Quantification of Fatty Acids in Human Calf Adipose Tissue and Muscle by ^{13}C MRS using J-refocused PRESS DEPT and ERETIC

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Introduction

Fatty acids serve as an important source of energy for muscular contraction and general metabolism [1]. Skeletal muscle fatty acid metabolism has been found in association with insulin resistance [2]. Compared to ^{1}H MRS, which enables distinction of intramyocellular (IMCL) and extramyocellular (EMCL) lipids, *in vivo* ^{13}C MRS offers a better separation of saturated and unsaturated fatty acid components and might allow for additional detection of omega-3, omega-6 and trans fatty acids, cholesterol and diglycerides [3]. Due to the intrinsic low sensitivity and large frequency dispersion of ^{13}C MRS, previous investigations were limited to subcutaneous adipose tissue using surface coil [4] or half-volume coil [5] localization and only relative percentages of different fatty acid subgroups were assessed.

In our work, a J-refocused proton PRESS localized DEPT sequence [6] is applied, to achieve reliably localized and SNR enhanced lipid signal detection by *in vivo* ^{13}C MRS using a dual tuned volume coil, and combined with optically transmitted and inductively coupled ERETIC as a reference for absolute quantification [7]. The proposed method allows for the first time to assess fatty acid concentrations from two metabolically distinct tissue types: adipose tissue (here: calf tibial bone marrow) and skeletal muscle. Relative concentrations of different lipid compounds as well as their absolute molar concentrations are evaluated and compared among omnivores, vegetarians and vegans.

Materials and Methods

The J-refocused PRESS DEPT sequenced is shown in Figure 1. Localization based on J-refocused PRESS and OVS (Outer volume suppression) is performed on the proton frequency and subsequently the polarization is transferred to ^{13}C via DEPT (a detailed description of all sequence parameters can be found in [6]). The synthetic ERETIC reference signal is injected simultaneously with proton decoupling and carbon signal acquisition (the setup is described in detail and validated in [7]). Twenty subjects in total were studied and divided into three age- and sex-matched groups (6 omnivores, 7 vegetarians and 7 vegans) with all BMI (Body Mass Index) in the normal range. Relative concentrations of various fatty acid compounds and their absolute molar concentrations were accessed. The absolute concentration in the unit of mM / L was calculated based on the one-time pre-calibrated ERETIC reference standard. T_1 and T_2 relaxation times during proton PRESS localization, corrected DEPT enhancement factors and the temperature were considered as correction factors.

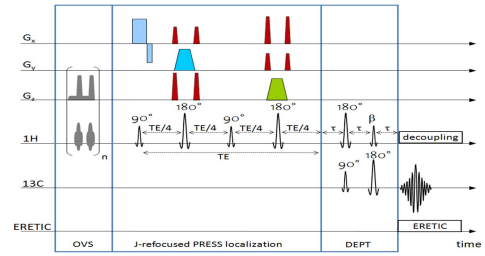

Figure 1 *J-refocused proton PRESS localized DEPT sequence, combined with ERETIC reference during the acquisition.*

Results and Discussion

Figure 2 shows the ^{13}C spectra of both saturated and unsaturated fatty acids from calf tibial bone marrow and skeletal muscle. Spectra with good SNR and a flat base line were obtained from both tissue types. The peak assignments are listed in Table 2. In Figure 2, spectra on the left are from an omnivore and on the right from an age-, sex- and BMI-matched vegan who has taken omega-3 supplement for two years. It can be seen that the signal intensity of poly-unsaturated fatty acids (PUFA; peak 2) is obviously higher in the vegan than in the omnivore in both bone marrow and muscle, which reflects the omega-3 supplement. Figure 3 plots the relative and absolute concentrations of all the fatty acid components in calf tibial bone marrow and skeletal muscle among the three dietary groups. In calf tibial bone marrow (Figure 3 top left), there are statistically significant differences between diet groups for peak 5 (middle methylene group), peak 1 – peak 2 (mono-unsaturated fatty acids; MUFA) and peak 2 (PUFA), which is not the case for skeletal muscle (Figure 3 top right). This indicates that the lipid profile from the bone marrow corresponds more to the general dietary group compared to the muscle. In skeletal muscle, the absolute molar concentrations of fatty acid components are about 10 times lower than in adipose tissue and both relative and absolute concentrations in the muscle have a large dynamic range in comparison to the adipose tissue. This might indicate that the lipid profile in the muscle is related to the short-term dietary intake and exercise which are subject- and time-dependent. It was interesting to find that there are significant differences between female and male from omnivores for almost all the concentrations except for the MUFA and PUFA concentrations in the muscle (Figure 4).

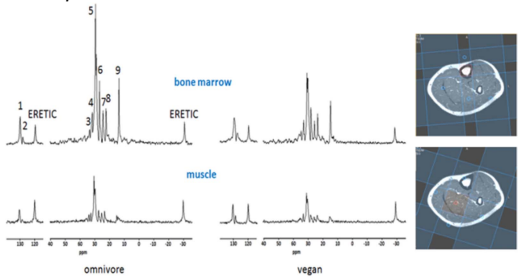

Figure 2 *^{13}C spectra from calf tibial bone marrow (top) and skeletal muscle (bottom) from an omnivore (left) and an age-, sex- and BMI-matched vegan (right). The ERETIC peak is shown in all the spectra and the peak assignments of fatty acids are listed in Table 1.*

Peak no.	Chemical shift (ppm)	lipid components	CSD*	T_1 relaxation time (ms)	T_2 relaxation time (ms)	Corrected DEPT factor
1	131.26	-HC=CH-CH$_2$-CH=CH-HC=CH-	96%	541.3	290.3	3.59±0.57
2	129.61	-HC=CH-CH$_2$-CH= CH-	99%	919.7	207.4	3.85±0.49
3	33.83	COO-CH$_2$-CH$_2$-R (C2)	89%	199.2	94.16	1.93±0.84
4	32.18	=CH-CH$_2$-CH$_3$ (ω3)	94%	1048.5	210.3	3.09±0.59
5	29.80	(CH$_2$)$_n$ envelope	99%	428.5	168.2	4.02±0.40
6	27.37	=CH-CH$_2$- (allylic α=cis)	92%	419.5	156.6	3.71±0.72
7	25.02	COO-CH$_2$-CH$_2$- (C3)	86%	270.8	104	3.32±0.30
8	22.86	-CH$_2$-CH$_2$-CH$_3$ (ω2)	80%	1658	196.6	3.40±0.20
9	14.11	-CH$_2$-CH$_3$ (CH$_3$)	99%**	2890	227.7	3.50±0.51

Table 1 *Peak assignments in the 13C spectra as well as the measured T1, T2 relaxation times in carbon frequency and DEPT enhancement factors after corrected for CSD, T1 and T2 relaxations of each carbon resonance.*

In conclusion, the results demonstrate the applicability of quantitative, SNR enhanced and localized ^{13}C MRS based on the combination of J-refocused PRESS DEPT and ERETIC for large scale noninvasive investigation of the impact of dietary intake, physical exercise or pathology on fatty acid metabolism and related diagnosis.

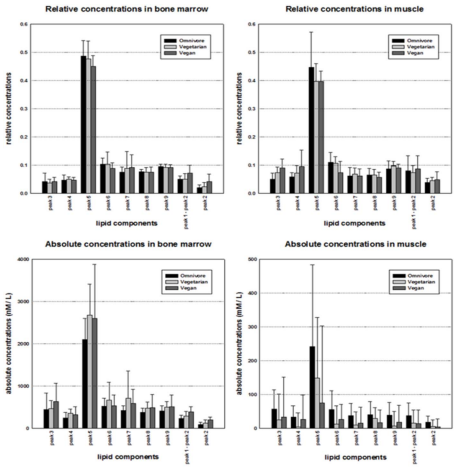

Figure 3 *Relative (top) and absolute (bottom) concentrations (mM / L) of each fatty acid component from the calf tibial bone marrow (left) and muscle (right) among omnivores (black bar), vegetarians (light bar) and vegans (gray bar). The mean value and the standard deviation are plotted in each dietary group.*

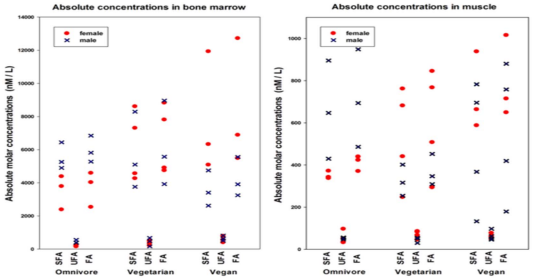

Figure 4 *Gender-marked absolute concentrations in mM / L of saturated, unsaturated and total fatty acids from the calf tibial bone marrow (left) and muscle (right) among omnivores, vegetarians and vegans.*

[1] Hwang JH. et al, NMR Biomed. 2003; 16: 160-167.
[2] Kelley DE et al, American Journal of Physiology-Endocrinology and Metabolism 1999;277(6):E1130-E1141.
[3] Fernandes JL et al, Magn Reson Chem 2012 Jun; 50(6): 424-8.
[4] Moonen CT et al, MRM. 1988; 6(2): 140-157.
[5] Thomas EL et al, Lipids 1996; 31(2):145-151.
[6] Chen X et al, ISMRM 2012: 1714
[7] Chen X et al, MRM 2012; 67(1):1-7.