MRI susceptometry measurements of murine brown and white adipose tissue

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Introduction

Characterization of the magnetic susceptibility (χ) of biological tissues may provide insight into their composition and microarchitecture. Despite their biological significance, there have been few reports of the magnetic properties of white adipose tissue (WAT)[1,2] and, to the best of our knowledge, none on brown adipose tissue (BAT). WAT is a major endocrine organ that stores triglycerides as energy substrates, while BAT is used for non-shivering thermogenesis[3]. There is renewed interest in BAT for its role in metabolic regulation. In this work, we use MRI susceptometry[4] to measure of χ of *ex vivo* murine BAT and WAT specimens. Using an MRI-based method allows for further investigation of the same BAT and WAT specimens with other MR methods *in situ*, such as chemical-shift imaging (CSI) that may provide additional chemical and structural information.

Methods

MRI susceptometry is based on a geometric model of an infinitely long cylinder perpendicular to B_0 which produces a dipole-shaped field perturbation external to the cylinder based on $\Delta\chi$ between the material inside and outside the cylinder[4]. A sample holder for a 5mm NMR tube was constructed out of Ultem PEI 1000 (McMaster-Carr), which has χ close to water[5], and the space outside the cylinder was filled with 1% agarose gel. The difference field map between a distilled water reference tube and the material of interest was fit to the field perturbation model, and $\Delta\chi$ with respect to water was computed. A difference field map removes effects of field inhomogeneities.

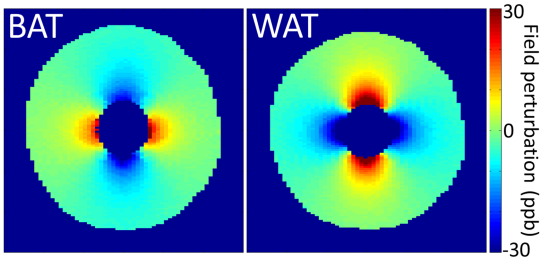

Fig 1. Sample field difference maps for BAT and WAT.

Female athymic nude mice (Harlan) at 11-12 (N=8) and 29-30 (N=7) weeks of age were euthanized. Immediately after sacrifice, interscapular BAT and peri-uterine WAT depots were harvested and immersed in ice-cold PBS until time of experiment. All tissue specimens were packed together in 5mm NMR tubes based on age group to provide sufficient sample volume (N=1 per age group).

All experiments were performed on a 9.4T Varian horizontal bore scanner. Field mapping was performed with a 2D multi GRE sequence: 128×64, FOV = 32×32 mm^2, number of acquisitions = 4, FA = 30°, echoes = 18, TE range = 5-64 ms, BW = 50 kHz, and TR = 75 ms. A 2D CSI scan was performed immediately after field mapping: 40×40, FOV = 32×32 mm^2, number of acquisitions = 1, FA = 90°, TE = 0.65 ms, BW = 5 kHz, number of readout points = 2048, and TR = 425ms. CSI data were processed offline to calculate a fat signal fraction (FSF) map of the sample[6].

Results and Discussion

Here, we report volumetric χ in SI units. The current MRI susceptometry method was validated by measuring χ of various oils (peanut/vegetable), which matched reported literature values (χ ~ -8.3ppm)[1,7]. We were also able to measure the reported molar χ of Gd-DTPA (χ ~0.026 cc/mol)[8].

Fig. 1 shows sample difference field maps of BAT and WAT specimens. The RMS residual of the fits was ~0.5 ppb, indicating close agreement to the dipolar field perturbation model. Table 1 lists the measured χ of BAT and WAT. There was little difference between the 11-12 and 29-30 week old age groups. WAT χ was measured to be more paramagnetic than water ($\Delta\chi$ ~0.3ppm) in rough agreement with reported χ of pure oils[1,7]. BAT χ was measured to be more diamagnetic than water ($\Delta\chi$ ~ -0.2ppm).

Fig. 2 shows sample average NMR spectra from CSI data of water, peanut oil, BAT and WAT. Inhomogeneous line broadening can be seen in the BAT and WAT spectra as expected. FSF can be calculated from the spectra by numerical integration, which is shown in Table 1. BAT and WAT FSFs are much lower than reported values in vivo values[9]. This is possibly due to excess surface water on tissue specimens, which could be significant given the small size of the specimens and number needed to fill the sample tube.

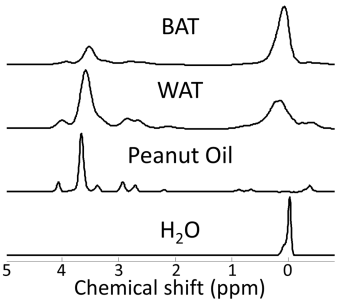

Fig 2. Average spectra from CSI data.

Given the higher water content of BAT, it would not be unexpected if the χ of BAT were between that of WAT and water. Although it is well known the iron content of BAT is much higher than that of WAT, the absolute concentration of iron is ~20 nmol/g of tissue[10], which is too low to increase the paramagnetism of BAT relative to WAT. BAT χ was unexpectedly measured to be more diamagnetic than even water, which an artifactually lower FSF cannot explain. One possible explanation is increased cholesterol content in BAT[11], which is more diamagnetic than water ($\Delta\chi$ ~ -0.2ppm)[12].

Table 1. Measured volumetric χ in SI units and FSF values of BAT and WAT

	WAT (11/12 wks)	*WAT (29/30 wks)*	*BAT (11/12 wks)*	*BAT (29/30 wks)*
χ (ppm)	-8.827	-8.693	-9.222	-9.218
FSF	0.667	0.709	0.324	0.237

Conclusion

For the first time, we report *ex vivo* MRI susceptometry measurements of murine BAT and WAT. WAT χ agreed with previous reported values, while BAT χ was measured to be even more diamagnetic than water. Further study is needed to elucidate the basis of this difference.

References: **1**. Hopkins JA , et al, *MRM*, 37:494 (1997). **2**. Sprinkhuizen SM, et al, *Magn Reson Mater Phy*, 25:33 (2012). **3**. Tilg H, et al, *Nat Rev Immunol*, 6:772 (2006). **4**. Weisskoff RM, et al, *MRM*, 24:375 (1992). **5**. Ravi KC, et al, *JMR*, 205:63 (2010). **6**. Chen L, et al, *JMR*, 158:164 (2002). **7**. de Rocheforte L, et al, *MRM*, 60:1003 (2008). **8**. Josephson L, et al, *MRM*, 22:204 (1991). **9**. Hu H, et al, *MRI*, 30:323 (2012). **10**. Joel CD, et al, *Biochem*, 1:28 (1962). **11**. Menschik Z, *Anat Record*, 116:439 (1953). **12**. Weast RC, et al, *CRC Handbook of Chem and Phys*, E-123–136 (1979). **Acknowledgements:** NIH T32 EB001628 and NIH R21 DK096282.