Investigation of intrahepatic fat and liver glycogen in diet induced fatty liver disease model

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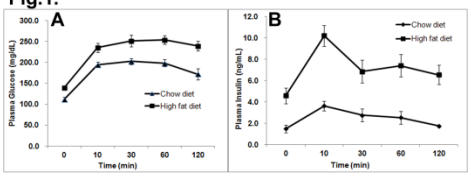
Target Audience: Authors interested in correlation of hepatic fat and glycogen in NAFLD

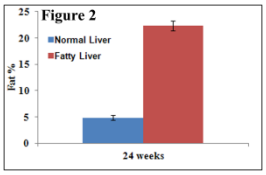
Introduction: Hepatic steatosis (non-alcoholic fatty liver disease-NAFLD) causes insulin resistance which acts as pathogenic link between obesity and its metabolic complications[1]. NAFLD is associated with metabolic syndrome involving obesity, dyslipidemia, hypertension, type-2 diabetes mellitus and cardiovascular diseases[2-3]. Evaluation and correlation between hepatic hyperlipidemia and hepatic glycogen content in insulin resistant non-alcoholic fatty liver model will help in understanding the early metabolic changes in liver metabolism. In this study we have evaluated the liver fat and glycogen content in non-alcoholic fatty liver using a diet induced insulin resistant rat model and compared with normal liver by in vivo and ex vivo MRS.

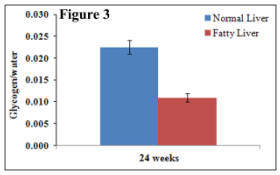
Animal model and Methods: All *in vivo* experiments were in compliance and approved by institutional animal care and use committee. Male F344 rats were fed with control chow diet (n=7) and high saturated fat diet (HFD), n=7, D12709B for 24 weeks. *In vivo* MR experiments with motion compensated respiratory gating were performed on a 7 T ClinScan MRI/MRS scanner (Bruker, Karlsruhe, Germany) equipped with a 72-mm volume resonator for RF transmission, in combination with a 20-mm surface receive-only coil. A volume-localized PRESS sequence was employed on liver with TR=4 s, TE= 13 ms, number of averages NA 128, voxel volume= 64 mm^3, spectral width= 3500 Hz, with 2048 complex points per free induction decay. Glycogen and fat content from in vivo spectra were estimated using LC Model software[4] . After the terminal in vivo experiments (24 weeks of age), ^{1}H high resolution magic angle spectra (HRMAS) of liver tissue samples were acquired using a 9.4 T Bruker Avance III spectrometer and a 4 mm HRMAS HX probe. Quantitative one-dimensional spectra were acquired with data points-32K, spin rate- 4K, sample temperature- 273 K, spectral width-7 kHz, relaxation delay-7 sec, transients-128 (with water presaturation). Concentrations of glycogen was estimated with reference to known concentration of 3-(Trimethylsilyl)-1-propanesulfonic acid sodium salt. All ex vivo data were analyzed using TOPSPIN 3.0 software. Fatty liver samples were histologically analyzed by Oil Red O and hematoxylin and eosin staining.

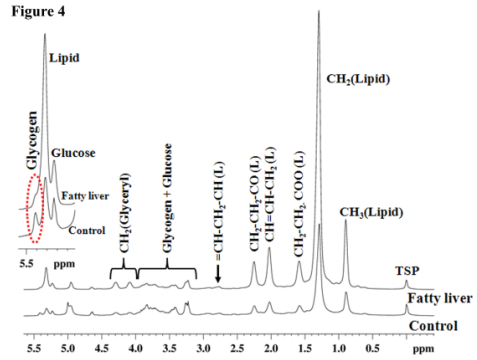
Results and Discussion: Oral glucose tolerance test (OGTT) measurements (**Figure 1**) confirmed the insulin resistant condition of the animals fed with high saturated fat diet (HFD). Blood glucose and insulin concentrations of HFD fed animals measured at 24th week were significantly (P<0.001) higher than chow diet fed animals confirming hyperinsulinemia and hypertriglyceridemia (Table 1). The liver fat from HFD (**Figure 2**) animals at 24th week was significantly higher (22.30±1.02%) compared to the control group (4.84 ±0.44 %) (P < 0.001). Hematoxylin-eosin and Oil Red O staining confirmed fatty liver condition of the HFD fed animals. Based on the scoring method[5], the HFD fed animals showed histopatholgical features of hepatic steatosis with a score of 3 indicating over 68 % of acini were occupied by lipid vacuoles compared to chow diet fed animals.

Fig.1.









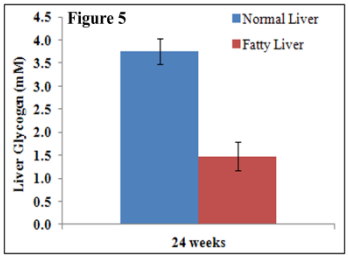
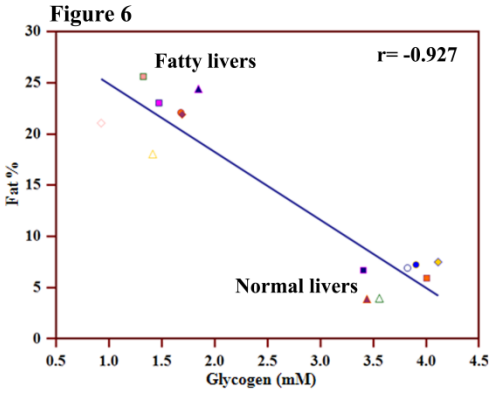


Table 1

Biochemical measurements	Control group	Fatty liver group
Cholesterol (mg/dL)	53.64	151.63
Triglyceride (mg/dL)	85.97	469.94
Glucose (mg/dL)	122	164
Insulin (ng/mL)	1.40	5.21
Body weight (gm)	342	487



HFD animals displayed histopathlgical features of hepatic steatosis. The *in vivo* glycogen content of fatty liver was significantly (P < 0.001) lower than (2 fold) normal liver (**Figure 3**). **Figure 4** shows the *ex vivo* HRMAS spectrum obtained from fatty liver and normal liver. The glycogen CH peak (expanded spectrum) at 5.4 ppm is well separated from the lipid CH=CH (5.3 ppm) and glucose CH (5.23 ppm) peaks. **Figure 5** shows the glycogen content in fatty liver and normal liver estimated from HRMAS spectra. Glycogen content in fatty liver (**Figure 5**) was significantly (P < 0.001) lower (2.5 fold) than normal liver. Glycogen content determined by *in vivo* and *ex vivo* were comparable. Insulin resistant condition down regulates the glycogen synthetase activity thereby causing reduction in synthesis of glycogen content[6-7]. Reduced glycogen content was reported in skeletal muscle of diabetic patients due to reduced glycogen synthase activity[7]. In the current study, the reduction in glycogen content (2.5 fold) in fatty liver compared to normal liver might be due to low glycogen synthase defects under hyperlipidemia and insulin resistant condition. The liver fat and glycogen were analyzed by regression analysis (coefficient of determination R^2= 0.86). The correlation coefficient was r= -0.92 (**Figure 6**) shows that the liver fat content is inversely correlated with glycogen.

Conclusions: Hyperlipidemia in fatty liver condition alters the glycogen synthesis. Liver fat content in fatty liver is inversely correlated with glycogen. Monitoring the early changes in liver fat and glycogen in the liver might be of potential in treatment of NAFLD.

References: (1). Dunn W, et.al., Am J Gastroenterol, (2008) 103, 2263; (2). Seppala-Lindroos A, et. al., J Clin Endocrinol Metab (2002) 87, 3023; (3). Ikai E. et.al., J Hum Hypertens, (1995) 9, 101; (4). Provencher et. al., *NMR Biomed.* (2001) 14, 260; (5). Wei Y, J Hepatol, (2008) 49, 417;(6). Cline GW, et. al., N Engl J Med., (1999) 341, 240; (7). Thorbun AW, et. al., J Clin Invest (1991) 87, 489.