Background: Omega-3 (n-3) fatty acids (FA) play an important role in neural development and other metabolic diseases such as obesity and diabetes. The knowledge about the in vivo content and distribution of n-3 FA in human body tissues is not well established and the standard quantification of FA is invasive and costly.

Purpose: To detect omega-3 (n-3 CH₃) and non-omega-3 (CH₃) methyl group resonance lines with echo times up to 1200 msec, in oils, for the assessment of n-3 FA content, and the n-3 FA fraction in adipose tissue in vivo.

Study Type: Prospective technical development.

Population: Three oils with different n-3 FA content and 24 healthy subjects.

Field Strength/Sequence: Single-voxel MR spectroscopy (SVS) with a point-resolved spectroscopy (PRESS) sequence with an echo time (TE) of 1000 msec at 7 T.

Assessment: Knowledge about the J-coupling evolution of both CH₃ resonances was used for the optimal detection of the n-3 CH₃ resonance line at a TE of 1000 msec. The accuracy of the method in oils and in vivo was validated from a biopsy sample with gas chromatography analysis.

Statistical Tests: SVS data were compared to gas chromatography with the Pearson correlation coefficient.

Results: T₂ relaxation times in oils were assessed as follows: CH₂, 65 ± 22 msec; CH₃, 325 ± 7 msec; and n-3 CH₃, 628 ± 34 msec. The n-3 FA fractions from oil phantom experiments (n = 3) were in agreement with chromatography analysis and the comparison of in vivo obtained data with the results of chromatography analysis (n = 5) yielded a significant correlation (P = 0.029).

Data Conclusion: PRESS with ultralong-TE can detect and quantify the n-3 CH₃ signal in vivo at 7 T.

Level of Evidence 1
Technical Efficacy Stage 1

OMEGA-3 (ω-3 or n-3) fatty acids (FA), together with n-6 FA, are essential polyunsaturated FA important for humans. n-3 FA are involved in neural development¹² and could be part of a novel therapy for nonalcoholic fatty liver disease (NAFLD).³ Today, several invasive methodologies enable the quantification of n-3 FAs; however, these methods are
time-consuming and costly. Knowledge about the in vivo content and distribution of n-3 FA in human body tissues is not well established.

Proton (1H) nuclear magnetic resonance (NMR) spectroscopy enables the detection of terminal protons of n-3 methyl (n-3 CH3) and non-n-3 methyl (CH3) groups in FA chains at two different frequencies (ν): 0.98 and 0.90 ppm. The methyl protons are coupled to two protons on the second carbon atom and form triplets in the NMR spectra. In the case of the n-3 CH3 group, the J-coupling constant is 7.5 Hz. The relatively adjacent base frequencies and signal splitting make the detection of n-3 FA in vivo challenging. The in vivo fraction of n-3 FA from the total FA chain is very low, -1:75 in adipose tissue. This complicates its detection and prolongs signal acquisition times.

As signal-to-noise (SNR) increases with magnetic field strength (B0), ultrahigh field (UHF) 7 Tesla (T) should provide higher SNR, which is beneficial for the detection of low-concentration n-3 FA. The nominal spectral resolution in Hz between CH3 and n-3 CH3 increases with the application of higher B0; however, the resolution of the methyl triplets remains constant. For a better understanding of this effect, the estimated spectral dispersion in vivo is illustrated in Fig. 1 for three clinical field strengths of 1.5, 3, and 7 T.

At magnetic field strengths of 1.5 and 3 T, the peaks of the CH3 triplet overlap the central and right outer n-3 CH3 peaks; however, no signal overlaps the left outer n-3 CH3 peak (1.08 ppm and 1.05 ppm, respectively) and can, in principle, be detected. The first detection of n-3 FA in vivo in subcutaneous adipose tissue (SAT) at a clinical field strength of 1.5 T with 1H MR spectroscopy (MRS) was carried out by Lundbom et al., in which the left outer n-3 CH3 peak was detected using a point-resolved spectroscopy (PRESS) sequence with a long echo time (TE) of 540 msec. This in vivo n-3 FA measurement was based on a long apparent transverse (T2) relaxation time of 478 msec for the central n-3 CH3 peak of linseed oil measured in that study. A method for assessment of the relative levels of n-3 FA content in oils, based on relative methyl peak linewidths with stimulated echo mode (STEAM) and PRESS sequences with TE = 160 msec at 3 T, was also presented. J-difference editing allowed for the detection of the left outer n-3 CH3 peak in SAT at 1.05 ppm with a TE of 199.5 msec at 3 T. However, at 7 T the left outer n-3 CH3 peak resonates at 1.00 ppm, which is only 0.02 ppm away from the central n-3 CH3 peak. To detect this peak with in vivo MRS in SAT using a whole-body 7 T MRI system and the usual B0 homogeneity is currently very difficult.

Nevertheless, the spectral dispersion at 7 T yields a better resolution of the central peaks of both methyl triplets, which have two-times higher intensity than their respective outer peaks.

All the aforementioned MRS methods used a medium TE (≥160 msec) that exploits the relatively long T2 relaxation times of n-3 methyl groups compared to the neighboring non-n-3 methyl groups and strong methylene ((CH2)n, or CH2) peak (3-5X longer), which resonates at a frequency close to 1.30 ppm. However, MRS measurements with a long TE have the disadvantage of heavy T2-weighting, which requires an accurate description of J-modulation and the calculation of T2 relaxation times for the correct assessment of the FA content. The effect of J-coupling interactions on the quantification and assessment of the T2 relaxation time of the CH3 group has been investigated in oils and bone marrow at 3 T with TE ≤200 msec. MRS at UHF is generally hampered by a higher specific absorption rate (SAR) and a larger chemical-shift-displacement error (CSDE). These obstacles can be overcome at the cost of worse localization and longer acquisition time by using Hermite pulses, which have lower RF power requirements, and by measuring with specific frequency offsets.

The aim of this study was to detect and characterize the relaxation behavior of CH3, CH3, and n-3 CH3 signals in oils, with an assessment of their apparent T2 relaxation times and their n-3 FA content, and to detect and quantify n-3 CH3 signal in vivo in SAT with a long TE at 7 T. For this purpose, we aimed to modify the PRESS sequence for UHF and validated the MRS data by gas chromatography analysis.

**Materials and Methods**

**Theoretical Considerations for n-3 CH3-specific Echo Timing**

FAAs have long aliphatic chains, which can contain ~10–30 carbons. The simulation of their relaxation behavior presents a complex problem. As Lundbom et al. pointed out, methylene and methyl protons at the end of non-n-3 FA chains are weakly coupled (Δν ≈ J), which allows for an approximation using a CH3 spin system. J-modulation of outer lines (S) of the n-3 CH3 triplet can then be described as a function of TE:

\[
S(TE) = S_0 \cos(2\pi f J TE) \exp(-TE/T_2) \quad (1)
\]

where \( S_0 \) represents the transverse magnetization of the outer triplet n-3 CH3 protons and \( J \) is the coupling constant (7.5 Hz).

Considering the limited B0 homogeneity in vivo MRS, the outer triplet lines of n-3 CH3 in antiphase may improve the spectral resolution of the central lines of CH3 and n-3 CH3 signals. Due to the expected low n-3 FA content in vivo, the n-3 CH3 signal acquired with TE < 200 msec will be overlaid by strong and broad signals from the CH2 and CH3 groups. Thus, TEs longer than 200 msec shall be used to detect the n-3 CH3 signal. As proposed by Škoch et al., the TEs for the measurements of the outer triplets in antiphase can be simply calculated according to:

\[
TE = \frac{1}{j} \left( \frac{1}{2} + k \right) \quad (2)
\]

where \( k = 0, 1, 2, 3, \ldots \). According to this function, the first eight applicable TEs ≥200 msec for n-3 CH3 detection will be: 200, 335,
The simulation of the T2 relaxation behavior of CH2 and CH3 protons with apparent T2 relaxation times of the CH2, CH3, and n-3 CH3 groups measured in oils with an estimated n-3 FA in vivo content is depicted in Fig. 2. As shown in the simulations, the shortest TE for n-3 CH3 detection with a negligible CH2 signal occurrence would be 1000 msec (ultralong TE).

Due to an imperfect signal quantification, B0 inhomogeneity, and the complicated J-modulations of all lipid resonances, the calculated relaxation times should not be considered as true, but rather, as apparent T2 relaxation times. For the sake of simplicity, we will use the term T2 (relaxation) times for measured apparent T2 relaxation times.

**RF Pulse Profiles for 7 T and n-3 CH3 Simulations**

The frequency profiles of the excitation and refocusing RF pulses in the standard PRESS sequence (Sinc pulse with duration = 2.6 msec, Mao pulse with duration = 6.0 msec) and excitation and refocusing Hermite pulses (excitation duration = 2.6 msec, refocusing duration = 3.0 msec) were measured in a silicone oil phantom using a birdcage extremity coil at 7 T, with a spin echo sequence and frequency encoding in the slice-encoding direction (transversal slab; thickness, 20 mm; field of view [FOV], 400 × 400 mm; TE = 230 msec; TR = 5 sec; bandwidth [BW] = 10 kHz).

The n-3 CH3-CH3 spin system was simulated and verified with a pulse acquired sequence with a 2 msec rectangular pulse and TE < 1 msec in the NMRScope-B tool in the jMRUI package.
The symmetric PRESS sequence with Hermite pulses and RF pulses centered at 1 ppm was simulated with TEs of 30 (shortest TE for PRESS) and 1000 msec (ultralong TE) for one spin, to replicate the effect of RF pulse bandwidths with ideal localization, and a 1 mL isotropic voxel, for realistic RF pulse effects and localization.

**MR Hardware and Spectroscopy Sequence**

All MRS measurements were performed on a 7 T Magnetom scanner (Siemens Healthineers, Erlangen, Germany) with a 28-channel knee coil (Quality Electrodynamics, Mayfield Village, OH).

The spectroscopic voxel was positioned according to T1-weighted scout images. The frequency was adjusted on the CH2 signal at 1.3 ppm and the homogeneity of the B0 field was first improved with automatic B0 shimming calculated from gradient echo images, then manually adjusted in an interactive mode. The reference voltage for RF pulses was automatically calibrated by the scanner. A frequency offset of –0.3 ppm was used for all measurements.

The PRESS sequence was equipped with the same Hermite pulses and durations as in the simulation. The signal was acquired with 2048 complex points and the receiver bandwidth was set to 3000 Hz.

**Oil Phantoms**

The oil measurements were performed on three samples with different n-3 FA content: Menhaden fish oil (high n-3 FA content), soybean oil (medium n-3 FA content), and corn oil (low n-3 FA content) (Sigma-Aldrich, St. Louis, MO, USA). The oils were poured into small plastic spheres (Ø 38 mm) and measured at room temperature.

The spectra for the assessment of the T2 relaxation times were measured from a volume of interest (VOI) of 0.5 mL with 60 TEs in a range of 30–1200 msec, with echo steps of 20 msec, TR = 5 sec, and number of acquisitions (NA) = 6.

**In Vivo Measurements**

The study protocol was approved by the Institutional Review Board and the Ethics Committee and written, informed consent was obtained from all participants prior to the study. In vivo MRS measurements were performed on 24 healthy subjects (14 females / 10 males, mean age: 35.7 ± 12.8 years, range: 26–68 years; mean body mass index: 24.7 ± 5.8 kg.m⁻², range: 18.8–49.5 kg.m⁻²). Except for two subjects on a vegetarian diet, none of the volunteers had any other dietary restrictions.

The typical VOI was set to 8 mL and placed in the SAT in the lower part of the thigh. The voxel was positioned just above the knee (3–4 cm), so the knee was at the inferior edge of the coil and the axial scout image was in the center. The in vivo spectra were measured with ultralong TE = 1000 msec, TR = 5 sec, and NA = 32.

**SAT Biopsy**

Five samples taken from the same anatomical SAT region measured with MRS were further analyzed by gas chromatography–mass spectrometry (GC-MS). Whole SAT samples from the knee region were obtained from three subjects who underwent either elective liposuction of the legs or elective arthroscopy of the knee joint. Subjects were excluded if they presented with leg ulcers or soft-tissue infection of the legs or suffered from any relevant systemic disease that would exclude patients from local anesthesia. SAT biopsies were performed at the end of the procedures, shortly before suture, by cutting a piece of SAT that did not exceed 3 g, or ~1 cm³, from a site along the incision line at the front of the knee. Samples from another two subjects were obtained under local anesthesia by the Bergström technique, as described in detail previously.²¹ All samples
were immediately snap-frozen and stored at −80°C until GC-MS analysis.

**Gas Chromatography–Mass Spectrometry**

About 1–2 mg of oil or tissue was homogenized in a 500 μL methanol:chloroform 1:2 solution. Each sample was spiked with C19:0 FA as an internal standard and lipids were extracted according to Folch et al. Lipid extracts were evaporated under a gentle stream of nitrogen. Then the samples were derivatized with 2 mL methanol:toluene 4:1 with 200 μL acetyl chloride at 100°C by continuous stirring. After 60 minutes, the samples were cooled to 4°C and 5 mL of 6% potassium carbonate solution was added. After centrifugation, the toluene layer was collected and used for GC-MS analysis.

A 6890 N/59730 N GC-MSD system (Agilent, Santa Clara, CA, USA) with a DB-23 30 m-column was used. Helium was used as a carrier gas with 1.0 mL/min flow in a solvent vent modus using a PTV-injector with a 250°C injection temperature. The initial oven temperature of 50°C was held for 4 minutes, then increased to 180°C at a rate of 10°C/min and held for 5 minutes, which was subsequently increased to 240°C at a rate of 5°C/min and held for 2 minutes and finally increased to 250°C at a rate of 3°C/min.

The mass spectrometer was run in the electron impact mode with 200°C injection temperature. The mass spectrometer was run in the electron impact mode with a DB-23 30 m-column was used. A mass spectrometer was used as a carrier gas with 1.0 mL/min flow in a solvent vent modus using a PTV-injector with a 250°C injection temperature. The initial oven temperature of 50°C was held for 4 minutes, then increased to 180°C at a rate of 10°C/min and held for 5 minutes, which was subsequently increased to 240°C at a rate of 5°C/min and held for 2 minutes and finally increased to 250°C at a rate of 3°C/min.

The mass spectrometer was run in the electron impact mode where the FA were detected in a scan mode of m/z 33–400. The source temperature was set to 230°C, the quadrupole temperature to 150°C, and the transfer line temperature was 260°C.

**MRS Data Postprocessing and Analysis**

The CH2, CH3, and n-3 CH3 signals were quantified in jMRUI with the AMARES algorithm. In oils, only the CH2 peak and central peaks of the methyl triplets were fitted with a single Gaussian line (oil prior knowledge). The T2 relaxation times were calculated in MATLAB (MathWorks, Natick, MA, USA), with the following monoexponential fitting function:

\[
S(TE) = S_0 \exp(-TE/T_2)
\]  

where \(S_0\) represents the signal (magnetization) at TE = 0 msec. The coefficient of determination (R^2) was used as an indicator of the quality of the fit.

In vivo, the CH2 and central n-3 CH3 peak were fitted with single Gaussian lines and the CH3 peak was fitted with two Gaussian lines (in vivo prior knowledge). Since the J-modulation of the CH3 group was unknown, outer triplet CH3 peaks were approximately fitted with a second Gaussian line to minimize a possible influence on the quantification of the relatively small n-3 CH3 peak.

Spectral quality was estimated according to the full width at half maximum (FWHM) of the CH3 signal measured at TE = 30 msec (FWHMCH3), the FWHM of the CH2 signal measured at TE = 1000 msec (FWHMCH2), and SNR, calculated as the ratio of the CH3 signal amplitude measured at TE = 1000 msec to the last 200 points of free induction decay. Exclusion criteria for in vivo spectra were a FWHMCH3 >30 Hz and SNR <15.

The n-3 FA content in oils was calculated as the ratio of the n-3 CH3 signal to the sum of both methyl signals measured with TE = 1000 msec after the T2 relaxation correction:

\[
\frac{I_{n-3CH3}}{I_{CH3}} \times 100\% \tag{4}
\]

where \(I_{CH3}\) and \(I_{n-3CH3}\) are the signal intensities after correcting for T2 relaxation, and are given in percent. To test in vivo prior knowledge, the n-3 FA content in oils was also calculated from line-broadened spectra adjusted to in vivo spectral quality.

The in vivo n-3 FA fraction in SAT (in arbitrary units) was calculated according to:

\[
\frac{I_{n-3CH3}}{I_{CH3} + I_{n-3CH3}} \tag{5}
\]

where \(I_{CH3}\) and \(I_{n-3CH3}\) are the signal intensities measured with TE = 1000 msec, and are given in arbitrary units (a.u.).

Quantification of FA with GC-MS was performed by correlating the integrated peak areas of FA with the integrated area of C19:0, which was used as an internal standard. The n-3 FA content by GC-MS was calculated as the ratio of n-3 FA to total FA. In vivo MRS data were compared to GC-MS with the Pearson correlation coefficient. All data are reported as the mean ± standard deviation (SD) and \(P < 0.05\) was considered statistically significant. The amplitudes of MRS signals are shown in a.u.

**Results**

**RF Pulses and Sequence Simulations**

The experimentally measured FWHM of the Sinc pulse was 3.4 kHz and the Mao pulse was 1.1 kHz. The FWHMs of the excitation and refocusing Hermite pulses were 1.6 kHz and 1.1 kHz, respectively. The flat frequency range of both Hermite pulses was ~500 Hz (1.7 ppm at 7 T). The frequency profiles of all RF pulses are shown in Fig. 3A,B. Accounting for the center of the RF pulses (1.0 ppm), this range covered nearly all the relevant chemical shifts of methylene and methyl protons, which is depicted in Fig. 3C. The simulation of the n-3 methyl group with the pulse acquired sequence confirmed our spin model, and is shown in Fig. 3D. The simulated imperfections of the Hermite pulses and PRESS localization are depicted in Fig. 3E,F for TEs of 30 and 1000 msec, respectively.

**Detection of n-3 CH3 and T2 Relaxation in Oil Phantoms**

The spectral triplet pattern of the CH3 and n-3 CH3 groups was clearly visible in the observed region by the application of a properly adjusted long TE in all oil phantoms. Reference spectra showing CH2, n-3 CH3, and CH3 signals from all three oils measured with TE = 30 and 1000 msec are depicted in Fig. 4. The n-3 CH3 signals in the measured spectra were in agreement with the simulations.

The maximum FWHM in all oils for the central CH3 peak was <4.5 Hz and, for the central n-3 CH3...
peak, <3.2 Hz. The T2 relaxation of all central peaks was influenced by the J-modulation from the outer triplet peaks.

The measured T2 relaxation behavior of the CH2 group, as well as the central signals of the CH3 and n-3 CH3 groups for the three oil samples, with calculated T2 times for the CH3 groups, are depicted in Fig. 5. The T2 relaxation times of all groups together with their R2 values are shown in Table 1.

Detection of n-3 CH3 In Vivo

The thighs of all subjects fit easily in the knee coil except for one case, when the MRS had to be performed in SAT in the calf due to an excessive amount fat in the thigh (BMI ~50 kg/m2). A typical localization of a voxel in the SAT is depicted in Fig. 6A,B. The spectral quality for the detection of the n-3 CH3 signal was sufficient in all volunteers; however, in two cases the very small amount of SAT in very lean and athletic males forced a doubling of the number of acquisitions. The n-3 CH3 resonance, measured with ultralong TE and NA = 32, was clearly resolved in vivo with the advantage of the suppression of the strong CH2 signal, as depicted in Fig. 6C. Signal fitting with Gaussian line-shapes yielded an acceptable fit with minimal residual signals, as shown in Fig. 6D.

FIGURE 3: Excitation and refocusing bandwidth of Sinc and Hermite pulses measured in phantoms (A,B) (500 Hz = 1.7 ppm at 7 T). Both excitation and refocusing pulses in the PRESS sequence used in this study could excite and refocus methylene and methyl protons in the n-3 FAs (blue) and the non-n-3 FAs (red) (C). The spin system of the n-3 CH3 used for simulation was demonstrated with a pulse acquire sequence (rectangular pulse with a duration of 2 msec) (D). The effects of standard Mao and Hermite pulses for one spin and for a VOI of 1 mL, respectively, were simulated for short and ultralong TEs (E,F). All simulations were apodized with a 2 Hz Lorentzian filter.

The mean FWHMCH3 in vivo was 26.9 ± 4.0 Hz, the FWHMCH3 for CH3 was 13.3 ± 4.1 Hz and, for n-3 CH3 was 11.5 ± 2.4 Hz. The mean SNR was 159 ± 111. There was no significant correlation found between the FWHMCH3 and SNR.

Based on the mean values of the FWHMCH3 measured from in vivo spectra, a line-broadening using a 12 Hz Gaussian filter was applied on oil spectra measured with a TE of 1000 msec. The mean FWHMCH3 after the line-broadening for CH3 was 13.4 ± 1.0 Hz, and for n-3 CH3 was...
11.0 ± 0.1 Hz. The example of a soybean oil spectrum before and after line-broadening, together with fitting with oil prior knowledge and in vivo prior knowledge, is depicted in Fig. 6E,F, respectively.

**Quantification and Validation of the n-3 FA Content in Oils and the n-3 FA Fraction in SAT**

The n-3 FA content in oils yielded a high correlation with data from the GC-MS analysis, which is shown in
TABLE 1. Individual T2 Relaxation Times of CH2, CH3, and n-3 CH3 Groups in Oils and Mean T2 Relaxation Times in Oils and In Vivo

|                | T2: CH2 [msec] | R2: CH2 | T2: CH3 [msec] | R2: CH3 | T2: n-3 CH3 [msec] | R2: n-3 CH3 |
|----------------|---------------|---------|----------------|---------|--------------------|-------------|
| Fish oil       | 90            | 0.996   | 318            | 0.949   | 651                | 0.877       |
| Soybean oil    | 53            | 0.990   | 331            | 0.977   | 644                | 0.946       |
| Corn oil       | 51            | 0.992   | 325            | 0.982   | 589                | 0.926       |
| Oils (n = 3)   | 65 ± 22       | 0.993 ± 0.003 | 325 ± 7 | 0.969 ± 0.018 | 623 ± 34 | 0.916 ± 0.036 |

All T2 relaxation times are shown with individual and mean R2 values.

Fig. 7A. The oil spectra after line-broadening correlated with GC-MS as well (Fig. 7B).

The n-3 FA fractions in vivo from all volunteers ranged from 0.085–0.252 and the mean fraction was 0.149 ± 0.042. The mean n-3 FA fraction in females (n = 14) was 0.140 ± 0.036, ranging from 0.085–0.228, and in males (n = 10) was 0.161 ± 0.046, ranging from 0.111–0.252. This difference was found to be not significant. No significant correlations were found between age, BMI, and n-3 FA fraction (in a.u., without T2 correction).

All five volunteers chosen for the SAT biopsy tolerated the procedure well. The calculated n-3 FA fractions from the SAT biopsy yielded significant correlation with the data from the GC-MS analysis (P = 0.029), and is depicted in Fig. 7C.

Discussion

A single-voxel PRESS sequence with TEs up to 1200 msec was used to detect n-3 CH3 in three oil samples with different n-3 FA fractions, and in vivo. An ultralong TE of 1000 msec, high spectral resolution, and an SNR of 7 T magnetic field enabled the detection of the n-3 CH3 signal in all three oil samples with different n-3 FA content and in the SAT of all 24 healthy subjects as well. In the presented work, the T2 relaxation behavior of the non-n-3 and n-3 CH3 groups was investigated in oils, with an assessment of their apparent T2 relaxation times. The calculated n-3 FA content in oils and n-3 FA fractions in vivo were validated by GC-MS analysis.

The spin-echo-based 1H PRESS single-voxel method is the sequence of choice due to its wide availability on MR systems. It provides higher SNR than the STEAM24 and requires less power than semi-LASER25 or LASER.26 In addition, PRESS is more sensitive to J-coupling than STEAM27; therefore, it has been used for n-3 FA detection in previous studies as well. Due to the increased SAR demands on 7 T, we used Hermite excitation and refocusing pulses,16,17 which require less power at the cost of a relatively narrow bandwidth.

The PRESS sequence is sensitive to chemical shift displacement error, which increases with B0. The Δν between the CH2 and the CH3 group is 0.4 ppm and between the n-3 CH2 and the n-3 CH3 group is 1.1 ppm, which makes the chemical shift displacement error relatively small. For the typical 8 mL cubical voxel, the displacement in one direction would be -12% and -33%, respectively. This relates to "four-compartment" chemical shift artifacts, which could decrease the spectral quality.28 This could explain the lower intensity of the outer n-3 CH3 signals in antiphase, as expected, by a 1:2:1 ratio with the central signal of the triplet, which was shown in the simulations. The frequency offset of the RF pulses could also be adjusted on the methylene resonance at 1.3 ppm or even further, eg., in the frequency between n-3 CH2 and n-3 CH3 (1.5 ppm), but, as the results of our prior experiments with oil phantoms have shown, this did not improve the spectral quality.

A spectral editing approach in SAT with oMEGA-PRESS was proposed by Skoch et al.,9 and later, with MEGA-sLASER by Lindeboom and de Graaf29 at 3 T and 4 T. Nevertheless, these techniques depend on the J-coupling, which we aimed to minimize, and they would also require an additional calibration for 7 T applications. Another localized method that uses the natural abundance of 13C to detect FA in SAT at 7 T was proposed by Lindeboom and de Graaf.29 This method enables the detection of several unique resonances for the assessment of FA saturation and the n-6/n-3 FA ratio, but requires a special double-tuned 1H/13C coil for the heteronuclear pulse sequence.

With regard to the issue of spectral line quantification, it was shown that the use of different algorithms can lead to different analytic results.30 AMARES quantification software offers an easy and robust solution for the quantification of MR signals without the simulation of metabolite basis sets, which, in the case of large molecules such as FA, could be very difficult. The ideal spectral line-shape for lipids could be characterized by the Voigt function;31 however, this line-shape is not included in the AMARES quantification. The Voigt line-shape can be approximated by a combination of Lorentzian and...
Gaussian functions. From our experience, the Lorentzian line-shape of the stronger CH$_3$ signal can underestimate the amplitude of the minor n-3 CH$_3$ signal, and using the Lorentz-Gaussian time domain filtering function may result in small, but unknown, variations in signal amplitudes.\textsuperscript{32} Instead of this combination and filter, two Gaussian lines with a fixed frequency were used to fit the CH$_3$ signal and their ratio was automatically estimated by AMARES.

The magnetic field of the whole-body 7 T scanner allowed for sufficient spectral resolution of the CH$_3$ and n-3 CH$_3$ triplets.

FIGURE 6: The typical position of the voxel (yellow cube) on axial (A) and coronal images (B) in SAT in a female volunteer. The corresponding in vivo proton spectrum (FWHM$_{CH_3}$ = 9.6 Hz, SNR = 227) measured with an ultralong TE of 1000 msec and NA = 32, showed a disappearing CH$_2$ signal and a clear separation of the n-3 CH$_3$ and the CH$_3$ signals (C). Peak fitting of methylene and both methyl signals with Gaussian line-shapes resulted in minimal residual signals (D). An example of soybean oil spectra fitted with oil prior knowledge (two Gaussians, E) and the same spectrum with a line-broadening filter fitted with in vivo prior knowledge (three Gaussians F).
in the oils. Our results confirmed the relatively long and different $T_2$ relaxation times of both methyl groups and the short $T_2$ relaxation times of the methylene groups, which enabled their better resolution and quantification with the ultralong TE, especially for the low n-3 FA-concentration corn oil.

$J$-modulations of the outer triplet peaks of the methyl groups influenced the measured $T_2$ decay of their central peaks; however, this effect did not hamper the calculation of the $T_2$ relaxation times. Because the central peaks were not affected by imperfections caused by the RF pulses and localization sequences, quantification with oil prior knowledge by fitting only the central peaks was feasible. This was demonstrated by the high correlation of their n-3 FA content with the GC-MS analysis.

The line-broadening of oil spectra caused the merging of the two methyl triplets into two partially overlapping peaks. These peaks contained signal from all the methyl protons. The quantification of the line-broadened signals with in vivo prior knowledge showed a high correlation with GC-MS analysis as well, and confirmed this approach for in vivo quantification.

The resonance frequencies of CH$_3$ and n-3 CH$_3$ are adjacent, and therefore, their distinction is extremely sensitive to $B_0$ field inhomogeneity. The in vivo signal measured closer to the skin experienced a slightly worse $B_0$ field due to the difference in magnetic susceptibility in the air–tissue boundary. In general, the FWHM of the CH$_2$ signal in magnitude mode during the $B_0$ shimming had to be less than 40 Hz in order to successfully resolve both methyl signals. Measurements with a TE of 67 msec could be chosen for n-3 CH$_3$ detection as well, but this would require a quite homogeneous $B_0$ with a small VOI (<1 mL). The suppression of the strong CH$_2$ signal could also be an option; however, this would require sequence optimization similar to water suppression techniques, which was not the aim of this study.

The assessment of n-3 FA is of great importance for understanding the metabolism of lipids. In the study by Ouldamer et al., GC of FA from the SAT extracts from rats showed high correlation with $^1$H MRS analysis. The n-3 FA fraction in the control group was 0.9%, and the n-3 FA content changed with dietary supplements. The n-3 FA fraction in SAT measured with in vivo MRS at 3 T has already been reported in several studies. Škoch et al. reported a content of 1.6 ± 0.5% in the dorsal waist assessed with the J-difference editing method with a TE = 199.5 msec. Lundbom et al. reported the n-3 FA fraction in the same body area measured with TE = 540 msec in a range from 0.09–0.2%, with a range from the GC analysis of 1.0–1.8%, which represented a significant agreement ($P < 0.05, n = 8$). There are three n-3 FA in human lipid metabolism: α-linolenic acid (ALA, 18:3n-3); eicosapentaenoic acid (EPA, 20:5n-3); and docosahexaenoic acid (DHA, 22:6n-3), and, although the exact composition of n-3 FA would be desired, current localized $^1$H MRS techniques are limited to the detection of only the n-3 CH$_3$ signal from the mixed content of those FAs.

Our GC-MS results, ranging from 0.8–1.5%, were in agreement with previously published data. The reported SAT n-3 FA fraction in this study should not be compared with the FA content because the signals were not corrected for $T_2$ relaxation. A rough estimation of n-3 FA content would be possible by using a linear regression analysis; however, this approach assumes constant $T_2$ relaxation times of methyl signals, which is questionable at this point. The proposed MRS method yielded a realistic validation of the aforementioned method and was in line with the literature.

Heavy $T_2$-weighting of methyl signals in vivo is one important limitation of this study. Although the assessment of $T_2$ relaxation times of methyl groups in vivo was not the aim of this pilot study, their knowledge is critical for future research into n-3 FA using long TEs. The SAT in vivo can contain various FAs with different saturations and can be site-specific. Oil phantoms are often successfully used as appropriate materials for proof-of-concept; however, for MRS measurements of subtle signals with ultralong TE, even small

### FIGURE 7: Scatterplots of the n-3 FA content in oils (A), the n-3 FA content in oils calculated from line-broadened spectra (B), and the n-3 FA fraction in subcutaneous adipose tissue (C) measured with a TE = 1000 msec and compared to GC-MS analysis.
variations in FA content in vivo could result in different relaxation behavior. Assessment of T2 relaxation times with TEs < 1000 msec will pose a problem for B0 homogeneity due to increasing overlap between CH2 and n-3 CH3 signals with shorter TE. There are several techniques for advanced B0 shimming,\textsuperscript{40,41} which could improve the spectral resolution and allow detection of n-3 CH3 signal with shorter TE. In addition, the T2 times could be calculated using a monoexponential function when TEs are selected according to Eq. 2. As already mentioned, the strong CH2 signal could be theoretically suppressed enabling using short TE (TE < 200 msec). The spectra were averaged online, which, in the case of movement of a subject, would be translated into broader linewidths. This effect can be resolved in frequency alignment and phase corrections for each transient in postprocessing in future studies. All our subjects had a sufficient layer of subcutaneous fat, but MRS in lean individuals with a small amount of fat would require very small voxels (<1 mL) and manifold NA, which could prolong acquisition time significantly. The SAT biopsy and the sample analysis is an invasive and costly technique, and, for a larger sample size, would require more time and resources, which were limited for this study. As we did not perform any test–retest measurements and we analyzed only five samples with GC-MS, further in vivo measurements of the SAT in different areas and biochemical validation from biopsy samples of a broader population are necessary.

Considering these benefits and limitations, this pilot work on direct n-3 CH3 detection in vivo at ultrahigh fields could be very useful for preclinical studies. Because of the simplicity of this method, it can be complementary to other metabolic MRS studies of SAT, muscles, or bone marrow.

In conclusion, the differences in T2 relaxation times between the CH2, CH3, and n-3 CH3 signals enabled the identification of well-resolved n-3 CH3 resonances, making PRESS with ultralong TE a method suitable for the direct detection of n-3 FA at 7 T. This work proposed a relatively simple approach for the reliable estimation of the n-3 FA fraction, in vivo, in a sample of healthy volunteers.

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