# Fat Compostion Determination via Combined $^{13}$C and $^{1}$H MRS at Ultra High Field

Sergey Cheshkov[1,2], Ivan Dimitrov[1,3], Karlos Moreno[1], and Craig Malloy[1,2]

[1]*Advanced Imaging Research Center, University of Texas Southwestern Medical Center, Dallas, TX, United States,* [2]*Radiology, University of Texas Southwestern Medical Center, Dallas, TX, United States,* [3]*Philips Medical Systems, Cleaveland, OH, United States*

**INTRODUCTION:** Studies indicate that composition of body adipose tissue, which is sensitive to diet, may predispose to cancer [1,2]. Utilizing the increased chemical shift dispersion, proton MRS at 7T has been proposed as a non-invasive tool for fat composition determination in human calf muscle [3] and breast [4] under the assumption that the detected fatty acids contain 0, 1, or 2 double bonds [3]. Compared to proton MRS, $^{13}$C with its large chemical shifts provides significant informational advantage in measuring multiple additional lipid fractions. An example is the ability of $^{13}$C MRS to measure the ω-6/ω-3 ratio [5,6] and *trans*-fats [7]. In this work we investigate a combined $^{13}$C and $^{1}$H MRS protocol, taking advantage from the high specificity of $^{13}$C and high signal-to-noise of $^{1}$H spectrum to perform more reliable lipid composition determination and to quantify some of the minor lipid components *in vivo*.

**METHODS:** Human data were acquired on a whole-body 7T scanner (Achieva, Philips Medical Systems, Cleveland, OH, USA) using a transmit/receive quadrature $^{1}$H/$^{13}$C calf coil under IRB approved protocol. Non-localized $^{13}$C spectra were acquired by averaging 64 acquisitions with TR 8 s for a total scan time of 11 min. One offset was used, centered on the $CH_2$ envelope of the fingerprint region (~ 29 ppm). Decoupling (WALTZ-16) with an 18 μT proton pulse centered at 1.3 ppm and NOE (10 μT at 5% duty cycle and a mixing time of 1.5 s) were used to simplify the spectra and enhance SNR. Scans were acquired with BW 16 kHz and 2k points. Proton SVS MRS was acquired from a 5x5x5 mm voxel with STEAM series of TE=27,28,29,30 ms, TR/TM=2500/23ms, BW 4kHz and 4k points, scan time of 3:30 min. A second acquisition followed with all selection and dephasing gradients inverted to eliminate frequency modulation sidebands caused by the large lipid peak at 1.3 ppm and eddy currents [4]. Additionally olive oil (in $CDCl_3$) phantom spectra ($^{1}$H and $^{13}$C) were acquired on the 7T scanner and on a high-resolution 600MHz spectrometer.

*Mathematical modeling*: We assumed that all fat content can be adequately represented by the following 5 fatty acids: palmitic (16:0; length of carbon chain : number of double bonds), palmitoleic (16:1), oleic (18:1), linoleic (18:2), and linolenic (18:3). The resolvable resonances in the fingerprint region of the $^{13}$C spectrum and the number of carbon nuclei contributing to each peak are listed in Table 1. Linear equations were solved via constrained (non-negative requirement on all fat fractions) least squares method in Matlab (MathWorks, Inc.): where the rows of matrix *A* are $\frac{1}{2}\min_x \|Ax-b\|_2^2$ formed by the columns of Table 1. Vectors x (non-negative) and b represent the unknown fatty acid fractions and the measured spectrum peak intensities (integrals), respectively. The information from the $^{1}$H spectrum is also included as additional rows in *A* e.g. palmitoleic+oleic=mono-unsaturated fat fraction; linoleic+linolenic=poly-unsaturated fraction.

**RESULTS AND DISCUSSION:** Proton-decoupled $^{13}$C spectra of olive oil (at a high resolution 600 MHz and at a whole-body 7T) and *in vivo* human calf are presented in Figure 1. Following [3] we obtained poly-unsaturated and mono-unsaturated fractions from the simultaneously acquired $^{1}$H spectra (Figure 2). This information was used together with the linear equations from $^{13}$C peaks to produce the fatty acid fractions shown in Table 2. They are in close agreement with the values for olive oil and normal human calf. For comparison purposes, the olive oil fat fractions at 600MHz (not possible *in vivo* at 7T) can be also independently determined by analyzing solely the omega-3 region (Figure 3) of the carbon spectrum. Olive oil values determined by the different methods are in reasonable agreement with each other.

**CONCLUSIONS:** The improved chemical shift dispersion at 7T enables quantitation of specific classes of fatty acids rather than description of the fraction of 0, 1 or 2 double bonds. Combined $^{1}$H and proton-decoupled $^{13}$C MRS presented here provides complementary and also over-determined information on the fat composition.

**REFERENCES:** [1] E. Cho et al., J. Natl. Cancer Inst. 2003;95: 1079-85. [2] P. Bougnoux et al., Cancer Epidemiol Biomarkers Prev 2006;15(3). [3] J. Ren et al., J Lipid Res. 2008 Sep;49(9):2055-62. [4] I. Dimitrov et al., Magn Reson Med. 67(1):20-26 (2012). [5] I. Dimitrov et al., Intl. Soc. Mag. Res. Med. 18, 320 (2010). [6] S. Cheshkov et al., Intl. Soc. Mag. Res. Med. 20, 4428 (2012). [7] I. Dimitrov et al., Intl. Soc. Mag. Res. Med. 18, 374 (2010).

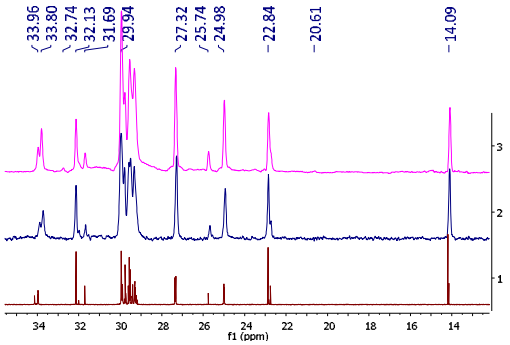


**Figure 1.** High spectral specificity seen in the fingerprint region of the $^{13}$C spectra: olive oil (600 MHz), olive oil (7T), human calf (7T) (bottom to top).

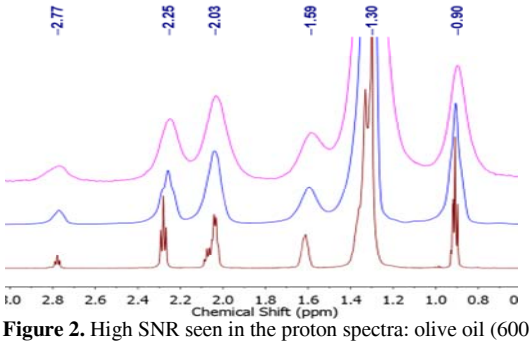


**Figure 2.** High SNR seen in the proton spectra: olive oil (600 MHz), olive oil (7T), human calf (7T) (bottom to top).

| peak | αCO | β-CH3 | β-CH3 | β-CH3 | $CH_2$ env. | allylic | diallylic | β-CO | α-$CH_3$ | $CH_3$ |
|---|---|---|---|---|---|---|---|---|---|---|
| ppm | 34 | 32.1(P+O) | 31.9(Po) | 31.6(L) | 29 to 30 | 27 | 25.5 | 25 | 23 | 14.1 |
| Palmitic (P) | 1 | 1 | 0 | 0 | 10 | 0 | 0 | 1 | 1 | 1 |
| Palmitoleic (Po) | 1 | 0 | 1 | 0 | 6 | 2 | 0 | 1 | 1 | 1 |
| Oleic(O) | 1 | 1 | 0 | 0 | 8 | 2 | 0 | 1 | 1 | 1 |
| Linoleic (L) | 1 | 0 | 0 | 1 | 5 | 2 | 1 | 1 | 1 | 1 |
| α-Linolenic (Ln) | 1 | 0 | 0 | 0 | 4 | 1 | 2 | 1 | 1 | 1 |

**Table 1.** Number of carbons contributing to each peak in the fingerprint region of the $^{13}$C spectrum for the fatty acids included in the analysis.

| Experiment/Fatty Acid % | Palmitic | Palmitoleic | Oleic | Linoleic | Linolenic |
|---|---|---|---|---|---|
| Olive Oil at 600MHz, w3 region | 23.6 | 6.2 | 52.3 | 16.4 | |
| **Olive Oil at 600MHz** | **25.8** | **5.6** | **52.3** | **16.4** | **0.0** |
| Olive Oil at 7T | 21.2 | 5.1 | 53.0 | 20.7 | 0.0 |
| **Human Calf at 7T** | **31.3** | **12.0** | **30.2** | **26.5** | **0.0** |

**Table 2.** Fatty acid fractions determined by combined $^{1}$H and $^{13}$C MRS. The high resolution olive oil spectrum also allows (not possible *in vivo*) fat composition determination solely based on the omega-3 region of the spectrum (see Fig.3).

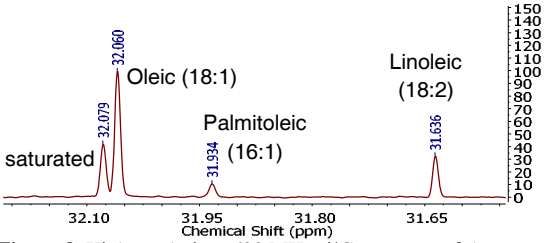


**Figure 3.** High resolution (600 MHz) $^{13}$C spectrum of the ω-3 region of olive oil allows for direct calculation of the relative amounts of the various lipid fractions.