ALTERATIONS IN THE HEPATIC LIPID PROFILE OF MICE FOLLOWING STREPTOZOTOCIN-INDUCED DIABETES

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PURPOSE: Alterations in the hepatic lipid content (HLC) and profile are associated with derangements in whole body metabolism. We used ^{1}H-MRS to follow longitudinal changes in the hepatic cytosolic lipids of mice after the induction of diabetes by streptozotocin (STZ). In addition, we assessed the total lipids by performing HR-NMR on liver extracts, thus gaining insight into the membrane phospholipids.

METHODS: C57BL/6J mice received an i.p. injection of STZ (180 mg/Kg) resulting in glycemia ≥ 15 mM. Mice were scanned before, 7 and 14 post-SZT, when they were sacrificed and the lipids extracted from the livers by the Folch method. Control mice were also scanned an immediately sacrifice. Mice were scanned under isofluorane anesthesia with a ^{1}H quadrature surface coil (two 13 mm-inner-diameter physically decoupled loops) over the abdomen, in a 14.1T-26 cm magnet. Breathing rate and body temperature were monitored through an MR-compatible system, which also delivered respiratory gating signals for all the MR acquisitions. Anatomical multi-slice GRE images (30×30mm^2, 128×128, TE 4.5 ms, minimum TR) were acquired and localized ^{1}H-MR spectra obtained from a 8 µl voxel confined to the liver with STEAM (TM, 20 ms; TR, 6.5 s; TE, 8; 32 scans). HLC was estimated as the area of lipid methylene protons (Lip1.3) relative to that of the water plus Lip1.3, with corrections for T_2 decay. Lipid spectra were acquired with STEAM (TM, 20 ms; TR, 5 s; TE, 2.8; 128 scans) and water suppression[1] without T_2 corrections due to the use of an ultra-short echo time[2] and by HR-NMR *in vitro* from the lipid extracts in a Bruker DRX-600 spectrometer with a cryo-probe tuned to ^{1}H (Fig 1). The lipid profile was derived from both measurements by using the indices shown in the equations in Fig 2. Data are mean ± SEM. Statistical significance was accepted for P<0.05 determined with repeated measurements 1-way ANOVA or paired student's *t* test.

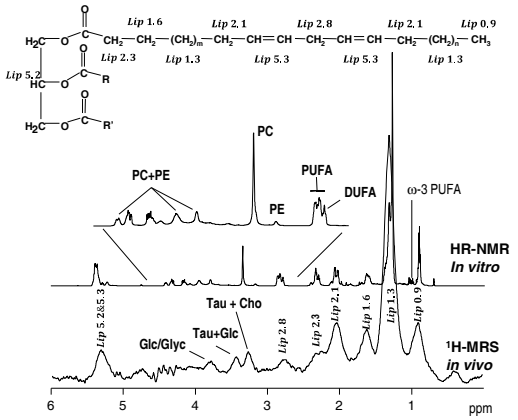

Fig.1 *Top: general structure of a glyceride; in triglycerides both R and R' are fatty acyl chains; in PC R' is a phosphate group linked to choline. Bottom: Spectra acquired from the mouse liver and the lipid liver extracts. Cho,cholines; Glc, glucose; Glyc, glycogen; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PUFA and DUFA poly- and di-unsaturated fatty acyl chains; Tau, taurine.*

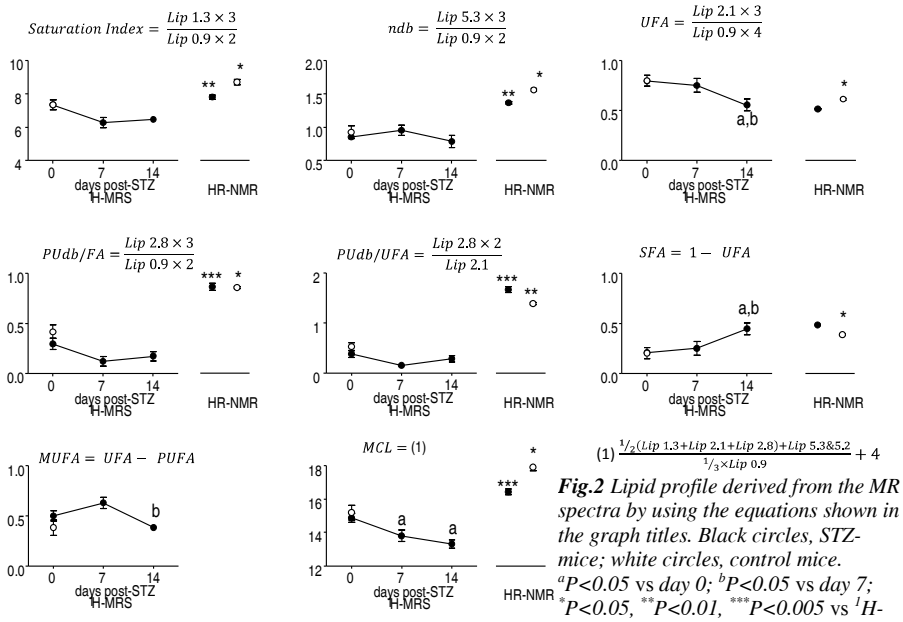

Fig.2 *Lipid profile derived from the MR spectra by using the equations shown in the graph titles. Black circles, STZ-mice; white circles, control mice. [a]P<0.05 vs day 0; [b]P<0.05 vs day 7; *P<0.05, **P<0.01, ***P<0.005 vs ^{1}H-MRS* in vivo *for the same group. ndb, number of double bonds; SFA, UFA and MUFA, saturated- unsaturated- and monounsaturated-fatty-acyl chains (FA); PUdb, poly-unsaturated double bonds; MCL, mean chain length.*

RESULTS AND DISCUSISON: HLC of STZ-mice before injection was 1.7±0.3%, similar to that of controls (1.4±0.1%), but decreased (P<0.05) to 0.6±0.04% 7 days post-STZ without further changes by day 14 (0.7±0.1%). Reduction of HLC was accompanied by alterations in the lipid profile with STZ injection as determined by ^{1}H-MRS (Fig 2): higher contribution from SFA and shorter chains. Compared to *in vivo* measurements, lipids in the liver extracts from both control and STZ-mice were composed of longer chains with a higher degree of poly-unsaturation, as confirmed by the presence of tri- an higher poly-unsaturated fatty acyl chains (PUFA) in addition to di-unsaturated (DUFA), depicted in Fig 1 (HR-NMR spectrum). This reflects the presence of membrane lipids which are composed of longer and highly poly-unsaturated fatty-acyl chains[3]. Accordingly, close to 1/3 of lipid chains in the extracts were from phosphatidylcholine, the principal membrane phospholipid, both in control and STZ livers. Compared to HR-NMR estimations in control mice, those performed in STZ mice reported shorter chains and a higher contribution from SFA, indicating that alterations in the cytosolic lipids were echoed in membranes. However, the PUdb/UFA was higher in the lipid extracts from STZ livers. This suggests an imbalance in membrane PUFAs, since that index didn't change in the cytosolic lipid pool with STZ injection. The overall alterations in hepatic lipids in STZ-mice agree with increased lipid breakdown for oxidation[4] and derangements in insulin-stimulated desaturase activity[5].

CONCLUSIONS: The profile of both cytosolic and membrane lipids is affected in STZ-diabetic mice that experience a reduction in HLC. Lipid-targeted interventions in this and other models of hepatic lipid depletion can be potentially followed by ^{1}H-MRS methods at high fields.

REFERENCES: [1]Tkac I et al. *Magn Reson Med.* 1999; 41:649. [2]Soares AF et al. *ISMRM Proc*. 2013: 4040. [3]Bohov P et al. *Ann. N. Y. Acad. Sci.* 1997; 827:494. . [4]Jourdan T et al. *J. Nutr.* 2009; 139:1901. [5]Wang Y et al. *J. Lip. Res.* 2006; 47:2028

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