LIVER INJURY FOLLOWING INTESTINAL ISCHEMIA-REPERFUSION DEMONSTRATED BY 31P MAGNETIC RESONANCE SPECTROSCOPY IN VIVO.

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Introduction
Intestinal ischemia-reperfusion (IR) injury is one of the most serious surgical conditions in paediatrics. Neonates may suffer necrotising enterocolitis (NEC), intussusception, midgut volvulus and strangulated intestinal hernia. The most common cause of morbidity and mortality in necrotising enterocolitis is multiple organ failure, especially involving the liver. It has been demonstrated that intestinal IR activates the release of inflammatory mediators and promotes leukocyte-endothelial cellular adhesion. Generalised microvascular injury is produced in both local and remote organs (1). A rat model of intestinal IR created by occluding and deoccluding the superior mesenteric artery has been used in several centres to study the pathophysiology of this condition, and a previous study of liver metabolites from our group by 31P MRS in vitro showed that 90 min of intestinal ischemia followed by 60 min of intestinal reperfusion significantly affected hepatic energy metabolism (2).

The aim of this study was to characterize the full time course of phosphorus metabolic changes in the liver throughout intestinal IR using 31P MRS in vivo.

Materials and Methods
Animals
Male Sprague-Dawley rats weighing between 190-230 g were used for this study. All animals were fed standard rat chow and water ad libitum and were given only water for 12 hours prior to the experiment. The animals were divided into three experimental groups with at least 6 rats in each group; A) sham operation plus 150 min of anaesthesia as a control B) 150 min of intestinal ischemia only and C) 90 min of intestinal ischemia followed by 60 min of reperfusion.

Surgical procedure
The animals were anaesthetised with oxygen and 1.5-2% halothane. Electrocardiogram (ECG) and rectal temperature were monitored. For group C and D the superior mesenteric artery (SMA) was isolated and a surgical clip was applied at its origin. A surface receiver circular coil 1 cm. in diameter was placed on the anterior surface of the liver. NMR spectroscopy was carried out in a 2.35 T horizontal magnet interfaced to an SMIS console. After 90 min of intestinal ischemia the clip was remotely removed. The anesthetised animal underwent monitoring for an additional 60 min during intestinal reperfusion. For group A, the animals underwent laparotomy and the SMA was isolated but no surgical clip was applied. For group B, the animals underwent laparotomy and the SMA was identified then the clip was applied through the assigned duration. The small intestine and liver were then freeze-clamped for tissue extraction. 31P NMR spectra were acquired every 4 min throughout ischemia and reperfusion.

In vivo 31P MRS measurement
Spectral changes were analysed by calculating the hepatic inorganic phosphate (Pi) to β-ATP ratio. Hepatic intracellular pH was also analysed.

Statistical analysis
Statistical analysis was performed using analysis of variance (ANOVA) with Newman-Keuls post hoc test. Significant differences were established at p < 0.05

Results
In group C, 5 rats died during the experiment, all in the intestinal reperfusion phase. Data from these animals were not included in the subsequent analysis. There was no mortality during the experiment in other groups.

Conclusion
The onset of liver injury occurred during the intestinal reperfusion phase and intestinal ischemia alone in this model does not impair liver energy metabolism. Hepatic hypoxia is not the cause of liver injury after intestinal IR. This study has shown that 31P NMR spectroscopy in vivo is capable of detecting early effects of intestinal IR on a remote organ and may be a valuable indicator of impaired hepatic function.

References

Fig. 1 shows example spectra of liver during intestinal ischemia (fig. 1.1) and during reperfusion (fig. 1.2), demonstrating an increase in the hepatic Pi/β-ATP ratio during intestinal reperfusion.

Fig. 2 shows the time course data for Pi/β-ATP in the three groups.

We analysed the slopes of individual trend lines for each animal during the initial 90 min (intestinal ischemia or control) and subsequent 60 min (ischemia, reperfusion or control) periods. There were no significant differences among any of the groups, except for the reperfusion phase in group C, which was significantly different to all other groups. There was no significant difference in hepatic intracellular pH among the groups, thus hypoxia is unlikely to be the cause of liver energy failure in this model.