

# Muscle group specific quantification of unsaturated fatty acids by localized DEPT-enhanced <sup>13</sup>C MRS and ERETIC

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## Introduction

<sup>13</sup>C MR spectroscopy *in vivo* enables to obtain metabolic information of humans not easily obtained by other non-invasive methods. The large chemical shift range of <sup>13</sup>C MR spectra allows the resolution of resonances from a large number of substances, and thereby the study of some metabolites which are difficult to resolve in <sup>1</sup>H MRS. One of them are unsaturated fatty acids, whose concentration is a valid tool to assess the dietary intake. In addition the ratio of poly- and monounsaturated fatty acids indicates some physiological diseases like cystic fibrosis [1]. However, the major drawback of <sup>13</sup>C MRS applied to humans is its low sensitivity, which is a consequence of the low natural abundance and the low gyromagnetic constant of the carbon nucleus. The attached protons cause multiplet structures which further decreases sensitivity. Under all these restrictions the quantification of metabolite concentrations of spatially specific localized <sup>13</sup>C spectra is still questionable. **In this work**, we propose a combined SNR enhancement by proton decoupling and ISIS-localized DEPT (Distortionless Enhancement of Polarization Transfer), aiming at muscle-group specific detection of unsaturated fatty acid in the calf muscle. SNR-enhancement was performed with a shielded transmit/receive <sup>13</sup>C/<sup>1</sup>H dual-tune volume calf coil (RAPID Biomedical GmbH) equipped with ERETIC (Electric Reference To access *In vivo* Concentration) [2]. With the chosen enhancement method *in vivo* calf muscle spectra from small volumes of interest in specific muscle fibre groups can be acquired and quantified using the ERETIC signal as reference.

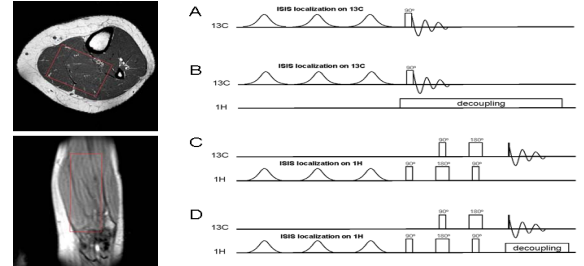
## Materials and Methods

Measurements were performed on a Philips scanner Achieva 3T (Philips Healthcare, Best NL). A shielded dual-tune <sup>13</sup>C/<sup>1</sup>H transmit/receive calf volume coil (RAPID Biomedical GmbH) was equipped with an adopted ERETIC setup [2] and enabled high sensitivity on the <sup>13</sup>C channel along with efficient and reproducible proton decoupling and polarization transfer. Adiabatic ISIS (Image Selected *In vivo* Spectroscopy) was used to localize specific volumes in the calf muscle area without subcutaneous fat as needed for ERETIC based quantification. It was implemented on the <sup>13</sup>C channel for direct acquisition with or without proton decoupling and on the <sup>1</sup>H channel for DEPT and combined proton decoupling and DEPT [Figure 1]. Continuous wave excitation was used to decouple a narrow range around the attached protons of unsaturated fatty acids at 5.5ppm in <sup>1</sup>H spectrum. Three block pulses were applied to realize short echo time DEPT at 6.45ms, which corresponds to the J-coupling value 155Hz of poly- and monounsaturated fatty acids [3]. The flip angle of the last pulse was set to 90 degrees [4]. Comparative measurements of all four localized SNR enhancement sequences were performed with the same repetition time to ensure the same T1 relaxation, the same receiver gain settings and averages in a large volume. Effective SNR enhancements were calculated and compared to theoretical expectations. ERETIC signal stability during proton decoupling was investigated. Finally the method with the highest SNR enhancement was combined with ERETIC signal injection and used to acquire a spectrum from a small volume inside single muscle fibre group. ERETIC signal amplitude and lineshape was scaled with respect to the fatty acid signal. It was placed at 105ppm where there is no metabolite and near the observed fatty acid signal in <sup>13</sup>C spectra. The direct acquisition and enhanced sequences with 1296 averages were performed.

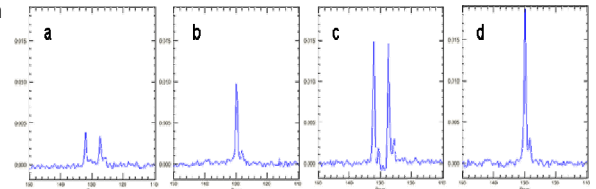
## Results and Discussion

The <sup>13</sup>C NMR spectra ranging from 110-150ppm acquired under the four sequences are shown in Figure 2, scaled to the same ordinate. The decoupled peak at 128.5ppm is from olefinic carbons adjacent to the intermittent methylene group ("inner" olefinic carbons) in polyunsaturated fatty acids, and the other peak at 130ppm is from "outer" olefinic carbons in polyunsaturated fatty acids as well as the olefinic carbons in monounsaturated fatty acids. Normally the signal at 130ppm is stronger than the one at 128.5ppm as shown in Figure 2, since the average poly- and monounsaturated fatty acid content is 17.8% and 44.8% respectively [1]. But this ratio can vary due to dietary intakes and differences in muscle metabolism. It is clear from the four spectra that decoupling fully collapses both doublets into single peaks and increases the signal height by a factor of two. DEPT transfers the polarization from attached protons to carbons and enhances the peak heights by the theoretical factor of four. If combining both techniques, higher enhancement is expected. The obtained results for narrowband decoupling and DEPT fulfil theoretical expectations [Table1, Figure 2]. The best SNR enhancement was achieved by the combination of decoupling and DEPT, but was slightly lower than predicted. Since the volume included only muscle tissue, we did not have baseline problem resulting from the subcutaneous fat signal. ERETIC signal intensity without (1.40±0.07) and with (1.40±0.06) proton decoupling as determined with TDFDfit [5] was identical. Muscle fibre group specific detection of unsaturated fatty acid from a volume size of 21.82mm\*16.02mm\*155.78mm [Figure 3] was possible in 13 min. With the ERETIC reference signal a reliable standard quantification is obtained.

**In conclusion**, ISIS-localized DEPT was implemented and combined with proton decoupling and the ERETIC reference standard for absolute quantification of unsaturated fatty acids in specific muscle groups. This technique can be easily extended to other muscle metabolites of interest for future physiological studies.



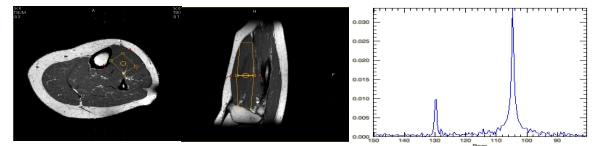
**Figure 1** Localized <sup>13</sup>C NMR spectroscopy using ISIS. Left shows the volume position (39.82mm\*56.83mm\*177.16mm); Right shows the sequence: A, only <sup>13</sup>C direction acquisition; B, direction acquisition with <sup>1</sup>H narrowband decoupling; C, spin-echo with DEPT enhancement; D, combination of proton narrowband decoupling and DEPT. All the four sequences are implemented with 512 sample points, 600ms repetition time, 2048 averages.



**Figure 2** Unsaturated fatty acid in the calf muscle of a healthy subject: a, direct acquisition; b, with proton decoupling; c, DEPT; d, proton decoupling and DEPT.

	Signal	Noise	SNR	SNR Enhanced
FID (single peak)	0.0366062	0.0156562	2.34	1.00
Decouple	0.108206	0.0134615	8.04	3.44
DEPT (single peak)	0.118363	0.0139386	8.49	3.63
Decouple&DEPT	0.192025	0.0139311	13.78	5.89

**Table 1** SNR enhancement with different methods. The noise is calculated as the average of three different spectra range (190-200ppm, 140-150ppm, 95-105ppm).



**Figure 3** Localization and <sup>13</sup>C NMR spectra of unsaturated fatty acid in a small volume (21.82mm\*16.02mm\*155.78mm) with ERETIC signal at 105 ppm.

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