In vivo detection and quantification of diet induced changes in adipose tissue composition by non linear NMR spectroscopy

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In vivo analysis of adipose tissue composition by NMR often requires water suppression, accurate voxel selection to avoid tissue interfaces, and the use of relatively small voxels and tedious localized shimming procedures to minimize local magnetic field inhomogeneities. In this report we show how intermolecular Zero Quantum Coherence (iZQC) signal can be used to acquire high resolution spectra of lipid depots spread over large (several cubic centimeter) volumes, without using localized shimming procedures or water suppression modules. We show how we calibrate and validate this method for samples containing known amounts of fatty acids, and how we use it *in vivo* to measure the total adipose tissue composition change induced by diet intervention in mice.

Methods: Four 4-weeks old C57 male mice were fed with either standard chow or with chow enriched with polyunsaturated fatty acids (PUFA) supplements. 2D iZQC spectra were then acquired after 4 and 12 weeks on a 7T small animal scanner using the sequence reported in[1]. The sequence is customized to select iZQC coherences between methylene protons (-CH2-) at 1.3ppm (P2) that are spatially coupled to the allylic protons at 2.03ppm (P4), the alpha-methylene protons (P5) at 2.25ppm, the diallylic potons (P6) at 2.77ppm, the glycerol backbone protons (P7-P8), and to olefinic protons (P9). Unwanted water-water coherences, as well as uncoupled water and fat components are dephased by a combination of gradient pulses, selective RF excitations, and RF phase cycling. The unsaturation and polyunsaturation level is then calculated for each animal, similarly to what is usually done for standard 1D ^{1}H spectroscopy[2] methods, by measuring the volume ratio between the allylic/methylene iZQC peak (P4-P2) and the alpha-methylene/methylene iZQC peak (P5-P2), while the ratio between the diallylic/methylene iZQC peak (P6-P2) and the alpha-methylene/methylene iZQC peak (P5-P2) is used to calculate the diunsaturation level. These values are then renormalized by using correction factors (1.3 for the (P4-P2)/(P5-P2) volume ratio and 0.45 for the (P6-P2)/(P5-P2) volume ratio) found experimentally by running the same experiments on samples of oleic, linoleic and linolenic fatty acids.

Results: Figure 1 shows an example of iZQC spectrum acquired from a 4cm slice across the entire abdomen of a mouse eating standard chow, as well as the standard 1D spectrum (fig. 1.b) acquired from the same slice. The iZQC spectrum shows impressive resolution enhancement compared to the standard 1D spectrum, and an excellent suppression of the large water signal coming from lean tissues. Figures 1c-d and 1f-g show a representative expansion of the (P4-P2) – (P6-P2) spectral region of the iZQC spectra generated from the 2 groups of mice. The peaks are all well separated, thus allowing their straightforward integration and use in the calculation of fatty acid composition. Changes in lipid composition in the group fed standard chow and PUFA supplements are clearly observed. In this group the P6-P2 peak is highly enhanced and increases over time, reflecting the change in lipid composition that occurs over time in these animals. By measuring the 0.5* volume((P4-P5)/(P5-P6)) for the two groups and using the correction factor of 1.3 found experimentally, we found a similar unsaturation levels for the two groups (~86±8%). However, when we compare the polyunsaturation level between the 2 groups, the difference between the standard diet and the PUFA diet group is quite dramatic. If we assume a level of 2% of triunsaturated fatty acids and a negligible level of higher polyunsaturated fatty acids, we can calculate a diunsaturation level for the normal diet group of 18±5%, which is in pretty good agreement with what was found by other groups[2]. However, for the PUFA diet group this calculation leads to misleading results. For these animals, if we assume a 2% level of triunsaturation, we come out with unreasonably high diunsaturation value, which after 12 weeks is larger than 100%. This clearly indicates that when the diet is supplemented with PUFA we cannot neglect the presence of higher unsaturated triglycerides.

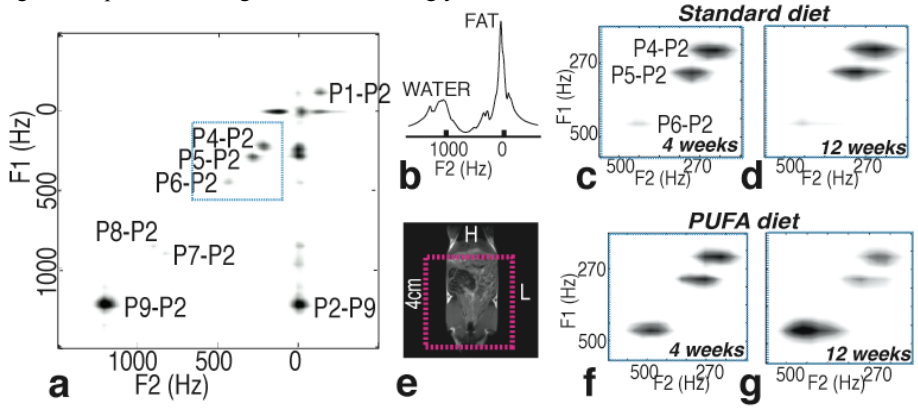

Figure 1: (**a)** iZQC spectrum acquired from a 4cm slice center in the abdomen of an obese mouse eating a standard chow. In addition to the coupling of the methylene protons with the methyl protons (P1-P2), coupling with the olefinic (P9-P2), allylic methylene (P4-P2), and diallylic methylene (P6-P2) protons are easily identified and resolved **(b)** Standard 1D spectrum acquired from the same obese mouse and from the same slice as in **(a)**. (**c-d and f-g**) Representative iZQC spectra from the two groups after 4 and 12 weeks, showing region highlighted in **(a). (e)** Coronal spin-echo images through the abdominal region of a mouse fed standard chow, showing (red-line) the selected area that we use to analyze fat composition changes with our method for all the animals.

Conclusions: We have demonstrated that 2D iZQC spectroscopy allows *in vivo* analysis of changes in adipose tissue composition upon diet intervention in mice. This method is naturally insensitive to magnetic field inhomogenities and it naturally suppresses water signal from lean tissues. This allows us to analyze fatty acid composition of lipid depots that are scattered over large volumes, or that surrounds internal organs, which are hard to analyze with conventional localized 1D ^{1}H spectroscopy.

References: [1]Branca et al."In Vivo Brown Adipose Tissue Detection and Characterization using Water-Lipid Intermolecular Zero Quantum Coherences". *Magnetic Resonance in Medicine* (in press). [2]Strobel, Klaus, van den Hoff, Joerg, and Pietzsch, Jens, Localized proton magnetic resonance spectroscopy of lipids in adipose tissue at high spatial resolution in mice in vivo. *J. Lipid Res.* 49 (2), 473 (2008).

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