Longitudinal hepatocellular lipid levels (IHCL) on ob/ob mice and the correlation to insulin levels

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Introduction: Quantification of hepatocellular lipid content (IHCL) with ^{1}H MRS non-invasively is attractive due to its potential association to multiple diseases, such as obesity, type 2 diabetes, and hepatic steatosis (1-2). For mechanistic studies and evaluating the value of potential MRS-based disease biomarkers, rodent models play an important role. Mouse models are of particular interest in view of the many genetically engineered lines available for mechanistic studies. In the current study we have used ob/ob mice, a well-established model for obesity related diseases (3). The purpose was to monitor IHCL level longitudinally in both ob/ob and ob/+ control mice and evaluate a potential link to blood plasma insulin levels.

Materials and methods: *Animals:* 8 ob/ob and 4 ob/+ male mice have been measured split into two (group A, B) for MRS. 8 ob/ob mice were used for only insulin measurement. The mice were anesthetized using isoflurane (1.5%-2.25%, to maintain respiration duration within the range of 0.5 - 1.5 s) in an oxygen-air mixture (150/400) throughout the experiments with a face mask. The body temperature was maintained with water heating and monitored. Respiration signal was obtained and used for respiration gating. Both ob/ob and ob/+ mice were examined between 12 and 24 weeks old. All animal experiments were approved by the Veterinary Office Zürich (Switzerland). *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. An IntraGate pulse sequence was used for anatomical reference images. The volume of interest of the subsequent proton spectroscopy was selected such that contributions from large blood vessels and subcutaneous fat were avoided. Single-voxel localized ^{1}H MR spectra were acquired using the PRESS sequence with additional outer volume suppression with the following parameters: voxel volume 3*3*3 mm^3, T_R=6 s, T_E=12,18,24,30 ms (for correcting the measured signal intensities for T_2 effect), band width=4006 Hz, number of sampling points=2048, acquisition time=511 ms, number of averages (NA) =40 (ob/ob) and 100 (ob/+). 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. *Determination of plasma insulin levels*: Blood plasma was collected one day before MRS and insulin levels were measured using a mouse insulin ELISA kit (Mercodia, Sylveniusgatan, Sweden). *Analysis of MRS data*: Spectroscopy data were processed using LCModel (Version 6.2-1Q, Stephen Provencher, Oakville, ON, Canada). Water concentration was used as reference for quantification. *Calculation of IHCL*: Different T_E values were applied to estimate the spin-spin relaxation time (T_2) of the peak at 1.3ppm (Lip13), which was used for IHCL quantification (1), within a region of interest in liver for individual scan. Quantification was done with T_2 correction and no correction for T_1 as at T_R = 6s, all resonances will be almost fully relaxed (4). *Statistical analysis*: All results are presented as mean ± SE. For statistical analysis OriginPro 8.1 (OriginLab, Northampton, MA, USA) has been used. The level of significance was set as α = 0.05.

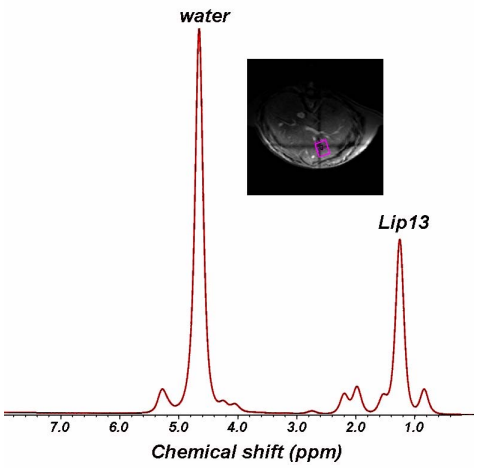

Fig 1: Typical hepatic ^{1}H MRS Spectra and the anatomical image of ob/ob mouse.

Results: Fig.1 shows a representative spectrum from ob/ob mouse without water suppression, location of the voxel is indicated on the anatomical image. Plotting quantitative data reveals a significant higher IHCL level in ob/ob than ob/+ mice at any timepoint (Fig.2). We did not observe an increase in ob/ob IHCL during the observation period, though there were considerable fluctuations in the values. In contrast, IHCL values in ob/+ mice increased as a function of age. The measured insulin level on ob/ob mice were shown in Fig.3. We observed a significant correlation between IHCL and insulin (Pearson correlations: 0.79, p<0.01, Fig.4).

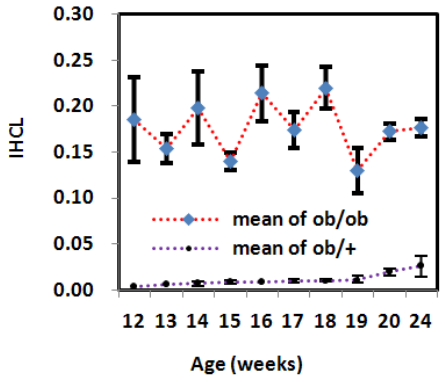

Fig. 2 IHCL levels on both ob/ob and ob/+ mice at all the ages.

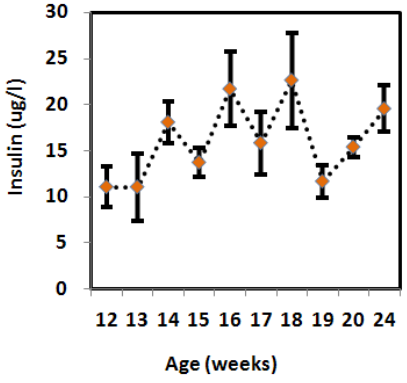

Fig.3 Insulin level of ob/ob mice.

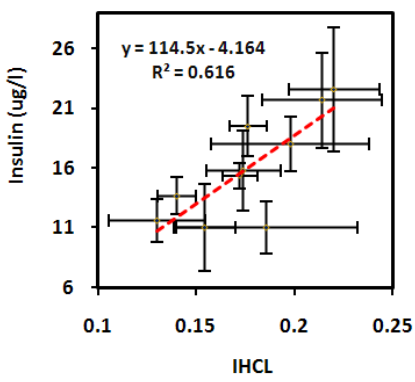

Fig.4 Correlation between insulin and IHCL of ob/ob mice.

Discussion and conclusion: Ob/ob mice showed significantly elevated IHCL levels as compared to ob/+ control mice at all the ages measured. Longitudinally, IHCL in ob/ob mice showed no obvious tendency, while in ob/+ mice, there was a slowly and continuous increase. The good correlation between insulin levels and IHCL in ob/ob mice may indicate that insulin signaling is involved in IHCL metabolism.

Reference: **1.** Corbin IR, et al, *Bba-Mol Cell Biol L*. 2009; 1791: 757-63. **2.** Van Herpen NA, et al, *Physiol Behav*. 2008; 94: 231-41. **3.** Lindstrom P, *Scientific World Journal*. 2007; 7: 666-85. **4.** Strobel K, et al, *Journal of Lipid Research*. 2008; 49: 473-80.