Changes in Choline Level and Lipid Profile in Rat Liver During Lipid Infusion Measured by Dynamic Proton MRS

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INTRODUCTION: Liver lipid content is known to be altered by the oral intake of polyunsaturated fatty acid (PUFA) and monounsaturated fatty acid (MUFA) [1]; however, the temporal relationship between the unsaturated lipid intake and liver lipid profile change is remains largely unclear. Choline is known for its essential role in the removal of the excessive lipid from liver, and the plasma choline concentration decrease is in conjunction with hepatic steatosis in patients receiving long-term lipid infusion (e.g., as parenteral nutrition) [2]. However, no study has determined the liver choline dynamics in response to the acute lipid intake. The liver proton MR spectroscopy (MRS) is typically used for quantifying lipid content [3, 4]. Previous MRS studies have reported the quantifications of liver lipid profile [5, 6] and liver choline *in vivo* [7, 8]. This study aimed to examine whether dynamic proton MRS can assess the time courses of liver choline level and lipid profile in response to the acute lipid intake.

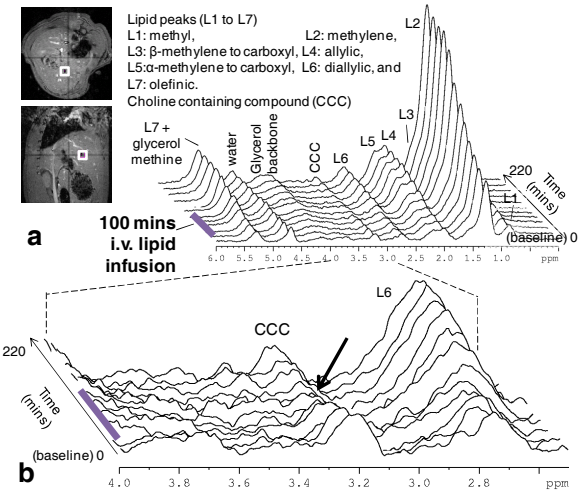

Figure 1 The stack plot of representative dynamic liver proton MR spectra acquired from a rat before, during and after the intravenous lipid infusion. (**a**) Eleven spectra (20-min acquisition time for each) were acquired with a typical voxel placement (white square) shown in anatomical images. Seven lipid peaks and the peak of glycerol backbone were increased drastically during and after the lipid infusion. (**b**) The enlarged spectra show the dip of choline peak amplitude (*arrow*) immediately after the lipid infusion.

MATERIALS AND METHODS: Animals: Male Sprague-Dawley rats (~350g, n=4) were infused with 0.9 ml heparinized saline followed by 10 ml 20% *Intralipid* (with 20U heparin/ml, 0.1 ml/min) via tail vein. During experiment, rats were anesthetized with 1.5% isoflurane, mechanically ventilated and kept warm at 37 ºC. **MR experiments**: All experiments were performed on a 7T MRI scanner. A stimulated-echo (STEAM) based single-voxel MRS sequence with echo time (TE) of 3 ms was used for spectra acquisition with respiration triggering. Note that such short TE was chosen to minimize T_2 weighting. Other parameters were TR=2 s, NEX=512, voxel size=4×4×4 mm^3. **Data Analysis:** Individual frequency realignment and phase correction were applied to the 512 FIDs before the average in order to mitigate the motion induced signal loss and spectral broadening. Spectral analysis was performed using the JMRUI software package. Ten spectral resonances were quantified by fitting the spectrum to a Gaussian line shape using the AMARES algorithm. Six fitted data points with SNR<1 or severe motion contamination were excluded from further analysis. For statistic analysis, a two-tail paired t-test was used.

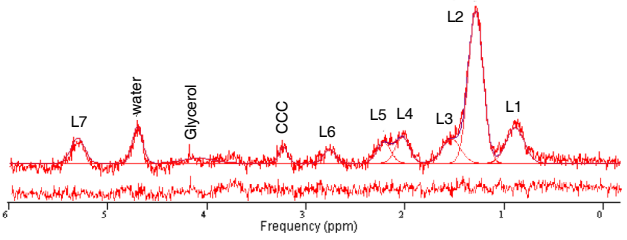

Figure 2 Ten spectral peaks were quantified from a typical rat liver spectrum that was acquired at the baseline. The original spectrum was overlaid with ten fitted peaks (*top*). The residual spectrum after fitting (*bottom*) was relatively flat, suggesting a reliable fitting.

RESULTS: Figure 1a shows the drastic increase of seven lipid peaks and the peak of glycerol backbone during and after the lipid infusion. Figure 1b shows the dip of choline peak amplitude immediately after the 100 mins lipid infusion. In Figure 2, the residual spectrum after fitting was relatively flat, suggesting good fitting quality. In Figure 3a, the hepatic lipid level drastically increased after the lipid infusion, i.e., from 49.5±9.8 at 0 mins to 171.1± 6.8 at 220 mins (p<0.001). In Figure 3b, the choline level significantly decreased from 3.3±0.7 at 0 mins to 2.6±0.9 at 120 mins (p<0.01) and 2.5±1.0 at 140 mins (p<0.05). The choline level was significantly increased after 140 mins, i.e., 3.5±0.9 at 180 mins vs. 2.6±0.9 at 120 mins (p<0.01). In Figure 3c, no apparent changes in the fraction of total unsaturated lipid were observed. In Figure 3d, the estimated fractions of PUFA consistently increased in four rats, while the MUFA deceased, as a result of the minimum changes of the fraction of total unsaturated lipid in Figure 3c. The fraction of MUFA was calculated by the subtraction of the fraction of total unsaturated lipid (0.5×L4/L5) and the fraction of polyunsaturated lipid (L6/L5). The fraction of PUFA after lipid infusion (i.e., after 100 mins) was found to be significantly larger than the fraction of MUFA, i.e., 0.55±0.05 for PUFA and 0.06±0.04 for MUFA at 220 mins compared with 0.36±0.14 for PUFA and 0.24±0.15 for MUFA at 0 mins.

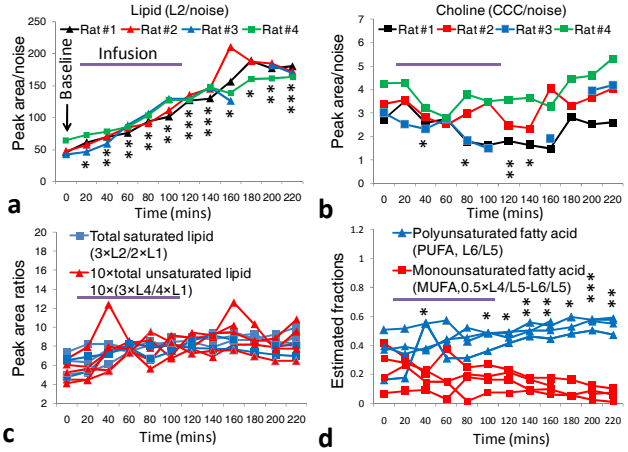

Figure 3 The time courses of lipid and choline levels and lipid composition parameters for each animal (n=4). 20% *Intralipid* was infused after baseline spectra acquisition and lasted for the first 100 mins. (**a**) Three-fold increase of hepatic lipid level was observed within 160 mins after lipid infusion was started. (**b**) Choline decrease during the infusion and choline recovery after the infusion were consistently observed in all rats. (**c**) The time courses of the total saturated lipid and the total unsaturated lipid largely overlapped after scaling, suggesting minimum changes in the fraction of unsaturated lipid during the infusion. (**d**) The estimated fractions of PUFA consistently increased in four rats, in conjunction with the MUFA decease. *p<0.05,**p<0.01,*** p<0.001.

DISCUSSIONS: The gradual decrease of choline content level after lipid infusion suggested the participation of choline in the liver lipid metabolism. The soybean oil based *Intralipid* emulsion contained ~60% PUFA. This may explain the increase of PUFA fraction after the infusion. The isoflurane anesthesia was used in this animal study, which may alter the liver lipid metabolism. Therefore, a control group without lipid infusion will be measured to verify if the anesthesia would affect the choline level and lipid profile.

CONCLUSION: Our preliminary results suggested that dynamic proton MRS could measure the changes of choline level and lipid profile in normal rat liver with acute intravenous lipid infusion.

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