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

Saman Hazany (samanh26@gmail.com)
USC Keck School of Medicine: University of Southern California Keck School of Medicine
https://orcid.org/0000-0002-4864-2710

Brittany DeClouette
USC Keck School of Medicine: University of Southern California Keck School of Medicine

Jessica Lowe
USC Keck School of Medicine: University of Southern California Keck School of Medicine

Paul E Kim
USC Keck School of Medicine: University of Southern California Keck School of Medicine

Stefan Bluml
USC Keck School of Medicine: University of Southern California Keck School of Medicine

Arthur Partikian
USC Keck School of Medicine: University of Southern California Keck School of Medicine

Research Article

Keywords: GSH, Glutathione, Epilepsy, MRS, MR Spectroscopy, Ketogenic Diet

Posted Date: May 13th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1627302/v1

Abstract

Background and Purpose: Ketogenic diet (KD) improves seizure control in patients with drug-resistant forms of epilepsy; however, the mechanism of action remains poorly understood. Increased mitochondrial levels of glutathione (GSH) might contribute to a change in seizure susceptibility suggestive of possible anticonvulsant role for GSH. Our goals were to quantify changes of absolute GSH levels in the brain by in vivo MR spectroscopy 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. Each patient received a baseline short TE single-voxel water-suppressed point-resolved spectroscopy (PRESS) 1 H MRS in parieto/occipital grey matter and parietal white matter on a 3 Tesla General Electric magnet prior to starting the ketogenic diet and a follow-up stury at 6 months. Multiple metabolites, including GSH, were quantified using LCModel.

Results: After being on ketogenic diet for 6 months, both patients (42-years-old male and 35-years-old female) demonstrated marked increases in absolute GSH level in both gray matter (0.1 to 1.4 and 0.1 to 0.7 IU) and white matter (0.65 to 1.5 and 0.8 to 2 IU), as well as 50% improvements in seizure duration and frequency. Other metabolites including ketone bodies, Acetone (Acn), Beta hydroxybutyrate (βHB), and acetoacetate (AcAC), did not demonstrate consistent changes.

Conclusion: 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.

Introduction

The ketogenic diet (KD) improves seizure control in patients with drug-resistant forms of epilepsy, however, the mechanism of action remains poorly understood. (5, 6) KD is a high-fat, low carbohydrate diet that mimics the fasting state by maintaining metabolic ketosis. Mounting evidence has implicated mitochondrial dysfunction contributing to both epileptogenesis and ongoing seizure susceptibility. Specifically, some literature suggests that increased mitochondrial levels of glutathione (GSH) might contribute to a change in seizure susceptibility and possible anticonvulsant role for GSH. GSH depletion has been shown in animal models of temporal lobe epilepsy and human epilepsy patients. (6, 8) 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. (4, 6)

In a cross-sectional study of 16 KD patients and 7 age-matched healthy controls (HC), Napolitano et al. demonstrated higher levels of brain GSH for KD patients (2.5 ± 0.5 mM) compared to HC (2.0 ± 0.5 mM). (9) 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 MR Spectroscopic studies. (13) To

our knowledge, our study is the first report of prospective longitudinal measurement of GSH in epileptic patients before and after intervention with the ketogenic diet.

Methods

In this prospective longitudinal pilot study, 5 cognitively normal adult patients with various forms of 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.

MRS acquisition and post-processing:

All MR spectroscopy studies were performed on a 3T clinical MR scanner (GE Healthcare) using single-voxel point-resolved spectroscopy (PRESS) with an echo time (TE) of 35ms, a repetition timer (TR) of 2s, and 128 averages. Voxels for MR spectroscopy 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 8cc whereas the volume for the white matter ROI was 6.7cc. Coronal fast spin echo T2-weighted and T1-weighted 3D fast spoiled gradient echo images were acquired for voxel placement. All spectra were processed with fully automated LCModel software {Provencher, 1993, Estimation of metabolite concentrations from localized in vivo proton NMR spectra} (11) 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 (BHB) and acetoacetate (AcAc) were added to the basis set to include these metabolites in the analysis.

Patients:

Patient 1: 42-year-old male with focal unaware seizures arising from left temporal lobe, status-post left temporal cortical resection in 2012 and currently on Levetiracetam, Lamotrigine, Lacosamide, Artisanal Cannabidiol mixture. The patient had 3–5 break-through seizures each lasting between 1 to 5 minutes before starting the ketogenic diet.

Patient 2: 35-year-old female with focal unaware seizures from right hemisphere with whole-cerebral spread status post long-term Vagal Nerve Stimulator (VNS) placement (implanted 6 years prior to the current study) and no prior surgical intervention currently on Lamotrigine, Carbamazepine, and Topiramate. The patient had 2–4 break-through seizures 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 (AP) and registered dietitian (JL) 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 per standard protocols for management of the ketogenic diet.

Each patient received formal nutrition education for the Modified Atkins Diet (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 one 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. Therefore, only two subjects completed the study and obtained baseline and follow-up imaging. After being on ketogenic diet for 4–7 months, both patients, a 42-year-old male (patient 1) and a 35-year-old female (patient 2) demonstrated marked increase in absolute glutathione (GSH) levels in both gray matter (0.1 to 1.4 and 0.1 to 0.7 IU) and white matter (0.65 to 1.5 and 0.8 to 2 IU), as well as at least 50% improvement in seizure duration and frequency (Table 1). Other metabolites including ketone bodies, Acetone (Acn), Beta hydroxybutyrate (BHB), and acetoacetate (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 two months into the program, but continued to consume about 2 tablespoons (2.5 oz) of MCT oil three times per day. His serum BHB 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 2 tablespoons TID as her diet was made more restrictive with additional reduction in carbohydrates in-order to maintain adequate ketosis. Her serum βHB also remained in the normal range between 0.10–0.18 mmol/L at her 6-month visit. The patients' clinical data and GSH and NAA brain metabolite levels are summarized in Table 1.

Table 1
Clinical and MR Spectroscopy data of patients (Pt). Abbreviations: GSH/tCr = Glutathione to total Creatine ratio; NAA/tCr = N-acetylaspartate to total Creatine ratio; IU: International Unit, GM: Gray Matter, WM: White Matter.

	Clinical Data					MR Spectroscopy Data		
Pt	Age (yrs)	Seizures (months)	Average seizure duration (min)	Diet Duration (Months)	Serum βHB (mM/L)	GSH (Parietal white matter) Absolute level (IU) GSH/tCr	NAA/tCr	βHB Absolute level (IU)
1 (Pre-KD)	42	3–5	1–5	4	0.15	GM: 0.12 (SD: 268%) 0.02	GM: 1.46	GM: 0.49
						WM: 0.65 (SD: 56%) 0.07	WM: 1.30	WM: 0.00
1 (Post-KD)		1–2	0.5		0.12	GM: 1.4 (SD: 35%) 0.33	GM: 1.57	GM: 0.07
						WM: 1.5 (SD: 31%) 0.19	WM: 1.28	WM: 0.02
2 (Pre-KD)	35	2–4	2–5	7	0.10	GM: 0.10 (SD: 370%) 0.02	GM: 1.39	GM: 0.68
						WM: 0.8 (SD: 39%) 0.12	WM: 1.42	WM: 0.25
2 (Post-KD)		1–2	0.5		0.10–0.18	GM: 0.70 (SD: 51%) 0.15	GM: 1.46	GM: 0.25
						WM: 2 (SD: 16%) 0.35	WM: 1.40	WM: 0.56

Discussion

This is the first longitudinal prospective study in human with in vivo MRS monitoring KD effect on brain metabolism. While only 2 out of 5 patients completed the study, the results are striking as both cases

demonstrated marked, 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. (10) 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. (1, 3, 9) Nuclear factor erythroid 2-related factor 2 (NRF2) 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. (7, 9) In addition, the following mechanism adopted from Norwitz et al (10), depicts the relationship between βHB, NADP+/NADPH ratio and antioxidants such as GSH. In summary increase in βHB leads to increased GSH concentration (Fig. 2). (10)

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 as a result of KD. This discrepancy is similar to the findings of Napolitano et al. where no correlation was observed between blood βHB and GSH. 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 (15). 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. demonstrated patients undergoing KD had higher levels of brain GSH compared to healthy controls. (9) Jarret et al. reported higher GSH concentration levels in the hippocampal mitochondria of rats under KD compared to controls by using High Performance Liquid Chromatography. (4, 9) The neuroprotective role of GSH against oxidative stress is well established. (9) Diseases such as multiple sclerosis and amyotrophic lateral sclerosis (ALS), have been association with GSH dysregulation. (2, 9, 16, 17) 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 echo time (TE) (9, 12, 18), which also can differentiate extended and closed GSH forms in the brain (9, 14). Our protocol was based on a clinical short echo time 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 of a variety of disorders (9).

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. 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 MR Spectroscopy and particularly the brain GSH measurement which it enables 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.

Conclusions

This is the first longitudinal prospective study in the scientific literature demonstrating marked increase in absolute level of glutathione (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. 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.

Declarations

Funding: Medical student award for Brittany DeClouette from the Radiological Society of North America (RSNA). No other funding.

o **Conflicts of interest/Competing interests (include appropriate disclosures):** "The authors have no relevant financial or non-financial interests to disclose."

o **Availability of data and material (data transparency):** The datasets generated during and/or analysed during the current study are not publicly available due to local collection of data, but are available from the corresponding author on reasonable request.

o Code availability (software application or custom code): All spectra were processed with fully automated LCModel software {Provencher, 1993, Estimation of metabolite concentrations from localized in vivo proton NMR spectra}.(11)

o **Authors' contributions:** All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Saman Hazany, MD, Brittany DeClouette, MD, Jessica Lowe, MPH, Paul E Kim, MD, Stefan Bluml, PhD, Arthur Partikian, MD. The first draft of the manuscript was written by Saman Hazany, MD and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

** Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals

o **Ethics approval (include appropriate approvals or waivers):** This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the IRB Committee of University of Southern California (7/25/2017/ HS-17-00373).

o **Consent to participate (include appropriate statements):** Informed consent was obtained from all individual participants included in the study.

o **Consent for publication (include appropriate statements):** The authors affirm that human research participants provided informed consent for publication of the images in all the Figures in the manuscript.

References

1. Cheng B, Yang X, An L, Gao B, Liu X, Liu S (2009) Ketogenic diet protects dopaminergic neurons against 6-OHDA neurotoxicity via up-regulating glutathione in a rat model of Parkinson's disease. Brain Res 1286:25–31 Epub 2009 Jun 25
2. Choi IY, Lee SP, Denney DR, Lynch SG (2011) Lower levels of glutathione in the brains of secondary progressive multiple sclerosis patients measured by 1H magnetic resonance chemical shift imaging at 3 T. Mult Scler J 17:289–296 Epub 2010 Oct 4
3. Hazany S, Hesselink JR, Healy JF, Imbesi SG Utilization of glutamate/creatine ratios for proton spectroscopic diagnosis of meningiomas.Neuroradiology. 2007Feb; 49(2):121–7. doi: 10.1007/s00234-006-0167-z. Epub 2006 Nov 4.
4. Jarrett SG, Milder JB, Liang L-P, Patel M (2008) The ketogenic diet increases mitochondrial glutathione levels. J Neurochem 106(3):1044–1051. doi: 10.1111/j.1471-4159.2008.05460.x. Epub 2008 May 5
5. Lionel AC, Monfared N, Scherer SW, Marshall CR, Mercimek-Mahmutoglu S (2016 Sep) MED23-associated refractory epilepsy successfully treated with the ketogenic diet. Am J Med Genetics A 170(9):2421–2425. doi: 10.1002/ajmg.a.37802
6. Milder J, Patel M (2012) Modulation of oxidative stress and mitochondrial function by the ketogenic diet. Epilepsy Res 100:295–303. doi: 10.1016/j.eplepsyres.2011.09.021
7. Milder JB, Liang LP, Patel M (2010) Acute oxidative stress and systemic Nrf2 activation by the ketogenic diet. Neurobiol Dis 40:238–244 Epub 2010 May 31
8. Mueller SG, Trabesinger AH, Boesiger P, Wieser HG (2001) Brain glutathione levels in patients with epilepsy measured by in vivo (1)H-MRS. Neurology 57:1422–1427. doi: 10.1212/wnl.57.8.1422
9. Napolitano A, Longo D, Lucignani M, Pasquini L, Rossi-Espagnet MC, Lucignani G, Maiorana A, Elia D, De Liso P, Dionisi-Vici C, Cusmai R, Metabolites (2020) Dec 10;10(12):504. doi: 10.3390/metabo10120504
10. Norwitz NG, Hu MT, Clarke K (2019 May) The Mechanisms by Which the Ketone Body D-β-Hydroxybutyrate May Improve the Multiple Cellular Pathologies of Parkinson's Disease. Front Nutr 14:6:63. doi: 10.3389/fnut.2019.00063
11. Provencher SW (1993 Dec) Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magn Reson Med 30(6):672–679. doi: 10.1002/mrm.1910300604

12. Sanaei Nezhad F, Anton A, Parkes LM, Deakin B, Williams SR (2017) Quantification of glutathione in the human brain by MR spectroscopy at 3 Tesla: Comparison of PRESS and MEGA-PRESS. Magn Reson Med 78:1257–1266. doi: 10.1002/mrm.26532
13. Seymour KJ, Bluml S, Sutherling J, Sutherling W, Ross BD (1999 Mar) Identification of cerebral acetone by 1H-MRS in patients with epilepsy controlled by ketogenic diet. MAGMA 8(1):33–42. doi: 10.1007/BF02590633
14. Shukla D, Mandal PK, Ersland L, Gruner ER, Tripathi M, Raghunathan P, Sharma A, Chaithya GR, Punjabi K, Splaine CA (2018) Multi-Center Study on Human Brain Glutathione Conformation using Magnetic Resonance Spectroscopy. J Alzheimers Dis 66:517–532. doi: 10.3233/JAD-180648
15. Simeone TA, Simeone KA, Stafstrom CE, Rho JM (2018) doi: 10.1016/j.neuropharm.2018.01.011. Epub 2018 Jan 8
16. Srinivasan R, Ratiney H, Hammond-Rosenbluth KE, Pelletier D, Nelson SJ (2010) MR spectroscopic imaging of glutathione in the white and gray matter at 7 T with an application to multiple sclerosis. Magn Reson Imaging 28:163–170. doi: 10.1016/j.mri.2009.06.008
17. Terpstra M, Henry PG, Gruetter R (2003) Measurement of reduced glutathione (GSH) in human brain using LCModel analysis of difference-edited spectra. Magn Reson Med 50:19–23. doi: 10.1002/mrm.10499
18. Weiduschat N, Mao X, Hupf J, Armstrong N, Kang G, Lange DJ, Mitsumoto H, Shungu DC (2014) Motor cortex glutathione deficit in ALS measured in vivo with the J-editing technique. Neurosci Lett 570:102–107. doi: 10.1016/j.neulet.2014.04.020

Figures

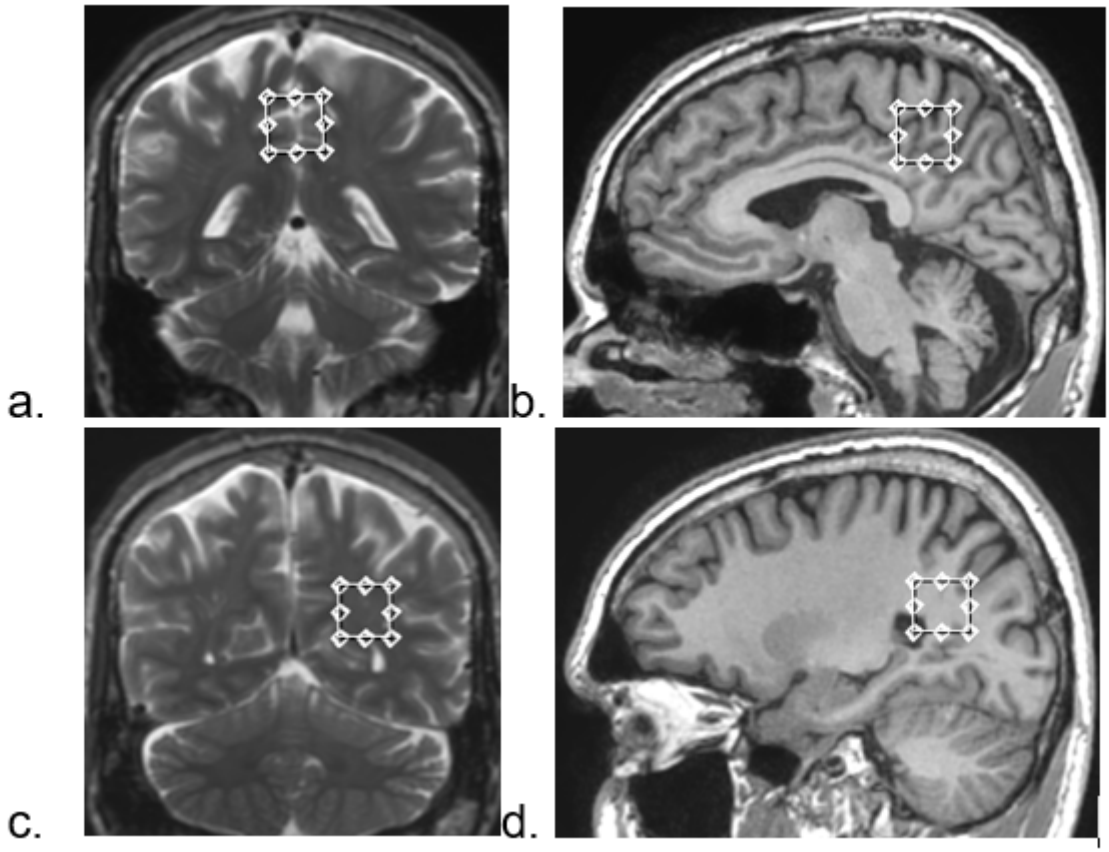

Figure 1

Demonstration of location of the MRS voxel in the parieto/occipital grey matter (a and b) and parietal white matter (b and c) on these coronal T2 (a and c) and sagittal 3 Dimensional Fast Spoiled Gradient Echo (3D FSPGR) (b and d) images of patient 1.

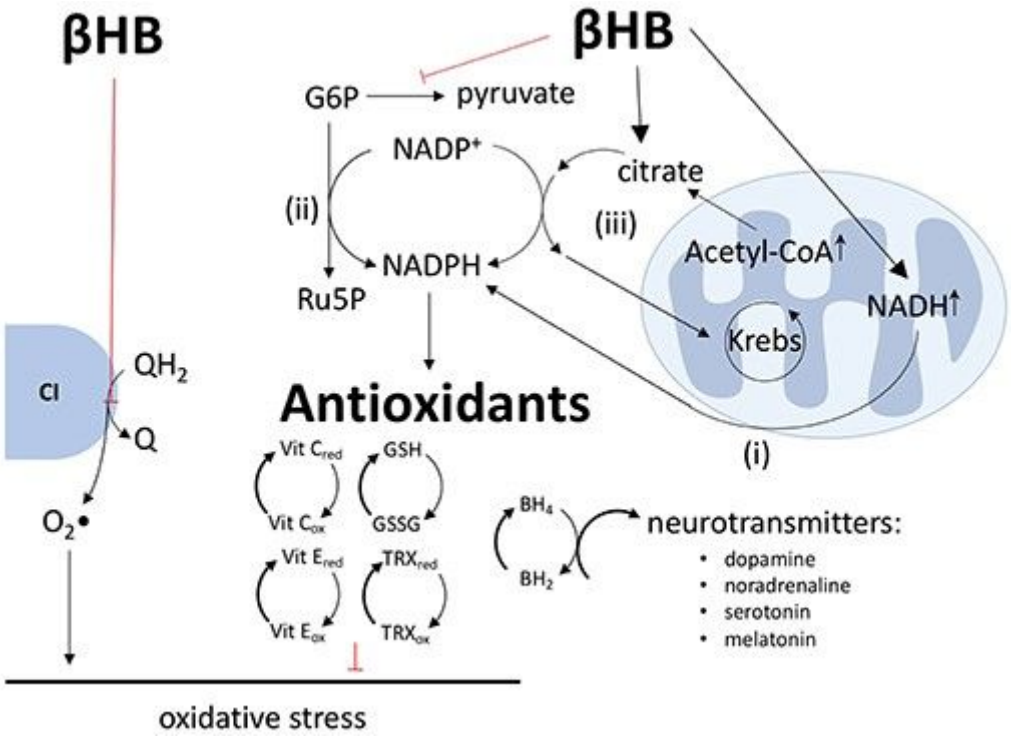

Figure 2

Adopted from reference 10 and depicts the relationship between Beta-Hydroxy-Butyrate (βHB), which supports antioxidant defenses, through improving (reducing) the glutathione (GSH-GSSG), thioredoxin (TRX), and vitamins C and E reduced to oxidized ratios.