Glucose and Intralipid Infusion in Rats: Comparative Quantification of Liver Steatosis by MRI, MRS and Histopathology

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Purpose: To assess the accuracy of 1.5 T dual-echo magnetic resonance imaging (MRI) and histology by using [1]H-magnetic resonance spectroscopy (MRS) as the reference standard for the discrimination of three rat phenotypes assigned to an experimental glucolipotoxic steatosis model or a control group.

Materials and Methods: Steatosis was induced in 2-month old Wistar (YW), 6-month old Wistar (OW) and 2-month old diabetic Goto-Kakizaki (GK) rats with a 72 h intravenous infusion of glucose and Intralipid fat emulsion and compared with control rats receiving a saline infusion. Dual-echo MRI measurements of hepatic fat content were compared with ^{1}H-MRS and histopathological steatosis grade. Histological evaluation of livers was performed by a pathologist according to the NASH scoring system. MR experiments were performed on a 1.5 T MR unit (Signa EchoSpeed version 9.1; GE Medical Systems, WI, USA). Dual-echo magnetic resonance imaging (MRI) was recorded on the whole liver using the 2D spoiled GRE sequence (TR/TE 150/2.2 and 4.5 msec, slice thickness/gap 2/0 mm, matrix 256 x 160, 90° flip angle). An additional dual-echo IP/IP sequence (TE 4.5/18.5 msec) was performed to calculate the T2* correction value. The fat content was calculated and corrected for T2* values. [1]H-MR spectra were obtained from a 1 x 1 x 1 cm voxel positioned at the center of the liver using a PRESS sequence (TR/TE = 1200/30 msec; 16 acquisitions) and treated with the LCModel software. In [1]H-MR spectra, L/(L+W) ratios were estimated, L being the sum of areas of the three lipid peaks (0.9, 1.3 and 2.0 ppm), and W, the area of the water peak (4.7 ppm) both being corrected for T1 and T2* values. The radiologist and pathologist were both blinded to the results of the other observer and to the group to which rats were allocated. The Pearson correlation coefficient was used to determine the correlations between two fat quantification techniques. Two-way ANOVA with Bonferroni post tests were used to compare rat groups according to phenotype and treatment.

Results: A significant correlation was found between dual-echo MRI and ^{1}H-MRS (r = 0.776, $P < 0.001$). A weaker correlation was found between histopathology and ^{1}H-MRS (r = 0.655, $P < 0.001$). MRS, MRI and histopathology results are summarized in Fig. 1. MRS and MRI accurately distinguished the rats receiving the infusion of glucose + Intralipid from those receiving the saline control whereas histology did not. Vacuolar degeneration was observed in 8/41 cases (19.5%) and poor morphology with central clear spaces in the cytoplasm of hepatocytes was observed in 13/41 rats (31.7%). These two factors affected the accuracy of steatosis quantification by histopathology (Fig. 2).

Conclusion: Dual-echo MRI showed higher correlations with ^{1}H-MRS than histopathology with ^{1}H-MRS in an experimental glucolipotoxic steatosis rat model. While many authors use histopathology as the gold standard, we suggest two factors which may limit the accuracy of steatosis quantification: vacuolar degeneration and glycogen. These results favor using MRI or ^{1}H-MRS for accurate steatosis assessment.
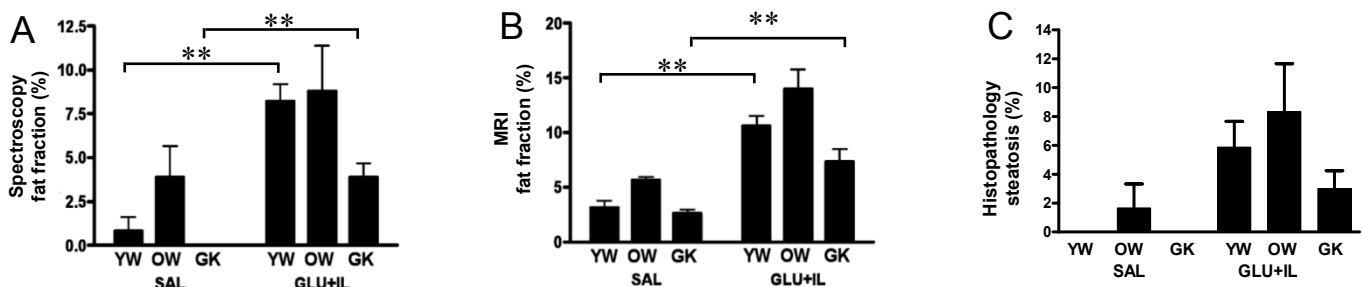
Figure 1. Bar graphs of rat liver fat fraction (%) as determined by ^{1}H-MRS (**A**), MRI (**B**) and histopathology (**C**) for the 3 rat phenotypes: young Wistar (YW), old Wistar (OW) and Goto-Kakizaki (GK) and 2 groups: saline control (SAL) and glucose + Intralipid fat emulsion (GLU+IL) infused rats. *$p<0.05$, **$p<0.01$.
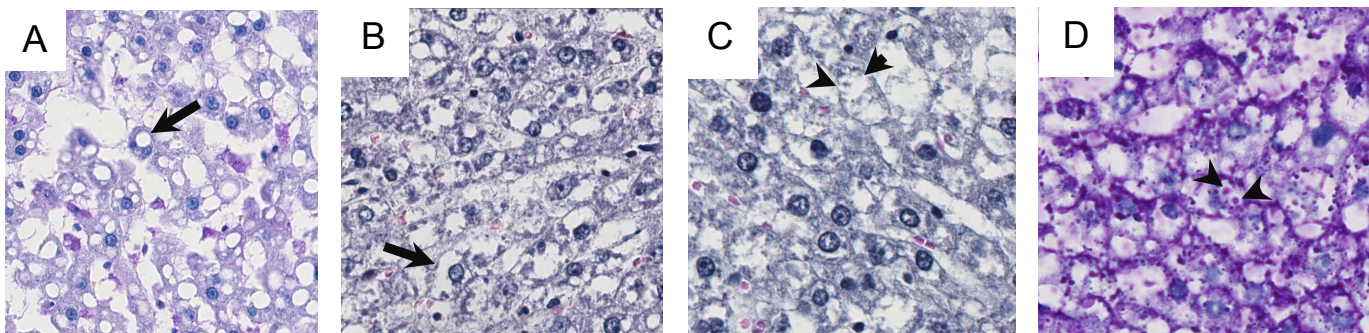

Figure 2. Histological micrographs (H&E, 40x magnification) illustrate 3 causes of clear spaces in the cystoplasm of hepatocytes which may affect the accuracy of macrovesicular steatosis quantification. Severe macrovesicular steatosis, estimated at 70% on histopathology (arrow) (**A**). Vacuolar degeneration (hydropic changes) of poorly preserved liver cell tracts significantly complicate histologic assessment of associated fatty liver changes (arrow) (**B**). Glycogen which has been dissolved in the aqueous fixative may also produce a vacuolated cytoplasmic appearance (arrowheads) (**C**). PAS coloration taken in the same zone and same animal show residual glycogen granules as red dots (arrowheads) (**D**).