Evaluation of hepatic lipid in high fat diet induced obese rats by in-vivo MRS and LC-MS techniques

Jadegoud Yaligar[1], Venkatesh Gopalan[1], Shui Guanghou[2], Lam B D Jenny[2], Tai E Shyong[3], Markus R Wenk[2], and S. Sendhil Velan[1]
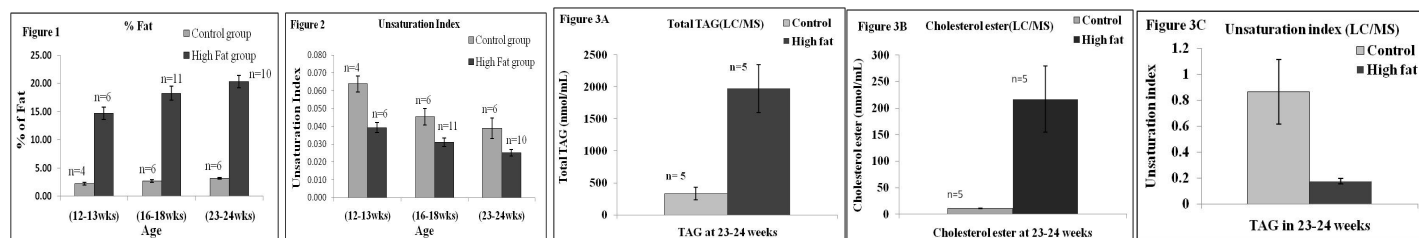
[1]Laboratory of Molecular Imaging, Singapore Bioimaging Consortium, Singapore, Singapore, [2]Department of Biological Sciences, National University of Singapore, Singapore, Singapore, [3]Department of Medicine, School of Medicine, National University of Singapore, Singapore, Singapore

Introduction: Excessive accumulation of fatty acids and triglycerides within the cytoplasm of the hepatocytes under non-alcoholic condition is non-alcoholic fatty liver disease (NAFLD). NAFLD is commonly associated with metabolic syndrome, obesity and diabetes. In Vivo Magnetic Resonance Spectroscopy (MRS) provides detailed information on the triglycerides including methyl, n-methylene, allylic methylene, biallylic methylene and olefine protons [1-2]. The MRS signals from the liver is averaged over all lipid chains and cannot be differentiated by respective chain lengths. On other hand in vitro Liquid Chromatography -Mass Spectrometry (LC-MS) has been developed and utilized to identify and quantify various lipid chains involved in anabolism and catabolism [3]. In this study we have employed both in vivo MRS and in vitro LC-MS approaches to investigate the hepatic lipid accumulation in high fat diet induced obese Rats.

Methods: All the *in-vivo* experiments are in compliance and approved by local Biological Resources Center (BRC). In this study the hepatic lipid was investigated in male F344 rats fed with control chow diet and high fat diet (HFD) D12709B. The composition of High fat diet included 62.4 % saturated fat, 30.7 % monosaturated fat, 6.9 % polyunsaturated fat content. All the animals were subjected to Magnetic Resonance Imaging (MRI) and Localized Magnetic Resonance Spectroscopy (MRS). Prior to in-vivo experiments animals were anesthetized with 1.5-2 % Isofluorane mixed with medical air and medical oxygen and in vivo experiments were performed using a 7 T ClinScan MRI/MRS scanner using a 72mm volume resonator for RF transmit and 20mm receive only coil. The longitudinal in vivo measurements were performed on both control group and HFD group animals at 12-13 weeks, 16-18 weeks, and 23-24 weeks of age. All experiments were motion compensated with respiratory gating during acquisition. Volume localized PRESS experiments were performed on a $2 \times 2 \times 2$ mm^3 voxel with TR=4 s, TE=13ms, 128 averages, and 2048 complex points were acquired with a spectral width of 3500 Hz. Lipid concentrations (saturated and unsaturated) for both control and high fat diet groups were estimated using LC Model software by using the resonances of methyl, n-methylene, allylic methylene, and the unsuppressed water signal [4]. After the terminal in vivo experiments (23-24 weeks of age) liver tissue samples were analyzed by both Oil Red O staining and LC-MS approaches. Ultra Fast Liquid chromatography (UFLC. Shimadzu) coupled with 3200 Q-Trap mass spectrometry (Applied Biosystems) by using Agilent Zorbax Eclipse XDB-C18 column i.d. 4.6×150 mm and the mobile phase chloroform:methanol:0.1M ammonium acetate(100:100:4) at flow rate of 0.25 ml/min [5]. Lipids in each class were quantified using appropriate internal standards. Unsaturation index in TAG was calculated using the ratio of level of TAG species with total double bond more than three (unsaturated) to that of more saturated TAG species (equal or less than three double bond).

Results and Discussion: Figure 1 shows the % of total fat in high fat and control groups of animals at different age groups. The total liver fat (%) was significantly higher in the animals fed with high fat diet at all age groups compared to their respective control groups. The unsaturation index was significantly higher for the control group compared to the high fat diet group at all measured age groups (Figure 2). The results were in agreement with the composition of the high fat diet D12709B. Figure 3A shows the total triglycerides (TAG) estimated using LC-MS approach in high fat and control animals at 23-24 weeks of age. The total amount of triglycerides (TAG) in high fat animals was 1975 ± 364 nmol/ml compared to its control group 331 ± 62 nmol/ml. Figure 3B shows the plot of cholesterol ester (CE) estimated using LC-MS in high fat and control animals. The Cholesterol Ester (CE) in high fat animals was 217 ± 62 nmol/ml compared to its control group 11 ± 0.5 nmol/ml. Figure 3C shows the decreases of unsaturation index in the liver tissue of high fat animals compared to its control group of animals. It is known that the desaturases enzymes might be defective in obesity and can reduce the unsaturated lipids [6].

Histopathology of liver: Figure 4 shows the Oil Red O stained sections of control and high fat diet animals after terminal experiments (23-23 weeks of age). Accumulation of excess lipid droplets (stained in red colour) in vesicles is noticed in high fat group of animals compared to control group of animals.

Figure 1: % Fat

Figure 2: Unsaturation Index

Figure 3A: Total TAG(LC/MS)

Figure 3B: Cholesterol ester(LC/MS)

Figure 3C: Unsaturation index (LC/MS)

Conclusions: The total liver fat content and unsaturation index in high fat fed and control animals estimated by in-vivo MRS technique is comparable with in-vitro LC-MS data. LC-MS techniques have been optimized to achieve detailed information on all lipid chains involved in anabolism and catabolism. Fraction of the unsaturated fat is lesser in HFD animals compared to control animals. The combined approach of using in vivo MRS and LC-MS techniques permits detailed understanding of the lipid content in the liver and can be utilized to improve the prior knowledge based quantitation of in vivo MRS data.

Figure 4   Oil Red O stained sections of the liver (Original magnification -20X)

Control animal   High Fat animal

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