

AXONAL INJURY AND MYELIN LOSS IN GLUTARIC ACIDEMIA TYPE I (GA-1) MOUSE MODEL OF DIET INDUCED ENCEPHALOPATHY

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INTRODUCTION: Glutaric Acidemia type I (GA-1) is an inborn error of lysine, hydroxylysine and tryptophan catabolism. Due to deficiency in glutaryl-CoA dehydrogenase (Gcdh), these amino acids can not be completely catabolized and lead to accumulation of glutaric acid in the brain, blood and urine. The world wide prevalence is approximately 1:30,000. The common findings at autopsy are severe neuronal loss in the caudate and putamen with spongiform white matter changes [1]. Treatment includes lysine restricted diet during the first few years of life, while it is not clear if individuals with GA-1 would benefit from life-long lysine dietary restrictions. In recent years, an increasing number of patients with late onset GA-1 are being recognized. Predominant MRI findings are very similar to leukoencephalopathy, with hyperintense signal restricted to supratentorial white matter on T2-weighted images [2,3]. We hypothesized that constant production of glutaric acid within the brain will lead to neurotoxicity, axonal injury and impaired myelin maintenance. To test this hypothesis we used Gcdh^{-/-} mice exposed to lysine enriched diet for 10 months.

METHODS: Gcdh^{-/-} mice and wild type (WT) controls (N=8 Gcdh^{-/-}, N=6 WT mice), both of mixed C57BL6/J X 129SvEv genetic background were used. The lysine-enriched diet was prepared by adding free lysine to a standard diet to achieve 4.8 % total lysine. MRI was performed on a 11.7 T Bruker system. Baseline scans were obtained prior to introducing the lysine-enriched diet at 8-weeks of age (N=6 Gcdh^{-/-} mice, N=4 WT mice). Mice were imaged again after 10-months of lysine-enriched diet. Prior to MRI mice were subjected to behavioral testing including acoustic startle response and open field tests. During MRI mice were anesthetized with 1-1.5% isoflurane (3% induction). To calculate T₂-values all mice were imaged using T₂-weighted multi-echo spin echo sequence (five 1 mm thick slices, TR/TE 2500/8.06-120.9 ms, 15 echoes, 156² μm² resolution, 2 NAX). To quantify magnetization transfer ratio (MTR), mice were imaged using 2D gradient recalled echo (GRE) sequence (1 mm single slice, TR/TE 190/2.85 ms, 78² μm² resolution, 4 NAX) with and without magnetization transfer (ten 8 μT Gaussian pulses, 500 Hz bandwidth, 3000 Hz frequency offset). ImageJ was used to calculate T₂-values and MTR. After 10-months of lysine-enriched diet, mice were sacrificed and examined for presence of additional lesions using high resolution MRI (3D GRE sequence, TR/TE: 120/6 ms, 14 NAX, 50³ μm³ voxel, N=4 Gcdh^{-/-}, N=3 WT). The remaining mice were sacrificed and the tissue was processed for histology. Statistical analysis was performed using one-way ANOVA, followed by a Holm-Sidak post-hoc test.

RESULTS: The baseline MR scans at 8-weeks of age did not demonstrate any significant difference in T₂-values or MTRs between WT and Gcdh^{-/-} mice. Following 10-months of lysine-enriched diet significantly increased T₂-values ($p < 0.05$) were present in the striatum of Gcdh^{-/-} mice (41.2 ± 3.05 ms) vs. WT (30.8 ± 2.7 ms), **Figure 1 A, B**. In addition, hypointense lesions were found in the thalamus of Gcdh^{-/-} mouse exposed to lysine-enriched diet, **Figure 1 C**. High-resolution *ex-vivo* MR images confirmed lesions in thalamus, **Figure 1 D**, and showed additional lesions in cerebellum (deep cerebellar nuclei) and pons in Gcdh^{-/-} mice on high lysine diet for 10 months. Reduced MTR were found throughout the brain of Gcdh^{-/-} mice, and significantly reduced MTR ($p < 0.05$) were found in the striatum of Gcdh^{-/-} (0.12 ± 0.03) vs. WT (0.21 ± 0.03) mice on lysine enriched diet, **Figure 1 E**. A specialized staining for neurofilaments (N52) revealed decreased number of axons, part of the fiber bundles passing through the striatum (striatal white matter patches), **Figure 1 F**. Behavioral testing following 10-months of lysine-enriched diet revealed significantly decreased ($p < 0.05$) acoustic startle response in Gcdh^{-/-} mice compared to age matched Gcdh^{-/-} maintained on the normal diet and WT on both lysine-enriched and normal diet, **Figure 2**. The open field test detected reduced locomotor activity and anxiety-like behavior only in Gcdh^{-/-} exposed to lysine-enriched diet for 10 months.

DISCUSSION: Increased T₂-values in the striatum of Gcdh^{-/-} mice exposed to lysine-enriched diet can indicate both axonal loss and myelin degradation in GA-1. Furthermore, both axonal loss and myelin degradation can contribute to reduced MTR, interfering with quantification of myelin degradation [4]. A previous *in-vitro* study demonstrated direct toxic effect of glutaric acid and its metabolites to immature oligodendrocytes, concluding this mechanism to be primarily responsible for leukoencephalopathy observed in GA-1 patients [5]. Thalamic lesions found in our study, open up the possibility that myelin degradation is secondary to axonal damage. We propose that thalamic lesions represent loss of neuronal cell bodies, and that damaged axons found in the striatum are part of the thalamo-cortical projection neurons. This specific population of thalamic neurons is likely at high risk for neurodegeneration in GA-1 due to high level of Gcdh expression previously reported for thalamus [6]. Behavioral testing suggests deficits in sensory motor integration, providing the insight into functional properties of these neurons. Careful examination of T₂-weighted images for hypointense thalamic lesions will help determine if the loss of thalamic neurons is present in humans with GA-1 or the white matter changes solely represent reduced myelin content. While the exact mechanism of myelin degradation in GA-1 remains to be clarified, presented data indicate a strong correlation between long-term lysine exposure and neuropathology in Gcdh^{-/-} mice, arguing for life-long dietary restrictions for GA-1 patients.

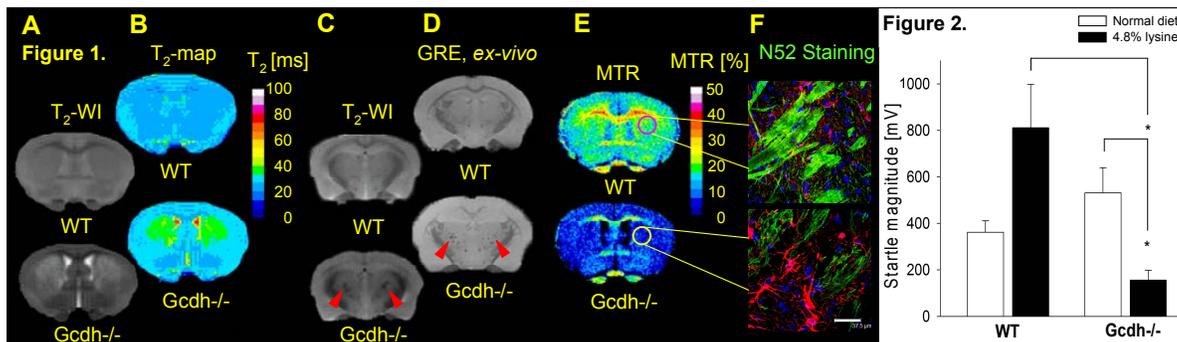


Figure 1. A, B Representative T₂-WI and T₂-maps for WT and Gcdh^{-/-} mice both on lysine-enriched diet for 10 months. Notice increased signal on T₂-WI and elevated T₂-values in the striatum of Gcdh^{-/-} mouse. **C** Representative T₂-WI at the level of thalamus (same mice), and **D** matching high resolution GRE images. Red arrowheads are pointing to the bilateral thalamic lesions in Gcdh^{-/-} mouse. **E** Calculated MTR images (same slice and mice as in A, B), and **F** corresponding histology and staining for neurofilaments (N52, green) and astrocytes (GFAP, red). Scale bar is 37.5 μm. Color scale shows different T₂-values and MTR percentages.

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Figure 2. Magnitude of acoustic startle response, measured as induced voltage. Significantly reduced startle response in Gcdh^{-/-} mice exposed to lysine-enriched diet compared to age-matched Gcdh^{-/-} on normal diet and WT on lysine-enriched diet (* $p < 0.05$, one-way ANOVA).

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