In vivo identification of a molecular marker for brown adipose tissue in NMR spectra of large volumes

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Introduction Brown Adipose Tissue (BAT) is though to have a large impact on long-term energy balance due to its ability to dissipate energy in form of heat. It is therefore a target of interest for obesity drugs[1]. Unfortunately, the BAT distribution in humans is difficult to study, since it requires either invasive biopsy or metabolic tissue imaging through FDG/PET. BAT has also been studied *in vivo* using localized single voxel spectroscopy[2] or fat-tissue fraction MRI[3]. Both of these methods are "per-voxel", and can therefore not be used to differentiate BAT from White Adipose Tissue (WAT) when the two tissues are mixed together, as is often the case. We present a new spectroscopic method that we use to detect BAT *in vivo* in large voxels (~ 50cm^3). This method selectively analyzes the hydration of adipose tissue at the cellular level by detecting the spatial correlation, on a microscopic scale, between fat and water. More specifically the spatial coupling, at cellular length scales, between methylene protons (-CH2-) at 1.3ppm and water (P2/W), and between methylene protons and olefinic protons (-CH = CH-) at 5.3 ppm (P2/P9) is detected by using the signal from intermolecular zero quantum coherences (iZQC)[4]. iZQC is intrinsically insensitive to magnetic field inhomogeneities allowing to resolve these couplings even in presence of large susceptibility gradients as those encountered in the human body. We demonstrate here that the ratio between these couplings reflects the structural difference between BAT and WAT, and, *in vivo*, provides a good marker for BAT. The straightforward extension of this method to detect BAT activation will also be discussed.

Methods A slice selective, iZQC spectroscopy sequence that isolates coherence between the methylene proton and the water-olefinic protons at the length scale of 90 μm is implemented on a 7T Bruker scanner. *In vitro* iZQC spectra from BAT and WAT tissue samples extract, and *in vivo* iZQC spectra from two different slices (neck (2cm), and abdomen (4cm)), are then obtained from normal (C57 and balb/c), nude (nu/nu), and obese (ob/ob, diet induce obesity (DIO), and yellow agouti A(y)) mice, at 6 weeks and at 2 years of age, before and after cold exposure (2h at 10°C) or norepirephrine (NE) administration.

Results The line narrowing capability of this method can be seen in Fig. 1 where the *in vivo* iZQC spectrum from a DIO mouse model was acquired over a large voxel that comprises the entire low abdomen. The nine different lipids can all be resolved in the iZQC spectrum. The iZQC spectrum of BAT tissue presents a P2/Water resonance frequency line which is absent in the spectrum of WAT extract (Fig. 2). This resonance frequency line represents a marker for BAT and is considerably higher in the *in vivo* spectrum of young mice, and in nude mice, while is almost absent in the spectra of obese mice (Fig. 3). The relative intensity of this peak with respect to the nearby P2/P9 peak depends on the age of the animal and on its metabolic efficiency, and is correlated with the relative BAT/WAT fraction, as shown by mouse dissections. Adrenergic stimulation (NE injection) and cold exposure also affect this ratio and the P2/Water linewidth.

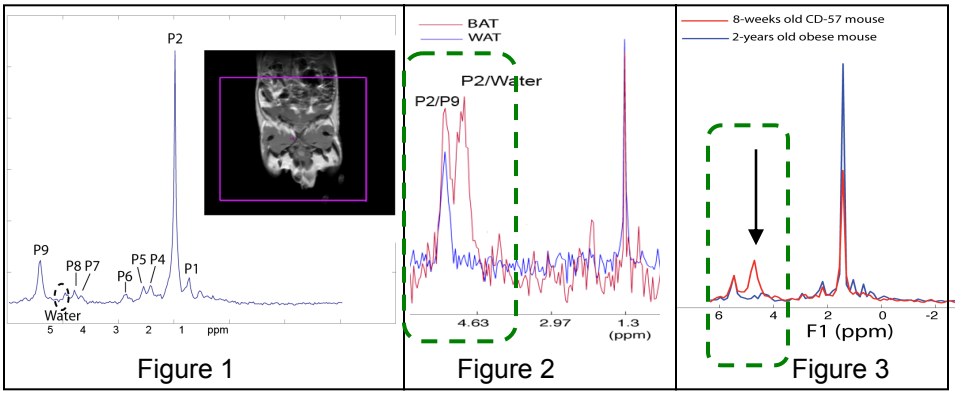

Figure 1 Figure 2 Figure 3

Fig.1: In vivo iZQC spectrum of the entire abdomen of a DIO mouse. Peaks are labeled as in ref.[2].
Fig.2 : IZQC spectrum of extract BAT (red) and WAT (blue) tissue. The methylene/water iZQC peak (P2/Water) is characteristic of the only BAT tissue.
Fig. 3: Comparison of the *in vivo* iZQC spectra from a 8 weeks old C57 mouse (red) and a 2 years old ob/ob mouse (blue). The methylene/water peak (arrow) is considerably reduced in the obese mouse spectrum.

Discussion Our *in vitro* data shows that the iZQC P2/W peak is characteristic only of BAT tissue, and is absent in spectra of muscle and WAT tissue. Our *in vivo* data shows that the (P2/W)/(P2/P9) ratio is high in nude and young normal mice and absent in obese mice, and that it changes upon adrenergic stimulation and/or cold exposure. It therefore seems to provide a good marker for BAT tissue and its activation. Since this method is intrinsically insensitive to magnetic field inhomogeneities, it does not require voxel selection and/or tedious shimming procedures, and allows the user to analyze large volumes (>50cm^3) *in vivo*. For these reasons, this method is optimal for detection of BAT depots *in vivo*, in small animal disease models as well as in humans, during the initiation, progression, and manifestation of obesity-related disorders.

References [1] Nedergaard J, et. al. Am J Physiol Endocrinol Metab 293:E444–E452, 2007. [2] Strobel K. et al, J Lipid Res 49(2), 2008. [3] Hu H.H. et al, Proceeding.ISMRM 210, 2009.[4] Galiana G. et al., Science, 332,2008.