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7T quantitative magnetic resonance spectroscopy of glutamate, GABA and glutathione in the PCC/precuneus in patients with epilepsy

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Abstract

Objective: The posterior cingulate cortex (PCC)/precuneus is a key hub of the default mode network, whose function is known to be altered in epilepsy. Glutamate and γ-amino butyric acid (GABA) are the main excitatory and inhibitory neurotransmitters in the CNS, respectively. Glutathione (GSH) is the most important free radical scavenging compound in the brain. Quantification of these molecules by magnetic resonance spectroscopy (MRS) up to 4T is limited by overlapping resonances from other molecules. In this study we used ultra-high-field (7T) MRS to quantify their concentrations in patients with different epilepsy syndromes.

Methods: 19 patients with temporal lobe epilepsy (TLE) and 16 with idiopathic generalized epilepsy (IGE) underwent MRI scans using a 7T research scanner. Single-voxel (8 cm^3) MRS, located in the PCC/precuneus, was acquired via Stimulated Echo Acquisition Mode (STEAM). Their results were compared to 10 healthy volunteers.

Results: Mean concentrations of glutamate, GABA, and the glutamate/GABA ratio did not differ between the IGE, TLE and healthy volunteer groups. The mean(±SD) concentration of GSH was 1.9(±0.3) mM in healthy controls, 2.0(±0.2) mM in patients with TLE, and 2.2(±0.4) mM in patients with IGE. One-way ANOVA with post-hoc Tukey-Kramer test revealed a significant difference in the concentration of GSH between patients with IGE and controls (P=0.03). Short-term seizure-freedom in patients with epilepsy was predicted by an elevated concentration of glutamate in the PCC/precuneus (P=0.01). In patients with TLE the concentration of GABA declined with age (P=0.03).

Significance: Patients with IGE have higher concentrations of GSH in the PCC/precuneus than healthy controls. There is no difference in the concentrations of glutamate, GABA, or their ratio in the PCC/precuneus between patients with IGE, patients with TLE, and healthy controls. Measuring the concentration of glutamate in the PCC/precuneus may assist with predicting drug-response.

Key Points

- The concentrations of glutamate and GABA in the PCC/precuneus are similar in patients with epilepsy and healthy controls.
- The concentration of glutathione in the PCC/precuneus is higher in patients with idiopathic generalized epilepsy in comparison to healthy controls.
- The concentration of GABA in the PCC/precuneus decreases with age in temporal lobe epilepsy patients but not in those with idiopathic generalized epilepsy.
- Elevated concentration of glutamate in the PCC/precuneus may predict short-term seizure-freedom in patients with epilepsy.

1. INTRODUCTION

Glutamate and γ-amino butyric acid (GABA) are the major excitatory and inhibitory neurotransmitters in the human brain, respectively.[1,2] Their concentration in the brain is affected by various antiseizure medications (ASMs) and it is believed that their ratio may reflect the propensity of the brain for hyperexcitable conditions such as epilepsy.[3] Glutathione (GSH), the main free radical scavenger compound in the brain, is metabolically related to glutamate and GABA and is involved in a wide range of neurological disorders.[4] Previous studies revealed a reduction in the serum level of GSH in patients taking certain ASMs and the activity of Glutathione Peroxidase in erythrocytes was found to be significantly lower in patients with idiopathic generalized epilepsy (IGE) when compared to a control group.[5,6]

Several studies have used magnetic resonance spectroscopy (MRS) to measure the concentrations of these metabolites in various brain regions in patients with epilepsy.[7–11] However, the accuracy of MRS has been limited by overlapping resonances from other molecules at magnetic fields up to 4T. In comparison, 7T MRI has enabled the acquisition of spectra with superior signal to noise ratios and chemical shift dispersion, thus improving the quantification of these metabolites.[12]

Indeed, in recent years several research groups have started using 7T MRS to study epilepsy. For example, GABA concentration was measured in the thalamus using 7T MRS and was found to be higher in two patients with well-controlled epilepsy as compared to six patients with poorly-controlled epilepsy.[13] In another study, levels of glutamate were acquired using a chemical exchange saturation transfer (CEST) technique at 7T in patients with non-lesional temporal lobe epilepsy (TLE) and found to correctly lateralize the seizure focus.[14] Our own group quantified glutamate concentrations at 7T in and around gliomas using both CEST and stimulated echo acquisition mode (STEAM) and demonstrated positive correlation between its level and parameters of tumor aggressiveness and epileptogenesis.[15]

The posterior cingulate cortex (PCC)/precuneus is the structural and functional core of the default mode network (DMN), the main resting-state network of the brain, which is known to be involved in many neuropsychiatric disorders including epilepsy.[16,17] However, MRS studies of the DMN in patients with epilepsy are scarce and limited by sample size and magnetic field strength. For example, a multi-voxel study at 3T demonstrated elevation of glutamate in various cortical and sub-cortical regions in four patients with IGE. No such changes were discovered in the cingulate cortex or the precuneus.[18] Notably, GABA and GSH were not measured in that study and we are unaware of any study measuring GSH concentrations via MRS in any brain region in patients with IGE. GSH was measured via MRS in patients with focal epilepsy and reductions in its concentration were measured in both hemispheres.[11]

The purpose of this study was to measure concentrations of glutamate, GABA, and GSH in the PCC/precuneus in patients with focal and generalized epilepsy at 7T and to compare these results with healthy controls. The clinical usefulness of finding distinct metabolic profiles in different groups derives from the potential application of such findings both for diagnostic purposes and for their use as biomarkers in epilepsy management, if future studies find changes in such profiles in response to therapy. As DMN alterations have been previously demonstrated with resting-state fMRI in both focal and generalized epilepsy, we expected such changes to be reflected by altered metabolite levels in both patient groups.[19–23] Our hypotheses were that both patient groups will demonstrate increased glutamate, reduced GABA, increased glutamate to GABA ratio and reduced GSH in comparison with healthy controls.

2. METHODS

2.1 Participants

Patients were recruited from the Comprehensive Epilepsy Programs of the Royal Melbourne and Alfred Hospitals in Melbourne, Australia. TLE and IGE were defined according to well-established ILAE criteria.[24] They were excluded if their epilepsy was related to a known brain lesion (other than mesial temporal sclerosis) or if they had contraindications for 7T MRI.

Nineteen right-handed patients (12 female) with temporal lobe epilepsy (TLE), ranging in age from 19-72 years (mean 41.9±13.9; median 42), and 16 right-handed patients (12 female) with IGE, ranging in age from 20-70 years (mean 39.2±15.3; median 33.5), were recruited. Of the TLE patients, nine had left-sided TLE, six had right-sided TLE, and four had bilateral seizure foci. The patients were matched with 10 healthy, right-handed, volunteers (six female). Their mean(±SD) age was 40.0±14.4 years (median 36.5). Mean(±SD) duration of epilepsy was 18.3±14.7 years in the TLE group and 24.6±18.4 years in the IGE group.

The age of participants was similar across the three groups (one-way ANOVA - $F(2,41)=0.13$; $P=0.88$) and there was no statistically significant difference of epilepsy duration between the TLE and IGE groups (Kruskal-Wallis H test - $\chi^2(1)=1.26$; $P=0.26$). The number of medications was also similar between the patient groups ($P=0.17$). Characteristics of all 35 recruited patients are provided in the supplementary material.

Seizure occurrence in the preceding 3 months was ascertained as a measure of drug-responsiveness to minimize recall bias. In patients who had started drug treatment for less than 3 months before the scan drug-responsiveness was verified three months later. Accordingly, short-term seizure freedom was defined as lack of self-reported seizures in the 3 months preceding the scan (other than seizures induced by medication withdrawal as part of video-EEG monitoring). Short-term seizure-freedom was reported by 11/19 patients with TLE (10/18 of the patients in the final analysis) and by 6/16 patients with IGE. Information about drug-treatment was collected from all patients. The study was approved by the Human Research Ethics Committee of Melbourne Health. All participants provided written informed consent.

2.2 Sample size estimation

Sample size estimation was limited by the lack of similar 7T studies of the aforementioned metabolites in patients with epilepsy. Nevertheless, considering the study of Pan et al, who measured the concentration of GABA scaled to N-acetyl aspartate (NAA) in the thalamus of healthy controls, patients with well controlled IGE, and patients with poorly controlled IGE, the smallest effect size existed between the healthy controls (0.053±0.012; n=8) and the poorly controlled group (0.038±0.009; n=6), amounting to Cohen's f=0.69.[13] Assuming α=0.05 and β=0.80, a three group ANOVA study looking for a similar effect size would need a total sample size of 24 participants (calculated by G*Power version 3.1.9.2).

2.3 Imaging protocol

A 7T research scanner (Siemens Healthcare, Erlangen, Germany) with a 32-channel head-coil (Nova Medical Inc, Wilmington MA, USA) was used. MRS scans were preceded by MP2RAGE (magnetization prepared 2 rapid gradient echoes), a three-dimensional T1-weighted sequence, for localization purposes with 0.9 mm isotropic resolution. Other parameters were TR=4900 ms, TE=2.9 ms, TI1=700 ms, FA1=5°, TI2=2700 ms, FA2=6°. Single-voxel ^{1}H MRS was acquired using STEAM with a 20 x 20 x 20 mm^3 cubical midline voxel (volume of 8 mL). The anterior inferior border of the voxel was just above the corpus callosum, its anterior superior border just dorsal to the marginal branch of the cingulate sulcus, and its posterior border ventral to the parieto-occipital sulcus, in a similar manner to the study of Kantarci et al – see Figure 1.[25]

Shimming was performed prior to MRS acquisition. The Siemens standard automated shimming was employed followed by manual fine tuning of gradients to optimize the line width. The TR was 8500 ms except for the scan of control number 5, who reached a specific absorption rate limit and therefore TR=9300 ms was used in his case. TE was 6 ms. In total, thirty-two averages were acquired for the water-suppressed sequence (acquisition time of 4:49 minutes), and four averages were used for the unsuppressed sequence (acquisition time of 51 seconds). The first spectrum from the healthy control scans was compared to the single spectrum obtained from the patients. A representative spectrum from each group (TLE, IGE, and controls) appears in Figure 2.

2.4 Quantitative MRS calculation

Metabolite concentrations were quantified using LCModel version 6.3-0B with a 7-Tesla basis set using scaling to unsuppressed water and Eddy-current correction.[26] The LCModel output included estimated concentrations (mM) and Cramér–Rao lower bounds (CRLB), which are estimated standard deviations, expressed in percent of the estimated concentrations.

For partial volume effect correction, we created binary masks for each MRS voxel using a MATLAB script created by Mr. Bartosz Kossowski from The Polish Academy of Sciences (https://www.nitrc.org/projects/rda2nifti/). MATLAB version R2017b was used (MathWorks, Natick, MA, United States). The ANTs toolbox (http://stnava.github.io/ANTs/) version 2.3.1 was used to transform the dimensions of the masks for consistency with MP2RAGE coordinates.[27] Skull-stripping and brain extraction of the MP2RAGE images was done using FMRIB's Brain Extraction Tool.[28] Each brain was then segmented using FMRIB's Automated Segmentation Tool (FAST) into partial volume maps for gray matter, white matter and CSF.[29]

Fractions of the partial volume maps within each MRS voxel were determined using the fslstats utility of the FSL suite version 5.0.10.[30] The NMR-visible water concentration (mM) in the voxel was estimated by $(43300f_{GM}+35880f_{WM}+55556f_{CSF}) / (1-f_{CSF})$, where f_{GM}, f_{WM}, and f_{CSF} are the volume fractions of gray matter, white matter, and CSF in the voxel.[31]

2.5 Statistical Analysis

Continuous variables (age of the participants, duration of epilepsy, the concentrations of glutamate, GABA, GSH, and the ratio of glutamate to GABA concentrations) were compared between healthy controls, patients with TLE, and patient with IGE. Shapiro-Wilk test checked the assumption of normality of these variables and Bartlett's test was used to check for homoscedasticity. One-way analysis of variance (ANOVA) with post-hoc Tukey-Kramer test for multiple comparisons was used to compare the means between the three groups when data passed these tests. In cases of normality without homoscedasticity Welch's ANOVA was used. In cases data did not pass the normality test Kruskal-Wallis H test

was used, adjusted for ties. In addition, the discrete ordinal variable of the number of medications was also compared between the patient groups using Kruskal-Wallis H test as well as the analysis of medication effects in subgroups due to very small sample sizes.

Mean concentrations of glutamate, GABA, the concentration ratio glutamate/GABA and GSH all followed a Gaussian distribution and passed the Shapiro-Wilk normality test with $P>0.05$. Other than the concentration of glutamate, all other MRS results that were the focus of this study also passed Bartlett's test of homoscedasticity and one-way ANOVA was used for comparison between the groups. The concentrations of glutamate were compared with Welch's ANOVA. The age of participants passed the Shapiro-Wilk normality test and Bartlett's test of homoscedasticity and was compared using one-way ANOVA across the three groups. Epilepsy duration, however, did not pass Shapiro-Wilk's test in the TLE group ($P=0.05$) and therefore Kruskal-Wallis H test was used for comparison of epilepsy duration.

Due to collinearity between metabolites and an overall small sample size exact logistic regression was employed for an exploratory analysis of the relationship between short-term seizure freedom and each of the metabolites this study focused on in the entire patient population. Multiple linear regression was used to study the effects of age and duration of epilepsy on metabolite concentrations in the patient groups. Beta regression was similarly used to study the effects of age and duration of epilepsy on the fraction of CSF in the region of interest as a measure of atrophy in all 35 patients. All statistical analyses were conducted with Stata 15.0 (StataCorp, College Station, TX, USA). Statistical significance was set at $\alpha=0.05$.

3. RESULTS

Metabolite concentrations and age of participants for patients and controls are summarized in Table 1. Results of multiple regression of metabolite concentrations in the volume of interest with age and duration of epilepsy in the patient groups are summarized in Table 2. Exact logistic regressions of short-term seizure-freedom with metabolite concentrations are summarized in Table 3. Tests of normality and homoscedasticity, composition of the volume of interest, analysis of drug effects, and clinical information for all the patients are summarized in the supplementary tables.

3.1 Quality Control

Values of CRLB for the various metabolites and Full Width at Half Maximum (FWHM) of the spectra were obtained from the LCModel output. Results with CRLB above 20% were unreliable and excluded from further analysis, resulting in the exclusion of a single GABA concentration from the spectrum of one patient from the IGE group (due to CRLB=44%).

Maximal CRLB for glutamate, GABA, and GSH were 2%, 11%, and 6%, respectively, in the control group. The mean(±SD) FWHM of the spectra was 0.034±0.003 ppm (10.1±0.9 Hz) in these subjects, confirming adequate quality.

In the TLE group, one patient's spectrum had poor quality with a FWHM of 0.099 ppm (29.4 Hz), which was above an established rejection cut-off of 0.070 ppm.[32] That spectrum was excluded from further analysis, resulting in maximal CRLB of 3%, 14%, and 6% for glutamate, GABA, and GSH, respectively, and a mean(±SD) FWHM of 0.034±0.005 ppm (10.1±1.5 Hz) for the remaining patients with TLE.

In the IGE group, after the exclusion of the single result mentioned above maximal CRLB were 4%, 18%, and 8% for glutamate, GABA, and GSH, respectively. Mean±SD FWHM of the spectra in the IGE group were 0.036±0.008 ppm (10.7±2.4 Hz).

3.2 Voxel Composition

Beta regression of the fraction of CSF in the volume of interest with age and duration of epilepsy showed a significant increase in the fraction of CSF with age in the TLE group P=0.02) with a marginal effect of 0.002 per year (95% confidence interval 0.0003-0.003). No independent effect of epilepsy duration was found on the fraction of CSF (P=0.19). In the IGE group, no effect of patient age or the duration of epilepsy on the fraction of CSF was demonstrated (P=0.69 and P=0.79, respectively). Considering the possibility of a differential effect of seizure focus laterality on this atrophic process in the TLE group, beta regression of the CSF fraction vs age was performed on patients with left-sided TLE (n=9) and

patients with right-sided TLE (n=6). For right-sided TLE the effect of age was not significant (*P*=0.89) whereas for left-sided TLE it was (*P*=0.009) with a marginal effect of 0.002 per year (95% confidence interval 0.001-0.004).

3.3 Glutamate

Welch's ANOVA showed no significant difference in the concentration of glutamate between the three groups. Multiple regression demonstrated no significant effect of age or the duration of epilepsy on the concentration of glutamate in the patient groups.

3.4 GABA

One-way ANOVA showed no statistically significant difference in the concentration of GABA between the three groups. Multiple regression of the concentration of GABA with age and the duration of epilepsy showed a significant reduction in GABA concentration with age in the TLE group (*P*=0.03) – see Figure 3B. The effect size, as measured by η^2, is 0.28 (Cohens' f=0.62). No such effect was found in the IGE group and no independent effect of epilepsy duration on GABA concentration was demonstrated in the patient groups.

3.5 The Glutamate to GABA Concentration Ratio

One-way ANOVA showed no significant difference in the ratio of glutamate to GABA concentration between the three groups. Multiple regression demonstrated no significant effect of age or the duration of epilepsy on this ratio in the patient groups.

3.6 GSH

One-way ANOVA demonstrated a statistically significant difference for the concentration of GSH between the groups, $F(2,41)=3.68$, $P=0.03$. Post-hoc Tukey-Kramer test for multiple comparisons revealed a statistically significant difference between the IGE and control groups ($P=0.03$). The effect size, as measured by η^2, is 0.15 (Cohen's f=0.42). The concentration was not significantly different between the IGE and TLE groups and between patients with TLE and healthy controls.

3.7 Other Metabolites

Figure 3A shows mean concentration and standard deviations of all metabolites with consistent CRLB<20% that were acquired from all groups: glutamate, GABA, GSH, glutamine, inositol, NAA, and total creatine (creatine + phosphocreatine). To facilitate comparison between studies, concentrations of glutamate, GABA, and GSH normalized to total creatine appear in the supplementary data (Table 2S). Notably, the stability of total creatine was checked among the patient groups and the controls once its normality and homoscedasticity were established. It was found not to be different between the groups, $F(2,41)=2.79$, $P=0.07$.

3.8 Effect of Metabolite Concentrations on Short-Term Seizure-Freedom

Exact logistic regression performed separately on glutamate, GABA, and GSH, evaluated their effect on seizure-freedom in the preceding 3 months before the scan in the entire patient population (16 of the 34 patients in the final analysis reported short-term seizure-freedom). While no effect of GABA, the ratio glumate/GABA, and GSH on short-term seizure-freedom was found, the concentration of glutamate was demonstrated to be a predictor of short-term seizure-freedom (odd ratio 2.14; 95% confidence interval 1.17-4.46; $P=0.01$) – see Table 3. In order to test whether patients who reported short-term seizure-freedom had indeed higher concentrations of glutamate in the PCC/precuneus, a t-test was performed after confirmation of normality, comparing the concentration of glutamate between the groups. In patients who reported short-term seizure-freedom,

the concentration of glutamate (mean±SD) was 10.6±1.4 mM, whereas in patients who did not the concentration was 9.4±1.2 mM, resulting in a one-tailed value of P=0.005.

4. DISCUSSION

The results of this study found a higher concentration of GSH on high field MRS in the PCC/precuneus in the IGE group in comparison with controls. No such difference was found for other metabolites that were examined in this study, which is in contradiction with our hypotheses and requires explanation. One possibility is that changes in excitatory and inhibitory neurotransmitters do exist in these types of epilepsy in comparison with each other and with healthy controls but are located in the epileptogenic networks themselves and not in the DMN. Another possibility is that no such changes exist.

However, we did find evidence that glutamate concentration in the PCC/precuneus may be a predictor of short-term seizure-freedom, possibly related to less DMN deactivation in these patients as a reflection of a more benign clinical course. There is evidence that task-induced deactivation in the PCC/precuneus correlates with the glutamate/GABA concentration ratio in this region.[33] The finding that glutamate, and not its ratio to GABA, was a predictor of short-term seizure-freedom suggests that DMN deactivation and metabolite concentrations in the PCC/precuneus may be related. Moreover, seizure-freedom in patients with MRI-negative TLE is known to be associated with ipsilateral elevation of glutamate concentration, which is speculated to reflect increased propensity for seizures on a background of preservation of tissue integrity.[34] It is possible that our results were underpinned by a similar mechanism.

Focusing on the age-related reduction in the concentration of GABA in the TLE group, we have also noted the increasing fractional component of CSF with age in patients with TLE as a measure of atrophy, a finding driven by patients with left-sided epileptogenic foci, and not demonstrated in patients with IGE. These findings could be related. Interestingly, in the worldwide ENIGMA project, MRI scans of 367 patients with IGE, 754 with mesial TLE with hippocampal sclerosis, 1026 with other forms of epilepsy, and 1727 matched healthy controls were compared. No changes

of cortical thickness were seen for the IGE group in the PCC and precuneus regions. In contrast, in TLE patients with hippocampal sclerosis the ENIGMA study revealed cortical thickness reductions of the bilateral precuneal cortices with left, but not right, seizure foci.[35] Our results are in line with this finding. It is possible, therefore, that the precuneus region undergoes atrophic changes in patients with left-sided TLE, affecting GABAergic more than glutamatergic neurons.

An important question to consider is whether the concentrations of the studied metabolites were affected by drug treatment. Some ASMs such as vigabatrin, gabapentin, lamotrigine, and topiramate are known increase brain GABA concentrations.[36,37] The effects of carbamazepine and valproate were also assessed in several small studies. Petroff and coworkers compared metabolite concentrations in the occipital cortex of 14 patients with epilepsy to 10 healthy controls using 2.1T MRS. They reported reduced GABA levels in 4/8 patients taking carbamazepine. Of the five patients taking valproate, one had reduced GABA levels, two had increased glutamate levels, and one demonstrated reduced glutamate concentration.[38] Garcia and coworkers used 3T MRS focusing on the parietal and occipital lobes to acquire the metabolic profile of patients with epilepsy taking valproate and compared them to healthy controls. While lower levels of glutamate were seen in the spectra of the parietal lobes in the patient group, the difference was not significant.[39] Finally, Doelken et al showed increased GABA levels in patients with focal epilepsy who responded to levetiracetam.[40]

The four most prevalent ASMs in our patient population were carbamazepine, valproate, levetiracetam, and lamotrigine. No effect of these medications on the levels of glutamate and GABA was found except for a reduction in the concentration of glutamate in patients with TLE treated with valproate (Cohen's d=1.9; see Table 4S in the supplementary data). However, only three patients in this group were treated with this medication, having little effect on the overall findings, and making it difficult to draw conclusions.

Could the higher levels of GSH in the PCC/precuneus in patients with IGE be related to drug effects? Ono and coworkers have shown that total plasma glutathione was reduced in epilepsy patients treated with phenytoin and carbamazepine alone or with multiple ASMs in comparison with

controls. No such results were seen in patients treated with valproic acid or phenobarbital monotherapy.[5] A similar study demonstrated a reduction in erythrocyte levels of GSH in children treated with valproic acid or carbamazepine.[41]

Animal models have shed light on the effects other ASMs have on GSH levels. Topiramate was shown to increase levels of GSH in erythrocytes in a rodent model of epilepsy, and oxcarbazepine was shown to decrease the brain levels of GSH in another model in mice[42,43] Lamotrigine was found to have both increasing and decreasing effects on brain GSH levels in rodent models of epilepsy and levetiracetam was shown to increase levels of GSH in the hippocampi of mice in a pilocarpine model of epilepsy.[43–45] No patient in our study was treated with phenytoin or oxcarbazepine, and only 2 of our IGE patients were taking topiramate (and none in the TLE group). As for carbamazepine, valproate, levetiracetam and lamotrigine, no effect of these medications on the concentration of GSH was found (see Table 4S in the supplementary data). As discussed above, the number of medications was similar between the patient groups. To summarize, no medication effect seems to have been responsible for the higher levels of GSH in the IGE group.

A possible explanation for this finding could be a response to oxidative stress. Numerous animal models have demonstrated the involvement of reactive oxygen species in the pathogenesis of epilepsy.[46] Similar mechanisms were shown in a range of neurodegenerative and psychiatric conditions, such as Huntington's disease, Parkinson's disease, Alzheimer's disease, and schizophrenia, with a compensatory initial elevation of GSH concentration eventually being overwhelmed.[46,47] GSH levels were shown to be reduced in the parietooccipital region of both hemispheres in patients with focal epilepsy in a previous MRS study.[11] However, we are unaware of a previous MRS study measuring GSH levels in patients with IGE. Notably, a 7T MRS study of metabolic changes in the hippocampi of rats after induction of status epilepticus by pilocarpine injection discovered gradual elevation of the GSH (scaled to creatine) over 7 days, remaining elevated in chronic epileptic rats.[48]

The study's main limitation is its relatively small sample size. While our results are exploratory in nature, they do suggest elevated GSH concentrations in the PCC/precuneus of patients with IGE. If the difference in GSH concentration between patients with IGE and other groups is found to be consistent in larger cohorts, it may have some value in the management of epilepsy patients in the future. For example, the exact

mechanism by which the ketogenic diet, which is used for treatment of intractable seizures, helps control seizures is not fully explained. Nevertheless, it was shown that a possible mechanism may be related to activation of pathways resulting in increased production of antioxidants such as GSH.[49] Monitoring serum and brain levels of GSH may prove to be important in guiding such modes of therapy.

The higher level of glutamate in patients who reported short-term seizure-freedom is another exploratory finding. If corroborated in larger cohorts, it may prove useful as part of a future model of drug-response prediction.

5. CONCLUSIONS

In conclusion, no difference was found in the concentrations of glutamate, GABA, or their ratio in the PCC/precuneus between patients with IGE, patients with TLE, and healthy controls. An age-related decline in the concentration of GABA in patients with TLE was demonstrated. Patients with IGE were shown to have higher concentrations of GSH in the PCC/precuneus than healthy controls. The concentration of glutamate in the PCC/precuneus may predict short-term seizure-freedom.

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Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Figure Legends

Figure 1 – T_1-weighted sagittal MRI with superimposed MRS 8 cm^3 voxel

Figure 2 – Representative spectra from each group (A - TLE; B - IGE; C – Controls)

Figure 3 – Results of metabolite quantification (A – Metabolic Panel of all groups with SD error bars; B - GABA vs age in patients with TLE)

Table Captions:

Table 1 – Comparison between the groups

Table 2 – Metabolites vs age and duration of epilepsy

Table 3 – Exact logistic regression of short-term seizure-freedom with metabolite concentrations

Supplementary Material Tables:

Table 1S – Tests of normality and homoscedasticity (*P*-values)

Table 2S – Metabolite concentrations normalized to total creatine (mean±SD)

Table 3S – Voxel segmentation

Table 4S – Analysis of drug effects (Kruskal-Wallis H test)

Table 5S – Clinical data

Table 1 – Comparison between the groups

	TLE	IGE	Controls	Statistic	*P*-value
Mean(±SD) age (years)	41.7(±14.3)	39.2(±15.3)	40.0(±14.4)	$F(2,41)=0.13$	0.88
Mean(±SD) glutamate (mM)	9.7(±1.0)	10.2(±1.8)	10.3(±0.9)	Welch's F(2,24.1)	0.24
Mean(±SD) GABA (mM)	1.5(±0.2)	1.6(±0.4)	1.5(±0.2)	$F(2,40)=0.45$	0.64
Mean(±SD) glutamate/GABA	6.4(±1.0)	6.6(±1.1)	7.0(±0.8)	$F(2,40)=0.95$	0.40
Mean(±SD) GSH (mM)	2.0(±0.2)	2.2(±0.4)	1.9(±0.3)	$F(2,41)=3.68$	0.03

Table 2 – Metabolites vs age and duration of epilepsy

Metabolite	*P* (model)	R^2	β (age)	*p* (age)	β (duration)	*P* (duration)
TLE						
Glutamate	0.10	0.256	-0.04	0.05	0.01	0.74
GABA	0.02	0.397	-0.01	0.03	0.00	0.56
Glutamate/GABA	0.18	0.208	0.02	0.31	0.02	0.36

GSH	0.18	0.206	0.01	0.10	0.00	0.91
IGE						
Glutamate	0.02	0.452	-0.07	0.18	-0.01	0.88
GABA	0.06	0.369	-0.02	0.09	0.00	0.66
Glutamate/GABA	0.22	0.226	0.07	0.09	-0.04	0.19
GSH	0.44	0.119	-0.01	0.43	0.00	0.81

Table 3 – Exact logistic regression of short-term seizure-freedom with metabolite concentrations

Metabolite	Odds Ratio	95% Confidence Interval	*P*-value (>= Model Score)
Glutamate	2.14	1.17 - 4.46	0.01
GABA	8.24	0.71 - 146.90	0.09
GSH	7.56	0.67 - 115.10	0.10
Glutamate / GABA	0.92	0.45 - 1.82	0.82