MRI and GC. However, the used MR-based methods differ from the ones used in this study.

Although not directly comparable due to data samples from different subject groups and adipose tissue depots, the reported FAC values in this study correspond well with previously published values. Based on the FAC reported in a review article summarizing the GC results of 19 studies including healthy volunteers,\(^{31}\) the corresponding mean \(f_{\text{SFA}}, f_{\text{MUFA}},\) and \(f_{\text{PUFA}}\) can be calculated as 0.29, 0.54, and 0.17, respectively. In another study, biopsies were taken from the abdominal subcutaneous fat of 10 subjects, and the mean \(f_{\text{SFA}}, f_{\text{MUFA}},\) and \(f_{\text{PUFA}}\) calculated from the GC data were 0.30, 0.58, and 0.12, respectively.\(^{32}\) In a study including 50 subjects, of whom 25 had an elevated risk of type 2 diabetes, the MRS data showed a mean unsaturated fatty acid fraction of 0.62 in the subcutaneous fat of the calf.\(^{10}\) Nemeth et al\(^{15}\) compared the abdominal FAC of 13 subjects using several methods including MRS, MRI, and mass spectroscopy–GC. From that study, they reported the following mean \(f_{\text{SFA}}, f_{\text{MUFA}},\) and \(f_{\text{PUFA}}\) values: 0.37, 0.44, and 0.19 (MRS); 0.38, 0.45, and 0.19 (MRI); and 0.38, 0.50, and 0.12 (GC).

Although a strong correlation was found between GC and especially MRI, the comparison between the healthy and edematous legs gave slightly different results. This may be caused by the artificial spatial variation of the frequency coding direction that is visible in the free estimated parameter maps.\(^2\) The reason behind this artifact has not yet been defined, but it has been speculated that it might be due to anti-aliasing filters\(^2\) or asymmetric frequency response.\(^{16}\) Similar spatial variation has been reported in MRS-based FAC quantification.\(^{13}\) Another possible explanation is that the FAC difference between the healthy and edematous legs in this study group is too small to be reliably detected by the MR-based methods, given their precision relative to the GC measurements. Furthermore, although most of the adipose tissue consists of triglycerides, other lipids can be found as well. For example, phospholipids are not a part of the MR signal but are included in the fatty acid profiles obtained from GC. Thus, some discrepancy between the measured values using GC and MR-based methods can be expected. However, due to the relative low amount of other lipids in adipose tissue compared with triglycerides, the bias is estimated to be small.

This study has two primary limitations. First, because the difference in FAC is expected to be quite small, a larger number of subjects might be needed for a full investigation of the difference in FACs between healthy and edematous legs. However, the main purpose of this work was to compare MR-based methods to GC, for which a smaller study population was sufficient. Second, the measured volume did not exactly match among the three methods, so the results might differ due to local variations of the adipose tissue. However, the \(f_{\text{SFA}}\) maps indicate a low regional variability in the subcutaneous compartment of the thigh.
5 | CONCLUSIONS

This study showed that FAC estimated with the MRI-based method correlated well with that of the independent gold-standard technique, GC, when ndb and nmidb were estimated independently. In contrast, only fSFA was reliably estimated using the constrained model based on the GC data from the patients in this study. For all tested FAC measures and both signal models, the MRI-based method showed higher agreement with GC compared to MRS. However, development is still needed to improve the precision and accuracy of the MRI-based and MRS-based methods. In the case of the subcutaneous FAC in lymphedema patients, GC showed significant difference in fSFA, fMUFA, and fPUFA between healthy and edematous legs.

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