Comparison of in vivo hepatic localized proton magnetic resonance spectroscopy at 9.4T on ob/ob and ob/+ mice

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Introduction: Liver plays a key role in lipid metabolism and constitutes a hub of fatty acid synthesis and lipid circulation through lipoprotein synthesis. Proton magnetic resonance spectroscopy has evolved as reliable method for measuring both the amount of hepatic lipids and their composition non-invasively. Hepatic lipids have been associated with the risk of multiple diseases including diabetes. The objective of this study was to assess hepatic lipids in ob/ob mice, an established murine model of obesity with mice displaying defective leptin signaling, heterozygous littermates (ob/+ mice) that do not develop a disease phenotype as the gene mutation is recessive, were used as controls.

Materials and methods: *Animals*: Twelve male mice were used: 8 ob/ob and 4 age matched ob/+ controls at 24 weeks of age. The mice were anesthetized using isoflurane (1.5%-2.25%) in an oxygen-air mixture (150/400) throughout the experiments with a face mask. The body temperature and respiration were monitored. All animal experiments were performed in strict adherence to the Swiss Law for Animal Protection. *MRS experiment:* All in vivo MRS measurements were performed on a Bruker BioSpec 94/30 (Bruker BioSpin MRI, Ettlingen, Germany) system using a combination of volume resonant coil and surface coil. IntraGate pulse sequence was used for anatomical reference images. The VOI was carefully placed on these images to avoid contribution from large blood vessels and subcutaneous fat. Single-voxel localized ^{1}H MR spectra were acquired using the PRESS sequence with additional outer volume suppression with the following parameters: VOI 3*3*3 mm^3, T_R=6s, T_E=12,18,24,30ms (for correcting the measured signal intensities for T_2 effects), band width=4006 Hz, number of sampling points=2048, acquisition time=511 ms, number of averages (NA) =40 (ob/ob) and 100 (ob/+) for sufficient SNR. For water suppression the VAPOR sequence has been used. An unsuppressed spectrum was recorded within the same voxel with number of average=40 (ob/ob) and 10 (ob/+) yielding the water reference signal. All spectral data have been corrected for T_2 relaxation, while long T_R values were used rendering T_1 correction unnecessary. *Analysis of MRS data:* All spectroscopy data were processed using LCModel (Version 6.2-1Q, Stephen Provencher, Oakville, ON, Canada). In ob/+, the spectra between 3.6ppm and 5.0ppm were cut to void the phase distortion of residual water peak. *Calculation:* Peak assignments were based on published data (1). Quantification was done with T_2 correction and no correction for T_1 was made since for a T_R of 6s, all resonances will be fully relaxed. The mean chain length of the hepatic lipids molecule (MCL) (2) and water proton fraction (WPF), which shows the percentage proton contribution from water compared to the total amount from water and hepatic lipid (1), and lipid composition(1, 3-4) were defined as literatures. *Statistical analysis:* All results are presented as mean ± STD. For statistical analysis OriginPro 8.1 (OriginLab, Northampton, MA, USA) has been used. Data (ob/ob vs. ob/+) were analyzed using the one-way ANOVA. The level of significance was set as $\alpha = 0.05$.

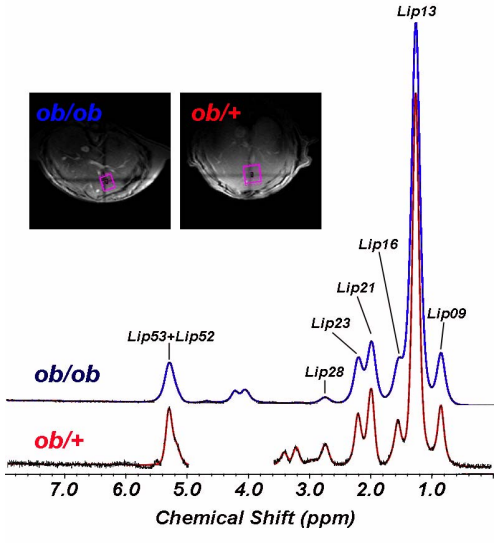

Fig1. Anatomical images and spectrum from ob/ob and ob/+ mice. Resonances are labeled according to their chemical shift values.

Results: In Fig.1, abdominal MR images and spectroscopy of ob/ob and ob/+ control mice are shown. Typically nine lipid resonances could be resolved. As shown in Fig. 2, seven lipid resonances were used for further processing (except for the two at 4.1ppm and 4.3ppm due to insufficient SNR). The derived averaged results from livers of the two mouse groups are shown in Table.1. As expected, lipid quantity (=Lip13/ (Lip13+water)) differs significantly between ob/ob and ob/+ mice (0.132 in ob/ob vs. 0.018 in ob/+). Significant increase could be observed in the saturated component (number of $-CH_2-$ structure at 1.3ppm per molecule) of ob/ob mice (10.167 in ob/ob vs. 7.060 in ob/+). No significant changes in the total unsaturated index, polyunsaturated bond index and total unsaturated bond index were observed. Furthermore, an increase in the mean chain length was observed in ob/ob mice compared to ob/+ mice (20.402 vs. 15.796). In addition, the water proton factor in ob/ob mice is around 0.786 vs. 0.965 in ob/+ mice.

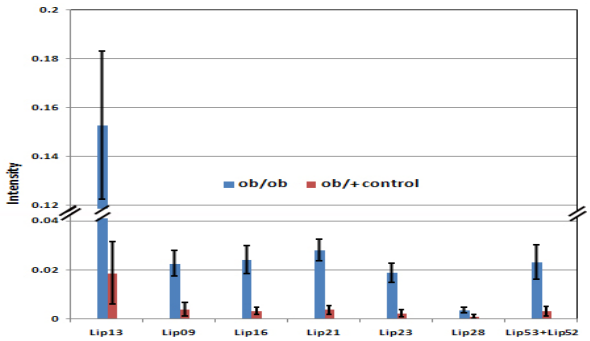

Fig 2. Mean levels and STD of the seven main resonances estimated for the ob/ob and ob/+ mice.

	ob/ob			ob/+		
lipid quantity	0.132	±	0.023	0.018	±	0.012
saturated component	10.167	±	0.922	7.060	±	0.816
total unsaturated index	0.956	±	0.185	0.770	±	0.212
polyunsaturated bond index	0.249	±	0.104	0.381	±	0.170
total unsaturated bond index	1.573	±	0.520	1.210	±	0.193
MCL	20.402	±	3.060	15.796	±	1.991
WPF	0.786	±	0.031	0.965	±	0.020

Table1. Hepatic lipid composition results.

Discussion and conclusion: The present study demonstrates that in vivo ^{1}H MRS can be an effective method to non-invasively detect accumulation of hepatic fat as well as potentially assess the fat composition on both ob/ob and ob/+ mice, which have attracted great research interest over the last few years, owing to their relation to insulin sensitivity, diabetes, and obesity. This may facilitate longitudinal monitoring of changes in lipid composition in response to diet, exercise, and disease.

References:

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