Western societies consume fat abundantly. The Paleolithic motto of “eat plenty when food is bountiful” is causing obesity rates of epidemic proportions in affluent societies. Hand-in-hand with excess calories come cardiovascular disease, diabetes and cancer. It is also becoming increasingly clear that what we eat matters as much as how much we eat. Predisposition to cancer [1, 2], type 2 diabetes [3-6] and heart disease has been attributed to diets high in saturated fats [7]. Current research has also focused on osteoporosis being caused by “bone obesity” [8]. Despite a considerable number of studies, the precise causal relationship between dietary lipids, adipose tissue composition and the risk of disease remains elusive and difficult to study, in part because of the traditional requirement for invasive biopsy. The ability to profile triglyceride composition non-invasively using proton spectroscopy would allow for simple integration of these tests with standard Magnetic Resonance Imaging (MRI) exams. A systematic analysis of fatty acid composition from 1H magnetic resonance spectroscopy (MRS) in humans has not been reported, likely because it is difficult to resolve the signals from protons on, or adjacent to, double bonds at 1.5, or even at 3T. Thus, the majority of MRS studies of lipid content in human tissues have focused on measuring fat fraction, calculated from the ratio of total triglycerides to water, in non-water suppressed spectra [9-11]. Alternatively, 1H-decoupled 13C MRS has been developed as a non-invasive alternative to biopsy for analysis of adipose tissue composition [12-16]. The chemical shift dispersion of 13C is a major advantage in measuring lipid signals from the various proton groups, but the need for a second RF channel and proton decoupling limits its clinical applicability. In order to achieve higher chemical shift separation, two-dimensional MRS has also been used at 3T for investigating muscle lipid metabolism [17]. However, MRS requires long acquisition times and may be of limited clinical acceptance.

In this article we report on a research method to measure in vivo lipid composition by 1H MRS at 7T. We also report the research for a non-invasive determination of iodine values of human fat. The iodine value of tissue shows the oxidative stability of the fat in the tissue and may prove to be an important index in determining predisposition to diseases involving oxidative metabolism.

There are two fundamental advantages of working at 7T that allow for fat composition quantification:

- The most important advantage is the larger chemical shift dispersion. Chemical shift dispersion is $B_0$. Given that in vivo signals have line widths of 20 - 30 Hz, a separation of at least 10 - 15 Hz is required in order to distinguish reliably between two peaks. At 1.5T this means that peaks cannot reliably be separated that are closer than 0.25 ppm. At 7T this translates to only 0.05 ppm! As an example, in Figure 1, while signals D and E are not distinguishable at 1.5T, and signal C severely overlaps signal B, no such complications are seen on the 7T spectrum.
• Higher signal-to-noise ratio (SNR), SNR = B0. For example, the 7T lipid protocol described below takes less than one minute to acquire. This reduces potential motion artifacts by significantly reducing the total examination time.

One of the biggest disadvantages at 7T – the higher field inhomogeneities (AB - B0) – is successfully mitigated on the Philips Achieva 7T platform with the availability of third-order shimming hardware. Furthermore, some of the high SNR can be traded for smaller voxels which will improve the shim. Although the SNR is - (voxel size)3, since the regional inhomogeneities dominate the determination of the line width, reducing the voxel dimensions will increase T2*, and this will partially compensate the SNR for the decreased voxel size. It has been shown [18] that the SNR falls only as - (voxel size)2, due to the increased T2*. Thus, decreasing the voxel size by a factor of two will reduce the line widths by two and the SNR only by four (not eight).

Methods

Single voxel 1H MR spectra of muscles in the calf were obtained from healthy adults on a 7T Achieva scanner (Philips Healthcare, Cleveland, OH, USA) using a partial-volume quadrature transmit/receive coil. The protocol was approved by the local Institutional Review Board, informed consent was obtained from the subjects and the exam was well-tolerated by all subjects.

It is well-known that 1H MRS is a sensitive reporter of the relative concentration of different lipid components. Depending on the specific fatty acids, deconvolution of the signal from a mixture of fats is feasible using high-resolution MRS. At low field strengths, and especially in vivo, shimming, motion and imperfect water suppression limit the chemical shift resolution of triglycerides to mostly that of the methyl groups at 0.9 ppm, methylene groups at 1.3 ppm and triglycerides to mostly that of the methyl groups at 5.3 ppm. In comparison, at 7T up to ten resonances can be distinguished, designated with letters A - J in Figure 1. The challenge therefore is to obtain maximal information about fat composition from these well-separated signals. Of the ten resonances, six contribute equivalent information about the total number of triglyceride molecules: the CH3 methyl protons (A); the CH2, methylene protons α-(E) and β-(C) to the carbonyl; and the glycerol backbone CH (I) and CH2 protons (G and H).

At the chemical shift resolution achievable at 7T, there are four other informative resonances to consider: 1) bulk CH3 methylene protons at 1.3 ppm (B); 2) allylic CH2 protons, α- to a double bond, at 2.02 ppm (D); 3) diallylic (also called bis-allylic) CH2 protons at 2.75 ppm (F); and 4) olefinic, double bond -CH=CH- protons, at 5.31 ppm (J), which partially overlap with the glycerol CH methine proton at 5.21 ppm (I).

The 1H chemical shift of in vivo fat resonances from bone marrow and subcutaneous tissue was assigned such that the methyl signal was at 0.9 ppm. Resonance areas were determined by fitting the spectra with Voigt shapes (variable proportions of Lorentzian plus Gaussian) after phasing and baseline correction. Peak areas for each individual resonance were corrected with its corresponding T1 and T2. After relaxation corrections, the lipid composition was evaluated as follows.

Although signals from a high-resolution 1H NMR spectrum of triglyceride mixtures may be resolved into most constituents, in vivo spectroscopy is constrained by limited chemical shift resolution. The problem in vivo can be simplified, without losing essential information, by assuming that all fatty acids contain either 0, 1 or 2 double bonds. These three types of fatty acids account for ~97% - 98% of total fat. Biologically, trilinolenic acid (18:3) is the only fatty acid that is excluded in this simplification but it contributes only ~0.5% of the total triglycerides [19]. With this assumption,

\[ f_{\text{sat}} + f_{\text{mono}} + f_{\text{poly}} = 100\% \]  

where \( f_{\text{sat}} \), \( f_{\text{mono}} \) and \( f_{\text{poly}} \) refer to the fraction of fatty acids that are saturated, monounsaturated and polyunsaturated, respectively. In the following, letters A - J represent the areas of the respective MRS signals. First, the signal unit area per proton can be arbitrarily assigned as \( U = E/6 \), since there are always six methylene protons α- to COO (resonance E) in every triglyceride molecule. There are alternative ways of determining the unit proton area from the spectra and one of them is utilized later in determining the iodine value (IV). The fraction that is polyunsaturated, \( f_{\text{poly}} \), can be calculated directly from the relative area of the resonance of the six “bridging” diallylic protons (resonance F):

\[ f_{\text{poly}} = \frac{F/6}{E/6} = F/E \]  

Once \( f_{\text{poly}} \) is known, \( f_{\text{mono}} \) can be evaluated from the relative area of protons α- to a double bond (resonance D) by:

\[ f_{\text{mono}} = \frac{(D/12)/(E/6) - f_{\text{poly}}}{0.5 * D/E - f_{\text{poly}}} \]  

where resonance D is divided by 12, since this is the number of protons α- to a double bond in

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Here 15.034 is the molecular weight of CH₃, 14.026 of CH₂, 173.1 of the glycerol backbone plus the three carbonyl groups (C₆H₅O₆) and 26.04 of CH=CH. In this calculation, the unit average proton area is designated as:

\[ U_{av} = \frac{[A/9 + C/6 + E/6 + (G + H)/4 + I]}{5} \]  

(6)

This averaging is done in order to minimize variations in signal intensity due to unknown underlying macromolecular peaks and possible rolling baseline.

**Results**

As seen in Figure 2, typically ten lipid resonances are observed in the 7T ¹H spectra from physiological fats, together with a small water peak. The lipid resonances are well-resolved at 7T, except for the double bond protons which partially overlap the methine proton of the glycerol backbone (\( \Delta \delta = 0.1 \) ppm). For lipid composition quantification, the signal intensities were corrected for the difference in T₁ and T₂.

Proton resonances at different structural positions have quite different values of T₁ and T₂, with T₁ in the range of 0.39 - 1.13 s and T₂ in the range of 40 - 66 ms. The study showed that for quantification of fat composition, the T₂ correction is much more important than the T₁ correction: for a typical echo time (TE) = 11 ms, the relative T₂ correction among the resonances D, E and F accounts for ~4%, but reaches 8% at TE = 20 ms and is as large as 18% at TE = 40 ms. This compares to a correction of only about 1% from the T₁ correction for the same three resonances at a recovery time (TR) = 2 s. The fractions of saturated, monounsaturated and polyunsaturated fat constituents were calculated as given by Equation 1-3: The composition of bone marrow and adipose fat were similar, with an average 27% saturated, 48% monounsaturated and 25% polyunsaturated fractions. These values are in agreement with values obtained by thin-layer chromatography [19].

The lipid IV index was also determined from the relative peak areas, as given in Equation 4, to be 75. The average lipid molecular weight was calculated to be 912. This is about 7% higher than the value of 855, which is the expected average lipid molecular weight measured by thin layer chromatography [20]. This overestimation is probably due to contamination of the peak intensities with additional intensities from macromolecular pools with chemical shifts overlapping the lipid resonances. In addition, the fitted data is seen to overestimate the bulk CH₃ protons signal for which a single Lorentzian/Gaussian does not truly represent its line shape. Also, the rest of the lipid signals were fitted as singlets, whereas it is known that
they are multiplets. Future work will incorporate this additional knowledge in fitting the *in vivo* spectra.

For the *in vivo* studies, a typical single voxel MRS of marrow and subcutaneous tissue only takes ~1 minute with excellent SNR which allows for rapid testing of instrumental and protocol reproducibility. This study showed that under repeated scans, the spectral variation in the same voxel was typically less than 5%. This is comparable to, or less than, the typical post-processing errors caused by fitting imperfect line shapes, which could be as much as 10% in total.

**Future work and conclusions**

In addition to measuring fat composition in subcutaneous fat and bone marrow, investigation is underway to profile muscle fat composition. The complexity of fitting *in vivo* human muscle spectra increases dramatically due to the effective “doubling” of the spectra, with signals from both extramyocellular (EMCL) and intramyocellular (IMCL) lipids [21]. Figure 3 shows the rich informational content of 7T *in vivo* human muscle spectroscopy. The additional information contained in the spectrum may be invaluable in evaluating diabetes progression, as the connection between insulin resistance and intracellular lipids is well-known [22]. What is not known is if the composition of the intracellular lipids matters and 7T MRS will be essential in answering such fundamental metabolic questions.

Recently, others have shown that high-quality 1H NMR spectroscopy of mouse adipose tissue is feasible at 7T [23]. The analysis used to quantify fat composition was similar to that described here. Like this earlier work, we chose to present the data as saturated, monounsaturated or polyunsaturated fractions. We believe this description is more likely to be accepted by clinicians than alternative classifications. This analysis also follows closely the widely popular nutritional facts information printed on food labels. Such similarity may facilitate further acceptance and understanding amongst the general population.

In conclusion, this 1H MRS study at 7T shows that it is possible to quantify the fat composition of human subcutaneous and bone marrow in a fast (~1 minute) and non-invasive manner. This has the potential to facilitate longitudinal monitoring of lipid changes in healthy and diseased tissues in clinical practice.

**References**


