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Brain Glutathione Increase and Seizure Burden Decrease in Patients with Intractable Epilepsy on Ketogenic Diet

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Background and Purpose: Ketogenic diet (KD) improves seizure control in patients with drug-resistant epilepsy. As increased mitochondrial levels of glutathione (GSH) might contribute to a change in seizure susceptibility, we quantified changes of absolute GSH levels in the brain by *in vivo* 1H magnetic resonance spectroscopy (1H MRS) and correlate that with degree of seizure control in patients on KD.
Methods: Five cognitively normal adult patients with drug-resistant epilepsy were initially included and 2 completed the study. Each patient was evaluated by a neurologist and registered dietitian at baseline, 1, 3, and 6 months for seizure status and diet adherence after initiation of a modified atkins diet. Multiple metabolites including GSH were quantified using LCModel (version 6.3-1P; Stephen Provencher, Oakville, ON, CA) on a short echo time single-voxel 1H MRS in parieto/occipital grey matter and parietal white matter on a 3 Tesla General Electric magnet prior to starting the ketogenic diet and at 6 months.
Results: Both patients (42-years-old male and 35-years-old female) demonstrated marked increases in absolute GSH level in both gray matter (0.12 to 1.40 and 0.10 to 0.70 international unit [IU]) and white matter (0.65 to 1.50 and 0.80 to 2.00 IU), as well as 50% improvements in seizure duration and frequency. Other metabolites including ketone bodies did not demonstrate consistent changes.
Conclusions: Markedly increased levels of GSH (7-fold and 14-fold) were observed in longitudinal prospective study of two adult patients with intractable epilepsy with 50% seizure improvement after initiation of ketogenic diets. This pilot study supports the possible anticonvulsant role of GSH in the brain. **(2023;13:1-6)**

Key words: GSH, Glutathione, Epilepsy, 1H MRS, MR spectroscopy, Ketogenic diet

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Introduction

The ketogenic diet (KD) improves seizure control in patients with drug-resistant forms of epilepsy, however, its mechanisms of action remain incompletely understood.[1,2] KD is a high-fat, low carbohydrate diet that mimics the fasting state by maintaining metabolic ketosis. Some mechanisms whereby KD might confer neuroprotection and antiseizure effects revolve around its impact on mitochondrial function. Ketones are more energy efficient than glucose and can stimulate mitochondrial biogenesis and enzymatic activity. By reducing the formation of reactive oxidant species, ketosis can also protect against glutamate-mediated apoptosis and necrosis. Ketones can also enhance the conversion of excitotoxic glutamate to the inhibitory gamma-aminobutyric acid (GABA) neurotransmitter.[3]

Mounting evidence has implicated mitochondrial dysfunction contributing to both epileptogenesis and ongoing seizure susceptibility. Specifically, glutathione (GSH) depletion has been shown in animal models of temporal lobe epilepsy and human epilepsy patients.[2,4] Subsequently, some literature suggests that increased mitochondrial levels of GSH might contribute to a change in seizure susceptibility and possible anticonvulsant role for GSH. In addition to other mitochondrial-related effects of the ketogenic diet, GSH biosynthesis increases after 3 weeks on KD, making it a target worth studying with respect to potential anticonvulsant effects.[2,5]

In a cross-sectional study of 16 KD patients and 7 age-matched healthy controls (HC), Napolitano et al.[6] demonstrated higher levels

of brain GSH for KD patients (2.5±0.5 mM) compared to HC (2.0±0.5 mM). Other cerebral ketone bodies such as acetone have also been suggested to potentially contribute to seizure control in epilepsy patients treated with ketogenic diet in prior magnetic resonance (MR) spectroscopic studies.[7] In this small pilot study, we report prospective longitudinal measurements of GSH in two patients with epilepsy before and after intervention with the ketogenic diet.

Methods

In this prospective longitudinal pilot study, five cognitively normal adult patients with various forms of drug-resistant epilepsy were initially included and two completed the study. For purposes of recruitment, drug-resistant epilepsy was defined as having at least two or more seizures per month despite at least two anticonvulsant medication trials. Each patient was evaluated by a neurologist and registered dietitian at baseline, 1, 3, and 6 months for seizure status and diet adherence. These time intervals were chosen since past clinical trials of the ketogenic diet including modified atkins diet (MAD) have demonstrated significant effectiveness in terms of reduction of seizure burden at such time intervals.

MR spectroscopy (MRS) acquisition and post-processing

All MR studies were performed on a 3 Tesla clinical MR scanner (GE Healthcare; Chicago, IL, USA) using single-voxel point-resolved spectroscopy (PRESS) with an echo time (TE) of 35 ms, a repetition timer of 2 seconds, and 128 averages. Voxels for MRS were placed in the mostly grey matter containing parieto/occipital region and the mostly white matter containing parietal region as shown in Fig. 1. Voxel volume for the grey matter region of interest (ROI) was 8 mL whereas the volume for the white matter ROI was 6.7 mL. Coronal fast spin echo T2-weighted and T1-weighted three dimensional fast spoiled gradient echo images were acquired for voxel placement. All spectra were processed with fully automated LCModel software (version 6.3-1P; Stephen Provencher, Oakville, ON, CA)[8] using the unsuppressed water signal (assumed water content=75%) as an internal reference for absolute quantitation. The standard basis set of metabolites provided with LCModel included GSH and the ketone body acetone (Acn). Simulated signals for beta-hydroxybutyrate (β HB) and acetoacetate (AcAc) were added to the basis set to include these metabolites in the analysis.

Patients

Patient 1 is a 42-year-old male with focal unaware seizures arising from left temporal lobe, status-post neo-cortical resection of the inferior-middle temporal gyri in 2012 and currently on levetiracetam, lamotrigine, lacosamide, artisanal cannabidiol mixture. The patient had 3 to 5 breakthrough seizures monthly, each lasting between 1 to 5 minutes before starting the ketogenic diet.

Patient 2 is a 35-year-old female with focal unaware seizures from right hemisphere with whole-cerebral spread status post long-term vagal nerve stimulator placement in 2013 (6 years prior to the current study) and no prior surgical intervention, currently on lamotrigine, carbamazepine, and topiramate. The patient had 2-4 breakthrough seizures monthly, each lasting between 2 to 5 minutes before starting the ketogenic diet.

Ketogenic diet administration

Prior to enrollment, each subject was screened by a board-certified neurologist/epileptologist and registered dietitian to determine appropriateness and safety of diet therapy for epilepsy using clinical management guidelines established by the International Ketogenic Diet Study Group published in 2008. Baseline comprehensive labs including screening labs for neuro-metabolic disorders were completed

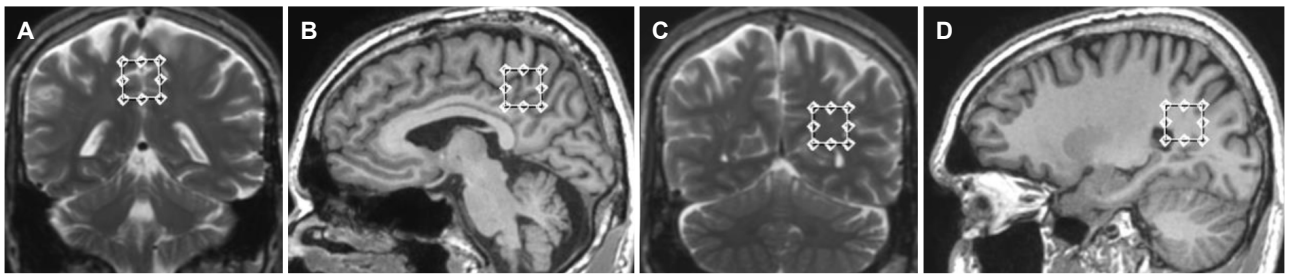

Figure 1. Demonstration of location of the MRS voxel in the parieto/occipital grey matter (A, B) and parietal white matter (B, C) on these coronal T2 (A, C) and sagittal three dimensional fast spoiled gradient echo (B, D) images of patient 1. MRS, magnetic resonance spectroscopy.

per standard protocols for management of the ketogenic diet. Based on such metabolic formal screening, none of the five subjects were found to have clinical or biochemical evidence of an inborn error of metabolism including possible mitochondrial disorders.

Each patient received formal nutrition education for the MAD supplemented with emulsified medium chain triglyceride oil. Each patient received a calendar where they documented daily seizures, morning and evening urine ketones and weekly weights. Within 1 week of diet education, the patients initiated MAD in their home, which was confirmed by a registered dietitian via phone. At the 1-, 3-, and 6-month clinic visits, seizure burden, potential side effects, and dietary adherence were assessed. Laboratory tests were repeated with the inclusion of serum beta-hydroxybutyrate at each clinic visit.

Results

Three out of five subjects either dropped out of the study, were lost to follow-up, or did not follow the dietary protocol. A 23-year-old female subject with multi-focal epilepsy status post vagus nerve stimulation device placement dropped out before the 3-month visit due to chronic mental health issues leading to hospitalization, in addition to some perceived gastrointestinal side effects of the diet and/or medium chain triglyceride (MCT) oil. A 26-year-old female subject with drug-resistant juvenile myoclonic epilepsy also dropped out before the 3-month visit as she did not follow the dietary protocol and, moreover, moved out of county. A 26-year-old male with multi-focal epilepsy with gliosis from presumed congenital toxoplasmosis infection did not correctly follow the dietary protocol and eventually dropped out of the study as well.

Therefore, only two subjects completed the study and obtained baseline and follow-up imaging. After being on ketogenic diet for 4 to 7 months, both patients, a 42-year-old male (patient 1) and a 35-year-old female (patient 2), demonstrated marked increase in absolute GSH levels in both gray matter (0.12 to 1.40 and 0.10 to 0.70 international unit [IU]) and white matter (0.65 to 1.50 and 0.80 to 2.00 IU), as well as at least 50% improvement in seizure duration and frequency (Table 1). Other metabolites including ketone bodies, Acn, βHB, and AcAC did not demonstrate any significant change as noted on MRS.

Patient 1 could not tolerate full MAD diet since he was vegan, so he was transitioned to a low glycemic index diet about 2 months into the program, but he continued to consume about two tablespoons (2.5 oz) of MCT oil three times per day. His serum βHB checked at his 6-month visit remained in the normal range of 0.12 mmol/L.

Patient 2 had gradual reduction of MCT oil consumption to about two tablespoons three times a day as her diet was made more restrictive with additional reduction in carbohydrates in order to main-

Table 1. Clinical and MR spectroscopy (MRS) data of patients

Patient	Clinical data					MRS data			
	Age (years)	Seizures (months)	Average seizure duration (minutes)	Diet duration (months)	Serum βHB (mM/L)	GSH (parietal white matter)		NAA/tCr	βHB absolute level (IU)
						Absolute level (IU)	GSH/tCr		
1									
Pre-KD	42	3-5	1-5	4	0.15		0.02	GM: 1.46	GM: 0.49
						GM: 0.12 (SD: 268)			
						WM: 0.65 (SD: 56)	0.07	WM: 1.30	WM: 0.00
Post-KD		1-2	0.5		0.12		0.33	GM: 1.57	GM: 0.07
						GM: 1.40 (SD: 35)			
						WM: 1.50 (SD: 31)	0.19	WM: 1.28	WM: 0.02
2									
Pre-KD	35	2-4	2-5	7	0.10		0.02	GM: 1.39	GM: 0.68
						GM: 0.10 (SD: 370)			
						WM: 0.80 (SD: 39)	0.12	WM: 1.42	WM: 0.25
Post-KD		1-2	0.5		0.10-0.18		0.15	GM: 1.46	GM: 0.25
						GM: 0.70 (SD: 51)			
						WM: 2.00 (SD: 16)	0.35	WM: 1.40	WM: 0.56

MR, magnetic resonance; βHB, beta-hydroxybutyrate; GSH, glutathione; IU, international unit; tCr, total creatine ratio; NAA, N-acetylaspartate; KD, ketogenic diet; GM, gray matter; SD, standard deviation; WM, white matter.

tain adequate ketosis. Her serum βHB also remained in the normal range between 0.10 and 0.18 mmol/L at her 6-month visit. The patients' clinical data and GSH and N-acetylaspartate brain metabolite levels are summarized in Table 1.

Discussion

This is the first longitudinal prospective study in human with *in vivo* MRS monitoring KD effect on brain metabolism. While only two out of five patients completed the study, the results are striking as both cases demonstrated marked improvement, up to 7- and 14-fold increase in absolute level of GSH in gray matter and about 2.5-fold increase in white matter, as well as at least 50% improvement in their baseline seizure severity.

The marked difference between gray matter versus white matter GSH absolute value increase is congruent with findings in prior literature and the proposed mechanisms that the brain response to the ketogenic diet is mainly at a cellular level located within the mitochondria of the gray matter.[9] Elevated GSH, a product of glycolysis and the citric acid cycle, as a result of KD, was demonstrated in a rat model of Parkinson's disease compared to controls, validating the idea that ketone bodies may influence GSH concentration.[6,10,11] Nuclear factor erythroid 2-related factor 2 transcription factor is a primary responder to cellular stress which promotes GSH biosynthesis. Its upregulation has been proposed as the underlying mechanism whereby GSH levels increase during KD treatment.[6,12] In addition, the following mechanism adopted from Norwitz et al.[9] depicts the relationship between βHB, nicotinamide adenine dinucleotide phosphate+ (NADP+)/NADPH ratio and antioxidants such as GSH. In summary, increase in βHB induced by the ketogenic diet leads to increased GSH concentration (Fig. 2) and that of other antioxidants.[9]

In conflict with the above proposed mechanism, we saw no evidence for increased intracerebral βHB absolute levels (Table 1) and no increase in their serum βHB levels in our two subjects as a result of KD. These discrepancies are similar to the findings of Napolitano et al.[6] where no correlation was observed between blood βHB and GSH levels. Furthermore, no consistent clinical correlation has been determined between serum βHB levels and seizure control in many studies, implicating antiseizure and neuroprotective effects of KD beyond those directly influenced by ketone bodies.[13] Other possible reasons for the absence of increased brain intracranial βHB levels in our study might be variability due to regional changes in the brain induced by KD, impact of concurrent antiseizure medications or MCT-oil consumption, or other unknown lifestyle factors.

In a cross-sectional study, Napolitano et al.[6] demonstrated patients undergoing KD had higher levels of brain GSH compared to healthy controls. Jarrett et al.[5] reported higher GSH concentration levels in the hippocampal mitochondria of rats under KD compared to controls by

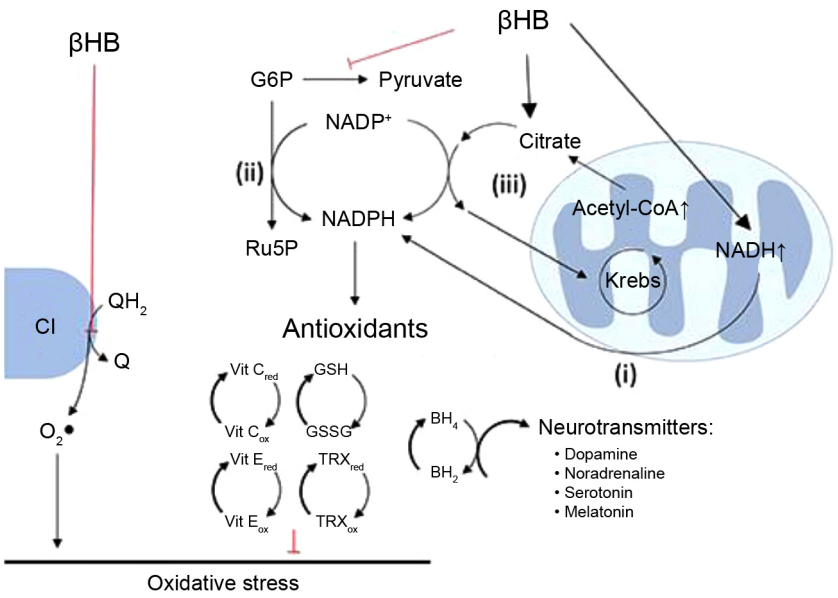

Figure 2. Adopted from Norwitz et al.[9] and depicts the relationship between βHB, which supports antioxidant defenses by improving (reducing) the glutathione (GSH-GSSG), thioredoxin (TRX), and vitamins C and E reduced: oxidized ratios. βHB, beta-hydroxybutyrate; NADP+, nicotinamide adenine dinucleotide phosphate; NADH, nicotinamide adenine dinucleotide; CI, confidence interval; GSH, reduced glutathione; GSSG, oxidised glutathione.

using high performance liquid chromatography.[5,6] The neuroprotective role of GSH against oxidative stress is well established.[6] Diseases, such as multiple sclerosis and amyotrophic lateral sclerosis, have been associated with GSH dysregulation.[6,14-16] These result support the notion that KD stimulates *de novo* GSH synthesis and improves the redox status of the brain likely through mitochondrial functional impact.

GSH is most accurately quantified using a J-edited MEGAPRESS sequence with a longer TE,[6,17,18] which enable the differentiation of extended versus closed GSH forms in the brain.[6,19] Our protocol was based on a clinical short TE PRESS sequence that can be obtained on any routine clinical MR scanner. Similar protocols have been widely used, reproduced and validated in quantification of GSH in multiple prior studies in a variety of disorders.[6]

The most important limitation of our study is the small number of patients included and the use of less stringent forms of the ketogenic diet, which did not achieve elevated serum ketone levels. Furthermore, we did not measure serum or brain acetoacetate levels, which has been shown to cross the blood-brain barrier more efficiently than βHB through monocarbocylic acid transporters. We also did not measure brain levels of glutamate or inhibitory GABA, which are neurotransmitters known to be impacted by the ketogenic diet in various animal models. Finally, regional differences in ketone body metabolism and downstream neuroprotective effects of the ketogenic diet likely exist and were not evaluated in this pilot study.[3]

Based on our promising preliminary results, we are planning to recruit more patients to confirm our findings regarding GSH increase and improved seizure control. In addition, we aim to investigate the potential role of MRS and particularly the brain GSH measurement in predicting which baseline biochemical signatures might best predict a positive response to planned ketogenic diets. Answers to such questions might shed light on the complex mechanism of action of KD and better inform patient selection for this major and challenging lifestyle modification.

This is the first longitudinal prospective study in the scientific literature demonstrating marked increase in absolute level of GSH in the brain (especially the gray matter) and 50% seizure improvement in two adult patients with drug-resistant epilepsy treated with the ketogenic diet and emulsified MCT oil. The findings of this pilot study bolster the possible anticonvulsant role for glutathione in the brain and increase evidence of the importance of this compound in metabolic intervention for seizures.

Conflicts of Interest

None.

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