# Prediction and prevention of encephalopathy in mouse model of glutaric acidemia

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**INTRODUCTION**: Glutaryl-coenzyme-A dehydrogenase (GCDH) activity is required for complete mitochondrial oxidation of lysine and tryptophan. GCDH deficiency results in accumulation of glutaryl-CoA and glutaric acid known as GA-1. Affected children may suffer acute striatal injury resulting in cerebral palsy within the first few years of life. Current treatment involves dietary lysine restriction and emergency administration of intravenous glucose for encephalopathy. We recently developed an animal model of GA-1 using a GCDH-deficient (Gcdh-/-) mouse. Although Gcdh-/- mice do not develop neuropathology spontaneously, dietary lysine supplementation results in age-dependent brain injury with striking similarity to the human disease [1]. Encephalopathy induced in these mice is progressive resulting in death between 3 and 6 days. Here we used brain extracts from normal and lysine supplemented Gcdh-/- mice to detect biochemical changes associated with early progression of encephalopathy between 48 and 60 hours of diet exposure. Additionally, we compared brain extracts from lysine-fed Gcdh-/- mice given glucose supplementation or homoarginine, which has been shown to block brain lysine uptake [2]. Our preliminary findings predicted that glutamate and glutamine changes with onset of encephalopathy might be detected non-invasively by $^1$H MRS. We present spectroscopy changes associated with progression of encephalopathy and with protective treatments.

**METHODS:** Gcdh-/- mice at 4-weeks of age, were placed in groups of ten on a normal diet or 5% lysine diet or 5% lysine diet with 5% glucose supplemented water or 5% homoarginine or both glucose and homoarginine. Animals and diets were checked daily for difference in weight and diet consumption. Four animals from each group were sacrificed at 48 and 60 hours. Amino acids were separated and measured from HPLC of brain extracts by UV detection after phenyl-isothiocyanate derivatization. MRI was performed on a 7.0 T Bruker system using a 2 mm birdcage coil, at day 48 h, 72 h, and 6 and 12 days following start of diets. Mice were anesthetized with isoflurane (1-1.5%) and imaged with a $T_2$-weighted multi-echo spin echo sequence (five 0.5 mm thick slices, TR/TE=3000/10.6-148.4 ms, 14 echoes, 117X117 $\mu m^2$ resolution, 2 averages). $^1$H MRS data were acquired between 48-72 h, using PRESS sequence, TR/TE= 2656/7 ms, 4.2 ml voxel covering the cortex and the striatum. Transverse relaxation time constant (T2) were calculated on a pixel-by-pixel basis from the corresponding exponential fits using CCHIPS software [3]. LCModel was used to process MRS data. Mann-Whitney U test was used for statistical analysis.

**RESULTS:** Glutamate and glutamine were significantly reduced by 50% and 25% respectively in the brain extracts of Gcdh-/- mice within 48 hours of lysine diet exposure (Fig. 1A). At 48 hours of lysine diet exposure, $^1$H MRS revealed a 38% decrease in the glutamate/glutamine to creatine ratio (Glx/Cr) and a 20% decrease in N-acetylaspartate to creatine ratio (NAA/Cr) in Gcdh-/- mice, consistent with HPLC findings (Fig. 1B). At this time point no striatal injury was apparent on T2-WI (Figure 1B). However the signs of massive brain edema evident as complete closure of lateral ventricles (Figure 2C) and dilation of the veins were present (Figure 2B arrows). Vascular dysfunction has been previously described in human GA-1 [4]. After this initial finding, animals that survived next 48-72 h begin to have increased signal on T2-WI (and elevated T2-values) in the deep cortical layer and striatum (Figure 2C). Animals on homoarginine, glucose and combined homoarginine and glucose therapy, did not have any signs of swelling however dilated vessels were present in some animals independent of treatment. There was no evidence of striatal injury in any of the treated animals, up to 12 days, however some animals had increased T2-values in the deep cortical layer independent of treatment (Figure 2D) probably corresponding to a cortical vacuolation (a common histological and MRI findings in *Gcdh-/-* mouse even on a normal diet, Figure 2A) [1].

**CONCLUSIONS:** We proposed that reduced glutamate/glutamine in GA-1 may be a consequence of Krebs cycle inhibition, as Glutaryl-CoA accumulation sequesters free CoA. Spectroscopy findings in live animals were consistent with biochemical findings in brain extracts of similarly treated mice. Reduction of glutamate and glutamine levels at 48 hours (determined both by HPLC and $^1$H MRS) preceded any evident neuropathology in the striatum. In addition reduced NAA/Cr ratio may further indicate onset of neuronal damage before it becomes apparent on T2-WI. These $^1$H MRS findings can translate to human GA-1 as a diagnostic marker of impending brain injury. Furthermore, our MRI and $^1$H MRS data strongly support combined homoarginine/glucose therapy as novel protective strategy for human GA-1.

**Figure 1**. (A) Glutamate and glutamine levels from brain extracts, measured by HPLC at 48 h following the onset of high lysine (or normal diet) for different experimental groups. (B) Glx/Cr and NAA/Cr ratio measured by $^1$H MRS for different experimental groups at 48 h. (*p<0.05, **p<0.01)

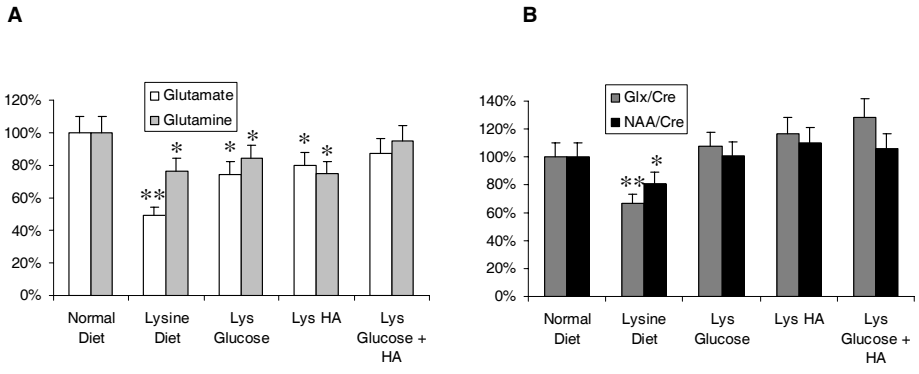
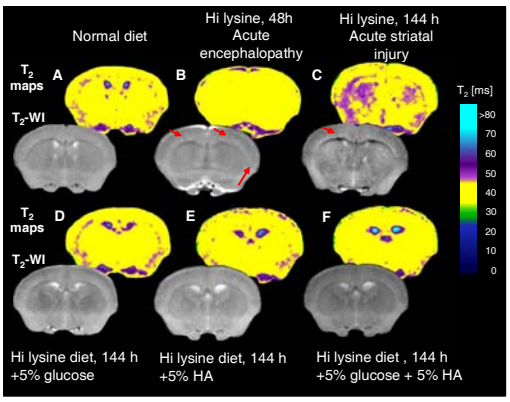



**Figure 2.** Representative T2-WI with corresponding T2 maps for Gcdh -/- mouse on normal diet (A) and corresponding treatments. Notice the enlarged draining veins on T2-WI for Gcdh -/- on high lysine diet for 48 h, during acute encephalopathy (B arrows) and at 144 h (C). Evidence of striatal injury is present at ~144h following the onset of high lysine diet (C). Animals that received glucose, homoarginine or both appeared protected (D, E, F)

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