Proton MRS of Changes of Lipid Unsaturation during Liver Regeneration

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Introduction
Partial hepatectomy (PHs) is a curative measure for hepatic malignancies, and has been performed more frequently with advances in diagnosis and surgical techniques1. Liver dysfunction is observed in postoperative period, and there is an increasingly important role of liver stem cells in liver regeneration2. A real time and specific monitoring method of liver regeneration is required. In a 1H-MRS study, energy depletion was observed and the ratios of adenosine triphosphate (ATP) to inorganic phosphate (Pi) decreased after PHx. The regeneration is at maximum at 24-48 h post-operation, which is supported by findings in DNA synthesis3. An increase in unsaturation of liver fatty acid was reported in rat liver after PHx ex vivo, and peaked at day 1 and day 5. Moreover, lipid metabolism is linked to a gene that found in liver regeneration4. However, data on the corresponding genetic models varied when correlating the gene with triglyceride to assess liver regeneration. This showed that triglyceride could be uncoupled from liver regeneration6. The use of 1H-MRS to monitor unsaturation of lipids could provide more information of lipid metabolism as compared with 31P-MRS, and could be a promising and alternative method to assess liver regeneration non-invasively. This could give us insight into the precise role of lipids in liver regeneration.

Methods
Animal Preparation: Male Sprague-Dawley (SD) rats (280-300 g; N = 3) were under investigation. A standard 70% hepatectomy was performed in which the left and median lobes of the liver were removed1. MRS was performed on the animals before and after PHs at 12h, 24h and 120h. In Vivo MRS: 1H MRS experiments were performed on a 7T Bruker MRI scanner with a 60 mm RF Tx/Rx quadrature resonator. Animals were anesthetized with ~1.5 % isoflurane, and body temperature was maintained at about 36.5°C with respiratory monitoring. Scout images were first acquired in three orthogonal planes with a FLASH sequence. A 5×5×5 mm3 voxel was placed on a homogeneous liver parenchyma, without involving large blood vessels. MAPPISHIM protocol was applied for imaging, and each voxel was shimmed by the first- and second-order localized voxel shimming based on field map technique. A FWHM linewidth of water signal of <30 Hz was achieved. The water signal was suppressed by variable power RF pulses with optimized relaxation delays (VAPOUR). Outer volume suppression (OVS) combined with respiratory-triggered point-resolved spectroscopy (PRESS) sequence was used for signal acquisition, with TR = 2 respiratory cycles (~2.0-2.5 s), TE = 15 ms, spectral bandwidth = 3 kHz, 2048 data points, 512 averages, and total scan time of ~15 min.

Data Analysis: MR spectra were processed using the MR spectroscopic analysis package provided by manufacturer. The raw data were zero-filled, apodized with a 2-Hz exponential filter, Fourier transformed, 0th- and 1st-order phase corrected, and baseline corrected. Major peaks with good SNR were evaluated, which including signal integrals of saturated lipid (–CH3, –CH2–, Sat), unsaturated lipid (–CH=CH–, Unsat) and choline (CCC) at 0.9-1.5, 5.3 and 3.2 ppm, respectively. The area under peak integrals of saturated lipid (–CH3, –CH2–, Sat), unsaturated lipid (–CH=CH–, Unsat) and choline (CCC) were manually quantified. The Sat and Unsat were measured by normalizing the peak area to CCC.

Results
In Fig. 1a, the 1H-MRS spectra of the liver before and after PHx at 12 h and 24 h were shown with Fig. 1b indicates the position of voxel. Peaks of Sat and Unsat lipids had changed at different time points. An increase in Sat and Unsat was observed at 12h and 24 h post-PHX, respectively (Fig. 2). Sat had a maximal increase at 12h (Fig. 2a), and Unsat had a maximal increase at 24h (Fig. 2b). Both lipids decreased at 120h post-PHX. The body weight of the rats decreased by 4% and 7% in average at 12h and 24h post-PHX, respectively, and increased by 4% in average at 120h post-PHX.

Discussion
In this study, we monitored the change in the three major components in the liver, which included Unsat, CCC and Sat. No significant change was observed in CCC, and total lipids increased. We compared Unsat:CCC with Sat:CCC, as the degree of unsaturation was reported to increase after PHX4. There was over 95% of the functioning hepatocytes enter the cell cycle, and DNA synthesis was found at 12h and reached a maximum at 24h post-PHX, which indicated the maximal liver regeneration2. A maximal increase in Sat was at 12 h and that in Unsat was at 24h. This could be ascribed to the initial phase of liver regeneration at 12h. When the liver regeneration reaches maximum at 24h, Unsat was the highest with Sat remained high. According to DNA synthesis studies, most of the liver regeneration takes place at day 3 and the mass of the liver restores at day 7 post-PHX9. At 5 days after PHx, level of unsaturation of liver fatty acid was at maximum ex vivo. Authors suggested that a high level of unsaturation could inhibit liver regeneration7. That is not much regeneration will be expected at day 5.

Conclusion
Proton MRS study of the change of unsaturated lipids in the liver after PHx allows us to monitor the liver regeneration. We found a high unsaturated lipid content at 24h post-PHX, at which liver regeneration is maximum. This could be related to liver regeneration. Hence, 1H-MRS could assess the liver regeneration in a multi-component manner.

References