Longitudinal evaluation of intramyocellular lipid (IMCL) in tibialis anterior (TA) muscle of ob/ob and ob/- control mice using a cryogenic surface coil at 9.4 T and correlation with insulin levels

Q. Ye[1,2], C. F. Danzer[3], A. Fuchs[1], W. Krek[3], and M. Rudin[1,2]

[1]Institute for Biomedical Engineering, Zürich, Switzerland, [2]Institute for Pharmacology and Toxicology, Zürich, Switzerland, [3]Institute of Cell Biology, Zürich, Switzerland

Introduction: The accumulation of lipid in non-adipose tissue closely relates to insulin resistance (IR) in type 2 diabetic [1]. The relationship between intramyocellular lipid (IMCL) levels and IR is not fully understood though a link to obesity as risk factor is established. Mouse models of diabetes/obesity are attractive tools for mechanistic studies. In this study, intramyocellular lipid (IMCL) levels in tibialis anterior (TA) muscle of ob/ob mice, a frequently used model of obesity, and their age matched ob/- controls were measured longitudinally from age 11 to 25 weeks using non-invasive ^{1}H MRS. In ^{1}H MRS of small animals, noise originating from sample is comparable to electronic noise. Use of a cryogenic transceiver RF coil was allowed to reduce electronic noise thereby increasing the sensitivity [2]. Aspects considered in spectrum analysis were reproducibility, spatial heterogeneity [3] and the influence of T_2 relaxation. IMCL levels derived from were correlated to plasma insulin levels.

Methods:

Study protocol: 16 male mice were used: 8 ob/ob and 8 age matched ob/- controls. Animals were anesthetized with isoflurane (2.0%), placed on a water heated cradle with the TA muscle aligned along the main magnetic field direction. The mice were examined longitudinally from age 11 to 25 weeks with ^{1}H MRS to monitor IMCL level in TA. In order to evaluate the reproducibility of ^{1}H MRS, 3 ob/ob and 2 age matched ob/- control mice were measured three times every other day at week 17, and T_2 relaxation was examined for each scan. Regional variation of IMCL in TA, was assessed in 5 ob/- control lean mice. All animal experiments were performed in strict adherence to the Swiss Law for Animal Protection.

In vivo NMR setup: All in vivo MRS measurements were performed on a Bruker BioSpec 94/30 (Bruker BioSpin MRI, Ettlingen, Germany) system using a cryogenic quadrature transceiver RF coil. A RARE pulse sequence was used for anatomical reference images: field-of-view=20*20mm^2, matrix dimension=256*128, TR=1.5s, TE=8ms, RARE factor=8. Single-voxel localized ^{1}H MR spectra were acquired using the PRESS sequence with additional outer volume suppression with the following parameters: voxel volume 1.28 to 3.2 mm^3, TR=2000ms, TE=25ms, number of averages=512. For water suppression the VAPOR sequence has been used. An unsuppressed spectrum was recorded within the same voxel with number of average=10. Regional heterogeneity of IMCL in TA was assessed by using different voxel sizes at the same location: 2.0*0.8*2.0 mm^3 versus 1.0*0.8*2.0 mm^3. T_2 was estimated from measurements with TR=2000ms, and TE=12, 24, 36, and 48ms.

Insulin measurement: Insulin levels were measured in ob/- and ob/ob mice at 12, 15, 20, 22, 24, 26 weeks old using the Mouse Insulin ELISA kit (Mercodia ,Sylveniusgatan Sweden).

Data analysis: All data were processed using LCModel for IMCL quantification. SNR and SHIM analysis was carried out using TopSpin (Bruker Biospin, Ettlingen) after applying 2Hz line broadening. Total creatine (I_{tCr} with tCr at 3.02ppm) was used as concentration reference, i.e. IMCL levels in TA are given as the intensity ratio I_{IMCL}/I_{tCr}.

Statistics: All results are presented as mean±SE. Two sample t-tests were used for statistical comparison.

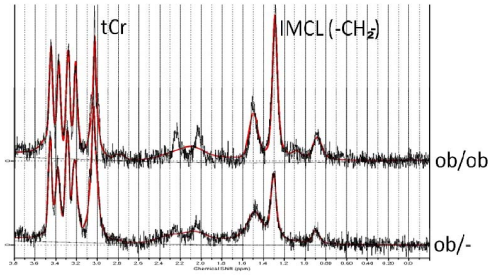

Fig 1. In vivo MR spectra of TA from ob/ob (upper) and ob/- control male mice at 17 weeks. Note significantly higher intensity of IMCL (-CH_2-) signal in ob/ob animals.

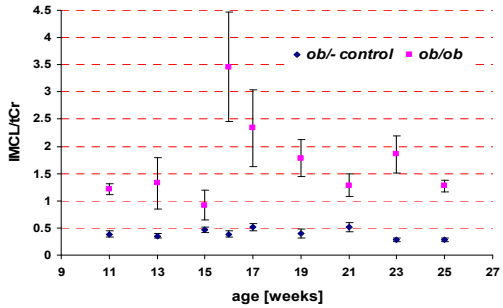

Fig 2. IMCL/tCr levels as a function of age in ob/ob and their lean control mice for the period 11 to 25 weeks. Data are presented as mean ± SE.

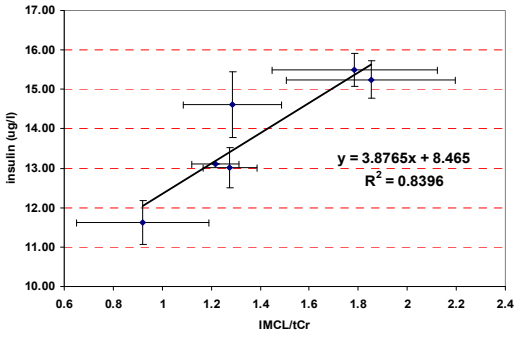

Fig 3. Correlation between plasma insulin levels and IMCL/tCr levels in mouse TA muscle.

Results:

The regional variation of IMCL distribution: There was no significant difference in IMCL/tCr levels when comparing data obtained from 3.2 mm^3 and 1.6 mm^3 (P>0.05), although there was apparently inter-individual variation.

T_2 correction: Determination of IMCL and tCr T_2 relaxation times allowed to estimate a correction factor that accounts for differences in relaxation at TE=25ms. Comparing corrected and uncorrected IMCL/tCr values yields a linear correlation (R^2=0.951).

Time course of IMCL levels in ob/- and ob/ob mice and correlation with plasma insulin levels: The ratios of IMCL/tCr in TA were significantly higher in ob/ob mice than in their age-matched ob/-lean controls at all ages studied (Fig.1, Fig. 2). IMCL levels of ob/ob mice increased from weeks 11 to 16, and then decreased from weeks 17 to 25, while their age-matched lean controls show stable IMCL level throughout the duration of the experiment. The highest IMCL levels in ob/ob mice were found at 16 weeks. During the observation period (11-25 weeks), a close correlation between IMCL/tCr and plasma insulin levels has been observed in ob/ob mice (Fig.3).

Discussion: As regional variation of IMCL within TA seems not critical for single-voxel spectroscopy we selected a voxel dimension of 2.0*0.8*2.0mm^3, which is almost the maximum area for single-voxel to located in mouse TA, for the longitudinal study. T_2 correction is mandatory for proper estimates of IMCL/tCr ratios. IMCL/tCr levels were found consistently higher in ob/ob animals than in ob/- control mice. The temporal profile shows a distinct maximum at an age of 16 to 17 weeks and then gradually declines until week 25 with values still being significantly higher than in control ob/- animals. The tight correlation between plasma insulin levels and IMCL/tCr hints a link between insulin regulation and lipidigenesis/lipid turnover.

Acknowledgement: We gratefully acknowledge funding by the Swiss National Science Fundation.

References:

1. Krssak, M., et al., Diabetologia, 1999. **42**(10): p. 1269-1269. 2. Ratering, D., et al., Magnetic Resonance in Medicine, 2008. **59**(6): p. 1440-1447.
3. De Feyter, H.M.M.L., et al., Magnetic Resonance in Medicine, 2006. **56**(1): p. 19-25.