ω-3 fatty acid detection by L-COSY in human bone marrow at 3T

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INTRODUCTION

ω-3 is an essential fatty acid (FA) that cannot be synthesized in the body and is obtained by diet. In the human body, essential FA serve multiple functions including neuroprotective functions, mood, behavior and prevents inflammation (1). ω-3 can down regulate inflammation in the presence of aspirin, affecting cellular signaling, and act on DNA by activating or inhibiting transcription factors. Deprivation in pregnant women can lead to developmental abnormalities (2). Decreased ω-3 levels were found in subjects with schizophrenia, bipolar disorder, major depression, and attention deficit disorder (2). It was also shown that ω-3supplements increase N-acetylaspartate, in patients with bipolar disorder (3). There are two types of ω-3 FAs: eicosapentaenoic acid (EPA) docosahexaenoic acid (DHA), both of these molecular structures end with R-HC=CH-CH_2-CH_3. The methyl group generates a triplet centered at ~1.0 ppm, with a scalar coupling of 7 Hz, while the methylene group resonates at 2.0 ppm. Recently ω-3 FA was detected in vivo in human adipose tissue at 1.5 T (4). We investigated possibility of detecting ω-3 in human bone marrow, at 3T, using localised one dimensional (1D) (PRESS) (5) and localized two-dimensional (2D) correlation spectroscopy (L-COSY) (6).

MATERIALS AND METHODS

MR data was collected on a standard Magnetom Tim Trio system (Siemens AG, Erlangen, Germany) with a 60 cm diameter bore (software version VB15A) and an eight channel knee coil (In Vivo Corp., Gainesville, FL) for phantom and in vivo tibial bone marrow studies.

1D spectra: were acquired using PRESS; TE of 135, 540, 675, 810 ms, no water suppression, 8 averages, acquisition vector of 1024 points, RF carrier frequency at 1.3 ppm (on resonance with $(CH_2)_n$), TR of 2 s, voxel size of 2x2x2 cm^3.

Spectra were acquired from cod liver oil (Arctic Cod Liver Oil, Nordic Naturals, Inc. Watsonville, CA) that contained 14% DHA, 9% EPA, 5% ω-3 FA, 13% non-ω-3 oleic FA). Localized, interactive shimming gave a line width at half maximum (LWHM) of ~ 13 Hz. Cod liver oil standard experiments were undertaken as follows: RF carrier frequency at 1.3 ppm, TR 2 s, voxel size: 2x2x2 cm^3, water suppression was disabled, spectral width=2000 Hz, increments size of 0.8 ms was used in 64 Δt1 increments giving an indirect spectral width of 1250 Hz, 5 averages per increment, 1024 data points were acquired. The L-COSY pulse sequence [90° - 180° – Δt1- 90° Acq] was used and thus, the delay between the last two RF pulses was incremented during the course of the experiment. Effectively, the first and second RF pulses generate a spin echo, which is then allowed to evolve before allowing for coherence transfer to occur by the terminal 90° RF pulse.
L-COSY data took 11 minutes to acquire.

In vivo 2D from the upper region of tibial bone marrow: spectral acquisition parameters as above with a voxel of 1x1x1 cm^3 generating a LWHM of ~24 Hz.

Spectral simulation was used to confirm the identity and shape of ω-3 2D cross peaks, a COSY spectrum of CH3-CH2-CH=CH- fragment was simulated using NMRSIM (version 5.0.1, Bruker BioSpin GmbH, Karlsruhe, Germany).

Two-dimensional spectral processing was the same for all 2D data sets and undertaken using Felix (7). Fourier transform was undertaken after linear prediction to 128 points and zero filling to 512 points in F1 and after zero filling to 2K along F2. Raw data was also weighted by multiplying F2 and F1 by skewed $sine^2$ and $sine^2$ apodization functions, respectively. Peak volume ratio was calculated for (A) CH_2-CH_3 (ω-3 cross peak at (0.94, 2.05)ppm)/$(CH_2)_n$ diagonal peak at 1.3 ppm and (B) diagonal peak at ~0.4 ppm/$(CH_2)_n$ diagonal peak at 1.3 ppm.

RESULTS AND DISCUSSION

Cod liver oil: Two weak low-frequency diagonal resonances, separated by 8 Hz, appear in the cod liver oil COSY spectrum at 0.4 ppm (high amplitude) and 0.46 ppm (low amplitude). Similar weak peaks are seen in the in-vivo 1D and 2D spectra of marrow but the closest assignment for these non-coupled diagonal peaks is a sterol resonance arising from stigmasterol and β-sitosterol at 0.6 ppm (8). A prominent peak at 1.05 ppm, due to the methyl group of ω-3 FA is seen in the phantom cod liver oil 1D spectrum (Figure 1). This peak is located between the methyl and methylene peaks from non ω-3 FA. It is noteworthy that in order to detect an in-phase triplet from the ω-3 FA methyl group, TE must be an integer multiple of 1/J=135 ms (Figure 1). This was the rationale behind the choice of the TE values (Figure 1). We found that the T2 value of the non ω-3 FA to be much shorter than that of ω-3 FA due to the fast decay on non ω-3 FA compared to ω-3 FA as can be seen in Figure 1. In the phantom, a doublet cross peak at (0.97, 2.05) ppm in the 2D spectrum was detected due to the CH_3 and the adjacent allylic CH_2 group of the ω-3 FA (Figure 3). The separation of the doublet peaks was15 Hz, which is double the value of reported J-coupling (4). The shape of this cross peak matches with the cross peaks obtained from the simulated spectrum (Figure 4), thus confirming identity. Phantom ratios A and B for were 8% and 0.4%, respectively.

Human tibial bone marrow: Resonances due to ω-3 FA methyl groups were detected in 1D spectra from bone marrow in vivo at long TE values (540 and 675 ms) as shown in Figure 2. Note that these spectra took 16 seconds to acquire from a 1 ml voxel. In the in-vivo 2D COSY spectra, the coupling of methyl and allylic methylene of ω-3 FA produced a single peak on both sides of the diagonal at (0.94, 2.05) ppm. where A and B were found to be 0.3% and 0.2%, respectively. Note that the value of A can be regarded as the fractional amount of w-3 FA in the examined specimen.
It is expected for A_{marrow} to be smaller than $A_{cod\ liver\ oil}$, since the oil is a health supplement. However, A_{marrow} can provide us with an internal indicator that can be used for treatment monitoring or health status. Assuming that B is due to sterols, the similarity between B value in marrow and oil needs to be supported by further studies. It should be considered that bone marrow may be physiologically a better medium to investigate ω-3 FA than subcutaneous adipose tissue.

CONCLUSION

Bone marrow is known to act as a long term storage depot for ω-3 FA. We show here that ω-3 FA can be detected in vivo from human tibial bone marrow. 2D COSY spectroscopy enables better detection and quantitation than 1D spectroscopy due to reduced peak overlap.

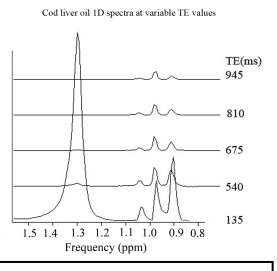

Figure1. Localized single voxel spectra at different TE values acquired from the cod liver oil phantom at 3T. Notice the rapid decline of 0.9 ppm peak from non-ω-3 FA, in comparison to the triplet at 1.05 ppm from w3 FA.

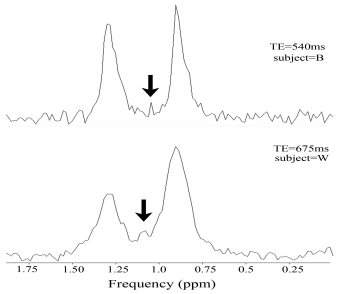

Figure 2. Localized 1D bone marrow spectra from two different healthy volunteers. Long TE values necessary to detect the ω3 FA peaks (arrow).

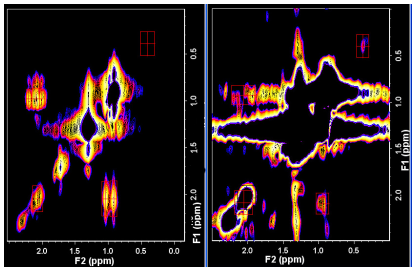

Figure 3.
Left L-COSY spectrum of cod liver oil. Right healthy volunteer. Cross peaks at (F2=1, F1=2) represent CH3-CH2 cross peak interaction from ω3 FA. Weak diagonal peaks at ~0.4 ppm may represent sterol

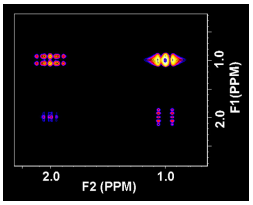

Figure 4.
Simulated COSY spectrum of the CH3-CH2- fragment of ω3 molecule. Cross peak at (F2=1, F1=2) ppm is showing as a doublet.

References:(1). Hashimoto M et al. Lipids 1999;34:1297-1304. (2) Berger GE et al. Neuropsychopharmacology 2008;33(10):2467-2473. (3)Frangou S et al. J Psychopharmacol 2007;21(4):435-439. (4) Lundbom J et al. J Magn Reson 2009. (5) Bottomley PA. Ann N Y Acad Sci 1987;508:333-348. (6). Thomas MA et al. Magn Reson Med 2001;46:58-67. (7) Felix. Felix NMR (Version 2007; 2007. (8). Guillen MD et al. Trends Food Sci Tech 2001;12 328-338.