

# Dynamic changes in brain metabolites and tissue water diffusion following oral amino acid challenge in Cirrhotics with Hepatic Encephalopathy

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## Introduction

Liver cirrhosis is a major health problem worldwide, killing more than 750,000 people each year. Almost 30% of patients with cirrhosis experience hepatic encephalopathy (HE), a debilitating neuropsychiatric complication of liver disease<sup>1</sup>. Cerebral edema is the likely cause of HE in cirrhotic liver disease<sup>2</sup>, but the mechanisms of edema development remain unclear. MR techniques have previously made fundamental observations regarding brain water and metabolite changes in HE. MR spectroscopy shows significant reductions in the osmotic metabolite *myo*-inositol<sup>3</sup>, while diffusion weighted MRI has been used to assess brain edema where apparent diffusion coefficient (ADC) was found to be elevated compared to appropriately matched control subjects<sup>4</sup>. Blood ammonia levels are proposed as a major driving factor in edema development<sup>2</sup>. Ammonia is neurotoxic and high tissue levels may disturb astrocyte cell volume homeostasis causing metabolic abnormalities in the brain<sup>2</sup>. When cirrhotic patients are given an oral amino acid challenge, blood ammonia levels increase and this is associated with brain electrophysiology and psychometric abnormalities<sup>5</sup>. We therefore hypothesized that if ammonia is a driving force in edema formation, induced hyperammonaemia would lead to transient changes in brain water distribution and metabolite levels detectable by diffusion tensor imaging (DTI) and MRS.

## Methods

**Patients:** Thirteen patients with liver cirrhosis, (10 with alcoholic liver disease and 3 with non-alcoholic cirrhosis, group age 53.3±2.3years) were recruited from the liver transplant waiting list in the North East of England. All had compensated liver function with a mean Child-Pugh score of 8.9 ± 0.5. Blood serum ammonia levels were determined before and following an oral challenge with a combination of 18g each of the amino acids glycine, serine and threonine contained within gelatine capsules. These amino acids were chosen as they have the highest ammoniagenic potential<sup>6</sup>.

**MR Protocol:** MRI was performed using a 3T Philips Achieva system equipped with an 8 channel head coil. Echo-planar imaging (EPI) based DTI were obtained with 6 diffusion directions and b-values of 0 and 1000 smm<sup>-2</sup> (24 × 6mm thick slices encompassing the whole brain, 2mm resolution, TR 2.5s, TE 71ms). T1 weighted anatomical images were also collected (3D T1w sequence, 1mm isotropic resolution, TR 9.6ms, TE 4.6ms). Single voxel proton spectra (PRESS sequence, TR 3s, TE 36ms, 128 averages, 8cm<sup>3</sup> voxel volume) were acquired from temporo-parietal white matter to measure brain metabolite concentrations. Tissue water measurements within the MRS voxel were also made as a reference for calculation of absolute metabolite concentrations (TR 10s, 16 echo times ranging from 40 to 1500ms). All scans were performed at baseline (scan 1) and at 135-175 minutes (scan 2) following the challenge. The study was approved by the local ethics committee and all patients gave written informed consent.

**Data Processing:** Following eddy current correction DTI data were processed to create apparent diffusion coefficient (ADC) images, figure 1 (left). The anatomical scans were segmented to create masks of normal appear white matter (NAWM), figure 1 (centre) and registered to the ADC data. These masks were then applied to the ADC scans on a slice by slice basis and the average NAWM ADC was calculated over all slices for each subject. Proton spectra were processed using the QUEST fitting method within the *jMRUI* software package<sup>7</sup> and levels of *myo*-Inositol, glutamate and glutamine were quantified. Peak areas from the water referencing spectra were fit to a biexponential decay to determine voxel tissue water fraction from which absolute metabolite concentration was determined. MRI measures and blood ammonia changes were compared using paired parametric statistical tests.

## Results

The amino acid challenge caused a mean (sd) rise in blood ammonia of 58.4 (± 40.9) µmol/l, which was accompanied by a mean increase in ADC of 9% (p=0.004). *myo*-Inositol concentration showed a significant reduction as a result of the challenge (p<0.005). Absolute increase in blood ammonia was significantly correlated with ADC in the individual patients, (Pearson r=0.6, p<0.04, figure 2), and also with change in glutamine levels (r=0.6, p=0.02). Change in *myo*-inositol levels between scans was significantly correlated with the difference in ADC across all subjects (r=0.6, p<0.03).

## Discussion

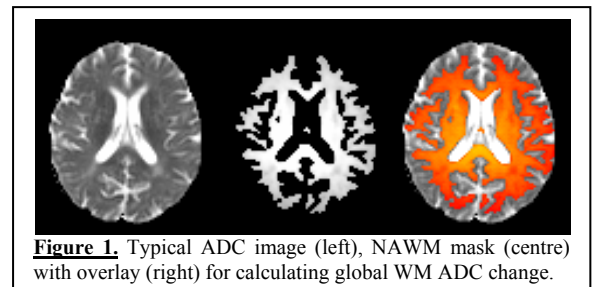
Inducing hyperammonaemia in patients with HE was found to be directly correlated with dynamic changes in both brain water diffusion and metabolite levels. Previously a comparable hyperammonaemic challenge has been shown to precipitate brain electrophysiology and psychometric abnormalities indicative of HE<sup>5</sup>. Our results strongly support the development of brain edema as a neurochemical mechanism for hepatic encephalopathy in patients with cirrhosis. In addition, the research show the potential to use MR measures as biomarkers for evaluating the effect of various therapies to reduce hyperammonaemia and curtail the development of cerebral edema in these patients.

## Acknowledgements

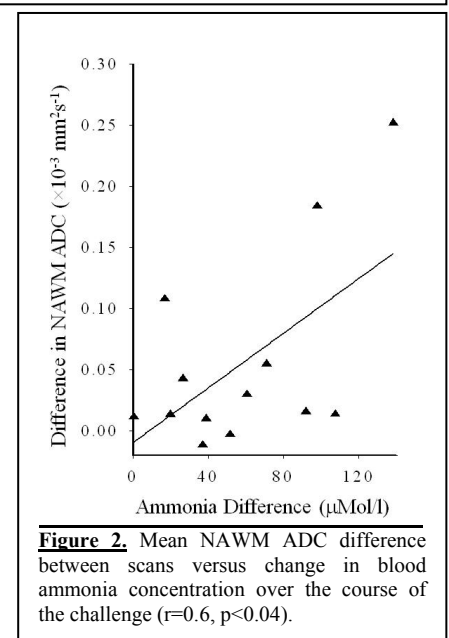
We acknowledge the Newcastle Healthcare Charity for funding this study.

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**Figure 1.** Typical ADC image (left), NAWM mask (centre) with overlay (right) for calculating global WM ADC change.



**Figure 2.** Mean NAWM ADC difference between scans versus change in blood ammonia concentration over the course of the challenge (r=0.6, p<0.04).