INTRODUCTION
Hepatic ischemia/reperfusion injury (IRI) occurs during liver transplantation, tumor resection, hemorrhagic shock and veno-occlusive disease, and is a major cause of acute liver failure which associated with high morbidity and mortality. IRI in liver is also responsible for early organ failure and increased incidence of both acute and chronic rejection after liver transplantation. Biochemical changes caused by hepatic IRI lead to hepatocellular remodeling, including cellular regeneration or irreversible programmed cell death. Proton magnetic resonance spectroscopy (1H MRS) has been increasingly employed to investigate in vivo liver metabolism noninvasively. However, in vivo study of hepatic IRI model using such technique has been limited. In this study, we aim to demonstrate that the alterations in the liver metabolism can be monitored with 1H MRS using an experimental hepatic IRI model in rats at 7 T.

METHODS
Animal Preparation: The rodent model of mild total hepatic IRI was performed as described previously in rats. Sprague-Dawley (SD) male rats (260-280 g; N = 6) were anesthetized with isoflurane/air using 1.0-1.5 % via a nose cone. In brief, the abdomen was shaved and a midline incision was made. The common portal vein, hepatic artery and bile duct in the hepatoduodenal ligament were clamped using a vascular clamp. The liver was inspected for ischemia for 2 minutes. After 30 minutes of hepatic ischemia, the clamp was removed initiating hepatic reperfusion. The liver was again inspected for restoration of blood flow, then the abdomen was closed and the animal was kept at ambient temperature of 37 °C. 1H MRS was performed at 1 day before injury, 6 hours, 1 day and 1 week after hepatic IRI.

MRS: All MRS experiments were performed on a 7 T Bruker MRI scanner using a 60-mm quadrature RF coil. Under inhaled isoflurane anaesthesia, the animal was kept warm under circulating water at 37 °C. Scout images were first acquired in three orthogonal planes with a FLASH sequence. For 1H MRS, a 5.0 × 5.0 × 5.0 mm3 voxel was placed over a homogeneous liver parenchyma with care avoiding large blood vessels. After first- and second-order localized voxel shimming with field map based shimming technique, a full-width half-maximum linewidth of water signal of ≤ 40 Hz would be achieved. The water signal was suppressed by variable power RF pulses with optimized relaxation delays (VAPOR). Outer volume suppression (OVS) combined with respiratory-gated point-resolved spectroscopy (PRESS) sequence was used for signal acquisition using TR = 2000 ms, TE = 15 ms, spectral bandwidth = 3 kHz, 2048 data points and 512 averages.

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. Signal integrals of lipid (integral sum of CH3, (–CH2–), and –CH=CH– at 0.9, 1.3 and 5.3 ppm, respectively), choline-containing compounds (CCC; at 3.2 ppm) were manually quantified by areas under peaks. The CCC-to-lipid ratio was measured by dividing peak area of CCC by that of lipid. The relative saturated and unsaturated fatty acid fractions were estimated by dividing peak areas of (–CH2–), and –CH=CH– by peak area of lipid, respectively. Paired one-way ANOVA was employed to compare differences in ratios of peak areas in different groups, with p < 0.05 considered as statistically significant.

RESULTS AND DISCUSSIONS
Figure 1 shows the typical liver 1H MRS spectra at 1 day before injury, 6 hours, 1 day and 1 week after hepatic IRI for the same animal. Figure 2 shows the CCC-to-Lipid ratio for all the animals studied. There were significant differences (p < 0.01) between different time points for CCC-to-lipid ratio. Note that free fatty acid remained relatively constant during hepatic IRI in an in vitro study, similar signal integrals of lipid were expected over different time points to the mild hepatic IRI in this study. The (–CH2–)to-lipid, and –CH=CH–to-lipid ratios showed no significant differences over different time points, as suggested by earlier study. The choline compounds resonance at 3.2 ppm include choline, phosphocholine, glycerophosphocholine, and taurine, which are thought to represent constituents in phospholipid metabolism of cell membranes. Elevation of CCC peak is believed to represent increased biosynthesis of membrane phospholipids, and hence as an active marker for cellular proliferation. The significant increase of CCC-to-lipid ratio at 6 hours after injury could arise from the hepatocellular regeneration within the first 6 hours after mild hepatic IRI. The CCC-to-lipid ratio then normalized afterwards suggesting that hepatocellular regeneration returned to baseline after 24 hours, which also correlates well with previous study. This CCC peak may be useful in evaluating the regeneration of hepatocytes upon hepatic IRI.

CONCLUSIONS
The experimental results of this study showed that alteration in the metabolism of choline-containing compounds is associated with hepatic ischemia/reperfusion injury. The higher CCC-to-lipid ratio is potentially a result of increased hepatocellular regeneration within the first 6 hours following reperfusion. From the well-controlled hepatic IRI model in this study, 1H MRS offers the promise as a robust tool to identify and quantify metabolic changes in liver with increased signal intensity and spectral resolution of metabolite resonances since signal-to-noise ratio and spectral resolution increase proportionally with B0.

REFERENCES