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Magnetic Resonance T₂-Relaxometry and 2D L-Correlated Spectroscopy in Patients with Minimal Hepatic Encephalopathy

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Abstract

Purpose—To evaluate T₂-relaxation changes in patients with minimal hepatic encephalopathy (MHE) using T₂-relaxometry and to correlate T₂ values with brain metabolites evaluated using two-dimensional (2D) magnetic resonance spectroscopy (MRS).

Materials and Methods—Eight MHE patients and 13 healthy subjects were evaluated using T₂-relaxometry, and 8 patients and 9 healthy subjects underwent 2D MRS in right frontal and left occipital regions. Whole brain T₂-relaxation maps were compared between MHE and control subjects using analysis-of-covariance, with age and gender included as covariates. T₂ values derived from the right frontal and left occipital lobes were correlated with the metabolite ratios.

Results—Multiple brain regions including anterior and mid cingulate cortices, right anterior and left posterior insular cortices, right prefrontal, medial frontal, and right superior temporal cortices showed significantly increased T₂ values in MHE patients compared to control subjects. MRS showed significantly increased ratios of glutamine/glutamate (Glx) and decreased ratios of myo-inositol, taurine, choline, and myo-inositol/choline (mICH) with respect to creatine (Cr_d) in patients compared to controls. Frontal Glx/Cr_d showed significantly positive correlation with T₂ values.

Conclusion—MHE patients showed significantly increased T₂ values in multiple brain regions reflecting increased free water content and T₂ values in frontal lobe correlated with the increased Glx/Cr_d ratio.

Keywords

Hepatic Encephalopathy; T₂-relaxation; Magnetic Resonance Imaging; Magnetic Resonance Spectroscopy

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INTRODUCTION

Hepatic Encephalopathy (HE) constitutes a spectrum of neuropsychiatric abnormalities, including cognitive deficits seen in patients with liver dysfunction (1). Minimal HE (MHE) refers to a state observed in patients with cirrhosis who have no gross clinical symptoms of brain dysfunction but have subtle changes in cognitive function, electrophysiological parameters, cerebral neurochemical /neurotransmitter homeostasis, cerebral blood flow, metabolism, and fluid homeostasis (2). Although a battery of neuropsychological tests is considered diagnostic for MHE, these tests are not very specific and do not reveal the underlying pathophysiology (1-3).

Brain magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) have revealed some characteristic findings in HE, and these modalities may complement the neuropsychological examinations (3-5). MRI of HE patients commonly showed bilateral, symmetrical hyper intensities of globus pallidus on T₁-weighted images (4,5). However, there have been conflicting reports about changes on T₂-weighted MRI in HE with many of the studies reporting no changes, albeit some studies reporting hyperintensities in patients with liver cirrhosis (5-7), and chronic HE (8), which would be reflective of prolonged T₂ values. One study however, reported T₂ and T₁ shortening in the basal ganglia and cortex in patients with chronic liver disease, which is contrary to observing hyperintensities on T₂-weighted MRI (9).

Measurement of T₂ provides a means for tissue characterization and helps to identify subtle pathology which may not be recognized visually (10,11). T₂-relaxometry measures mainly free water content in the tissue in absence of diamagnetic and paramagnetic substances (12). T₂ values increase with increased free protons compared to bound protons, which occurs with increased free water content, myelin, axonal, and cell injury (10,13-17). The procedure has been used in several central nervous system conditions including in hippocampal sclerosis, multiple system atrophy, multiple sclerosis, traumatic brain injury, and congenital central hypoventilation syndrome (10,11,16,18).

A remarkable marker of HE as shown by one-dimensional (1D) proton MRS has been a triad of changes in cerebral metabolites including the increased ratios of glutamine/glutamate (Glx), and decreased ratios of myo-inositol (mI) and choline (Ch) with respect to creatine (Cr) (19-21). The overlap of metabolite peaks in 1D MRS caused by many factors, including J-modulation leading to varying phase artifacts with echo time (TE), making quantitation difficult for many metabolites. Two-dimensional (2D) MRS overcomes this problem by adding a second frequency dimension to each spectrum by acquiring multiple 1D spectra with incrementally longer TEs and applying double Fourier transform on the set of spectra to produce a 2D spectrum (22).

Our aim was to assess whole brain regional alterations in brain T₂ values and metabolite changes in right frontal and left occipital regions using 2D localized correlated spectroscopy (L-COSY) in patients with MHE compared to control subjects and to correlate the metabolic changes with the T₂ values derived from these locations in MHE patients.

MATERIALS AND METHODS

Subjects

Eight patients with MHE (age range = 45-65 years, mean age $\pm$ SD = 49.6 $\pm$ 14.7 years, 4 males) and thirteen control subjects (age range = 28-68 years, mean age $\pm$ SD = 50.1 $\pm$ 10.8 years, 5 males) were studied with MRI, and 8 MHE patients and 9 control subjects completed 2D MRS. The diagnosis of low grade encephalopathy was based on the West

Haven criteria (23) and abnormal neurological scores. All patients were on lactulose therapy as a part of the treatment of HE. Exclusion criteria of HE patients were age less than 18 or more than 75 years, active alcoholism during past three months, HE grades from II-IV, gastrointestinal bleeding or infection during past week, and any brain shunt procedures before the study. All HE patients with additional neurological or psychiatric diseases or with treatment with anti-psychotic or other drugs that may alter neuropsychological functions were excluded. Control subjects were without any neurological and metabolic disorders and were recruited through advertisements. Control and HE subjects with contraindication for MRI scanner environment were also excluded from the study. The study protocol was approved by the Institutional Review Board, and all subjects gave written consent prior to the study.

MRI and 2D MRS

All brain MRI studies were performed using a 1.5 Tesla Avanto MR scanner (Siemens Medical Solutions, Erlangen, Germany) while subjects lay supine. Foam pads were used either side of the head to minimize head motion during the study. Proton density (PD) and T₂-weighted images [repetition time (TR) = 7500 ms; echo-time (TE₁, TE₂) = 17, 134 ms; matrix size = 256 × 256; field of view (FOV) = 230 × 230 mm²; slice thickness = 4.0 mm; interslice gap = 0.0 mm; turbo factor = 5; flip angle (FA) = 150°] were acquired in the axial plane covering whole brain, using a dual-echo turbo spin-echo pulse sequence. High-resolution T₁-weighted images were collected using a magnetization prepared rapid acquisition gradient-echo pulse sequence (TR = 1660 ms; TE = 3.87 ms; inversion time = 900 ms; FA = 10°; matrix size = 256 × 256; FOV = 230 × 230 mm²; slice thickness = 1.2 mm; number of slices = 176).

We used a quadrature body coil for “transmission” and a dual surface coil for “reception” for MRS. One surface coil was placed directly on the right forehead and the second coil on the left occipital regions of the subject. Magnetic resonance spectroscopy was performed with 2D L-COSY sequence, over two locations: prefrontal dorsolateral white/gray matter and occipito-parietal white/gray matter. The sequence consisted of three slice-selective radio frequency (RF) pulses (90°-180°-90°) for the volume localization of a 30 × 30 × 30 mm³ voxel, with the last 90° RF pulse also enabling the coherence transfer necessary for 2D L-COSY (22). The spectral encoding for the second dimension was inserted between the second and third slice-selective pulses. A WET-based sequence was used for global water suppression (24), and spectra were recorded using the following parameters: TR = 2000 ms, TE = 30 ms, total number of scans = 768, scan time = 25 minutes (96 Δt₁ increments and 8 NEX/Δt₁), spectral width, F₁ = 625 Hz and F₂ = 2000 Hz. Global water suppression was done using the algorithm provided by the manufacturer (Siemens Medical Solutions, Erlangen Germany) after achieving a line-width of 8-10 Hz by manual shimming in all the subjects. By optimizing the static field (B₀) homogeneity, the cancellation of overlapping antiphase cross-peak multiplets was minimized.

Data Processing

All brain MRI images of individual subjects, including T₁, PD, and T₂-weighted images were assessed for any major brain lesions before T₂ calculation. PD and T₂-weighted images were also examined for motion artifacts to ensure that images were acceptable for subsequent data processing.

The statistical parametric mapping package SPM5 (Wellcome Department of Cognitive Neurology, UK), and Matlab-based (The MathWorks Inc, Natick, MA) custom software were used for the data processing.

T₂ Relaxation Quantitation—Using PD and T₂-weighted images, T₂ values were calculated pixel-by-pixel (18). The average noise level outside the brain was obtained from PD and T₂-weighted images, and was used as a threshold to exclude non-brain regions. The same threshold was applied to all images and for all subjects. The following equation determined pixel-by-pixel T₂ values:

$$T_2 = \frac{(TE_2 - TE_1)}{\ln\left(\frac{SI_1}{SI_2}\right)},$$

1

where TE₁ and TE₂ were the echo times for PD and T₂-weighted images, and SI₁, SI₂ were the signal intensities from the PD and T₂-weighted images, respectively. T₂ maps were generated using the T₂ values calculated for each pixel.

Post-processing of 2D MRS—The 2D raw matrices (1024 complex points along the first dimension and 96 points along the second dimension) were transferred to SGI™ O₂ workstation (Silicon Graphics Inc, San Jose, CA) for post processing. All the 2D spectra were processed using a Felix-2000™ package (Felix NMR Inc., San Diego, CA). Before Fourier transformation, all matrices were filtered using skewed squared-sine bell functions along both dimensions, linear predicted in the second dimension to 128 increments from the acquired 96 increments, and then zero-filled in both dimensions to 2048 × 256. The resulting spectra were constructed in magnitude mode and displayed as contour plots. The volumes under the 2D diagonal and cross peaks were calculated using manual peak picking on contours. The area of integration for each peak was predefined and was saved in a volume table. The areas for integration in the table were determined from the theoretical calculation of the spin system, and later fine tuned by the phantom experiments. By overlaying this volume table on all the spectra, errors due to changes in the areas of integration were minimized.

Data Analysis

Voxel-based- relaxometry—We used voxel-based-relaxometry procedures, which allow comparison of T₂ values across the entire brain and show T₂ value changes or differences between the two groups (10). T₂ maps of MHE and control subjects were normalized to standard Montreal Neurological Institute (MNI) space. T₂-weighted images of each subject were normalized to the MNI template, using a *priori-defined distribution* of tissue types, and the resulting parameters were applied to the corresponding T₂ maps. The normalized T₂ maps were smoothed using a Gaussian filter (full-width at half-maximum = 10 mm). The normalized and smoothed T₂ maps were compared between MHE and control groups using analysis of covariance at each voxel (p < 0.001, uncorrected), with age and gender included as covariates.

Brain sites with significantly increased T₂ values in MHE patients compared to control subjects were displayed in glass brain view with projections across the 3D volume onto 2D axial, coronal, and sagittal views for structural identification.

Quantification of Metabolites—The raw volume integrals of the diagonal peaks of choline, creatine, and N-acetyl aspartate were measured and denoted as Ch_d, Cr_d and NAA_d, respectively. Among the cross peaks, eleven peaks were chosen as summarized in Table 1. 2D cross peaks of some metabolites overlap with others such as threonine and lactate (represented as ThrLac), and one of the two myo-inositol cross peaks and free choline (represented as mICh). All peaks were selected from the region below the diagonal peaks; cross peaks above the diagonal were not chosen due to the asymmetry effect which is

stronger for peaks close to the water resonance. In addition, simulation of 2D cross peaks was also used to double-check the peak assignments. We calculated the ratios of the diagonal and cross peaks of different metabolites with respect to Cr_d ($F_2 = F_1 = 3.0$ ppm) using the 2D peak volumes. Compared to the absolute concentration of metabolites, the 2D metabolite ratios minimized errors due to the fluctuation of the scanner's magnetic field and hardware, head size, and coil positioning.

Region-of-interest Analysis—Using information of voxel locations for 2D MRS, region-of-interest (ROI) masks were created for both regions, including right frontal and left occipital lobes. These masks were used to derive T_2 values from normalized T_2 maps of individual subjects. T_2 values for these locations were calculated from only those MHE and control subjects who had good quality spectra. T_2 values calculated for these brain regions were compared between groups using independent samples t-tests.

Statistical Analysis—The numerical demographic variables were compared with independent samples t-test and categorical values were compared with Chi-square test. The mean and standard deviations (SD) of the metabolite ratios and the T_2 values were calculated and compared between the MHE patients and control subjects using two tailed independent samples t-test. Pearson's correlation test was used to evaluate the correlation between brain metabolite ratios and T_2 values derived from the same locations. Within-group comparisons were performed between frontal and occipital variables (i.e. metabolite ratios and T_2 values) using the two tailed paired t-test.

RESULTS

There were no significant differences between patient and control groups with respect to age ($p = 0.33$) or gender ($p = 0.95$). Multiple brain sites in MHE patients showed increased T_2 values, suggesting abnormal areas, compared to control subjects (Figure 1). No brain regions emerged with higher T_2 values in control subjects compared to MHE patients. Brain regions with increased T_2 values in MHE patients appeared in anterior and mid cingulate cortices, right anterior insula extending to surrounding white matter and left posterior insula, right prefrontal, medial frontal, and right superior temporal cortices.

T_2 values calculated from right frontal and left occipital lobes are summarized in Table 2. T_2 values, derived from right frontal white matter, significantly increased in MHE patients compared to control subjects ($p = 0.005$). The left occipital lobe showed a trend towards increased T_2 values in MHE patients but failed to reach statistical significance.

Different brain metabolite ratios of MHE and control subjects are summarized in Table 3, and representative 2D L-COSY spectra of MHE and control subjects acquired from the left occipital lobes are shown in Figure 2A and 2B respectively. Several metabolite ratios including Ch/Cr_d, mIch/Cr_d, Glx/Cr_d, and mI/Cr_d significantly differed between MHE patients and control subjects in both the frontal and the occipital regions. The mean metabolite ratios including Ch/Cr_d, mIch/Cr_d, and mI/Cr_d of frontal and occipital lobes were significantly lower in MHE patients compared with control subjects. The Glx/Cr_d ratio was significantly higher in MHE patients in both the right frontal and the left occipital lobes compared with control subjects. The Tau/Cr_d in frontal lobe and NAA/Cr_d in occipital lobe showed significantly reduced metabolite ratio in MHE patients compared to control subjects.

T_2 values and Glx/Cr_d ratio acquired from right frontal lobe showed significantly positive correlation in MHE patients ($r = 0.81$, $p = 0.014$, Figure 3A). In contrast, Ch/Cr_d and T_2 values correlated with negative trend for the same location ($r = -0.69$, $p = 0.059$, Figure 3B).

None of the metabolite ratios showed significant correlation with T_2 values acquired from the occipital white matter in MHE patients.

Comparing the significantly altered frontal and occipital metabolite ratios revealed no significant differences within both the control and the patient group ($p > 0.05$). For the T_2 values, there was no difference between the frontal and occipital T_2 values in the control group ($p = 0.23$) whereas in the patient group, frontal T_2 values were significantly higher than the occipital values ($p = 0.018$).

DISCUSSION

In this pilot study, we observed increased T_2 values in several regions of the brain in MHE patients as compared to healthy controls. In addition, MHE patients had increased Glx/Cr_d which correlated with the increased T_2 values in the frontal lobe. Our findings suggest increased free water content in several brain regions in MHE patients and that, accumulation of glutamine (a detoxification metabolite of ammonia), contributes to these changes in the brain water content in these patients.

The observed increase in T_2 values could be due to several reasons which lead to increased free protons, such as increased tissue water content or in case of neuronal injury or demyelination or gliosis (10,13-17) out of which any or all may be present. Development of HE in patients with chronic liver disease has been postulated to be closely associated with the increase in astrocyte hydration without an increase in intracranial pressure, described as low grade cerebral edema (25). Prior imaging studies in patients with cirrhosis or HE have included conventional T_1 - and T_2 - weighted imaging, magnetization transfer ratio (MTR) imaging, diffusion weighted imaging (DWI) and diffusion tensor imaging (DTI), and water content mapping (26-29). No previous study has however, utilized T_2 relaxometry to evaluate brain tissue characteristics in MHE patients. Only few previous studies have reported changes on T_2 weighted imaging in patients of HE (6-8). T_2 hyperintensities were observed in the cerebral white matter in and around the corticospinal tract in cirrhotics which were seen to normalize after successful liver transplantation (6,7). These hyperintensities were also seen to relate to functional abnormalities (6). On histopathological correlation in two autopsy proven cases of chronic HE, cortical laminar T_2 hyperintensities were shown to reflect pseudolaminar spongy degeneration of the deep layers of the cerebral cortices associated with numerous Alzheimer type II astrocytes and T_2 hyperintensities in the cerebral white matter reflected loss of myelin and axons, which was possibly due to longstanding edema caused by chronic hepatic encephalopathy (8). Rovira et al. observed a significant decrease in the MTRs in frontal and parietal white matter of cirrhotic patients which they supposed could be due to low grade astrocytic swelling (28). These changes were shown to be reversible after liver transplantation consistent with the development of low grade cerebral edema (6). Using DWI and DTI, increased interstitial brain water content has been reported in various white matter and grey matter locations in low grade HE (26,27). A recent quantitative assessment of cerebral water content showed the presence of low-grade cerebral edema in HE patients (29).

Thus, a more plausible explanation for T_2 changes in the current patient group appears to be an increase in free water. Alternative explanations such as neuronal injury or demyelination are not suggested by the current and previous MRS reports in MHE patients which should have then respectively shown decreased NAA and increased choline levels in those circumstances. In the presence of gliosis, myo-inositol has been shown to increase in diseases such as hippocampal sclerosis (30) and Wilson's disease (31) which is not the case in our patients. Also, if gliosis was the cause of the observed T_2 changes, then a correlation

between myo-inositol as an astro-glial marker and T_2 changes would have been expected which was not observed in the current study.

Consistent with the previous 1D MRS studies reporting significant decline in mI and total choline, 2D L-COSY data showed a significant decrease in mI cross peak and choline diagonal peak. In addition, a strong and significant decrease of mICh peak was seen, which was expected, as this cross peak is created by the overlap of mI and Ch. This peak is the best single marker in the 2D spectra for distinguishing healthy volunteers from patient with MHE. It has been demonstrated that several J-coupled metabolites, such as Asp, Tau, threonine and GABA, are better resolved in a 2D L-COSY spectrum. Also, the cross peaks of glutamate/glutamine were better separated from other overlapping metabolites in 2D L-COSY compared to 1D studies. The 2D MRS technique is able to separate PCh from the total choline peak observed in 1D MRS studies and also identifies a clear cross peak of PE not clearly detectable in the 1D MRS studies.

In our pilot study, significant increase in Glx/Cr_d showed a strong positive correlation with increased T_2 values in the frontal region suggesting that the T_2 relaxation changes in MHE patients are, at least partially mediated via the increased ammonia and increased glutamate/glutamine and they can be related to cerebral edema (25). Astrocytes are the main site for ammonia detoxification via conversion of glutamate to glutamine. One of the hypotheses for explaining pathophysiology of HE is that increased ammonia in cirrhotic patients leads to increased formation of glutamine which exerts an osmotic effect leading to astrocytic swelling and initiating compensatory osmoregulatory attempts via depletion of osmolytes like myo-inositol, taurine, etc. (32). Further, this low grade astrocytic swelling may lead to selective alterations of blood brain barrier function, increased GABA-ergic tone, glycogen deposition and alterations of neurotransmitter processing in the astrocytes (25,33). The increased interstitial water seen using DWI/DTI has been postulated to be due to the accumulation of extracellular glutamate and shift of osmolytes into the extracellular space (26,27,34). Studies have supported the elevation of T_2 values during development of cytotoxic edema (35,36) as well as vasogenic edema (37,38). Whether Glx refers to intracellular or extracellular, glutamate or glutamine is not possible to differentiate using in vivo magnetic resonance and similarly, whether these T_2 changes refer to purely intra- or extra- cellular water changes or both is difficult to infer (13,15). However, chronic liver failure is more consistent with development of vasogenic edema as compared to cytotoxic edema superimposed on vasogenic edema in acute on chronic liver failure (26,34).

Ch/Cr_d showed an inverse correlation with T_2 values in the frontal region in MHE patients though it did not reach statistical significance ($p=0.059$); limited number of patients may have restricted reaching a significant level. Choline is known to be reduced in cirrhotics with and without MHE and is one of three components of the MRS findings in HE (20,21). Also, a component of the choline peak, glycerylphosphorylcholine is considered to be an osmolyte. The negative correlation with T_2 value changes points towards an osmoregulatory role of choline metabolites. Since the glutamine and choline levels show no correlation, whereas both independently correlate with the T_2 changes, it can be possible that changes in both occur independent of each other and then affect the brain water equilibrium independently rather than one following the other.

While metabolite ratios between the two regions did not differ in either controls or patients, frontal T_2 values were significantly higher than the occipital values in patients. Whereas this result may be a consequence of small number of patients studied, this could imply that similar changes occur all over the brain at the metabolic level whereas at the structural level, these changes vary regionally in severity or chronological development in early grades of HE. This is consistent with prior reports of the pattern of cerebral dysfunction in HE.

suggesting that functional dysfunction is restricted to some brain regions in the early stages of the disease (39,40).

A small number of patients is one of the limitations of the study. Another limitation is that MRS was performed in two locations allowing comparisons in only those two locations with the T_2 changes. The metabolite ratios in the current study were obtained using operator defined volume contours of the metabolite peaks in a 2D MR spectrum. More accurate quantification could have been done using prior knowledge fitting algorithm for 2D MRS such as ProFit (41).

In conclusion, T_2 values obtained using voxel based approach, are increased in several regions in the brain of patients with minimal hepatic encephalopathy and reflect increased free water. Increased Glx in these patients is strongly associated with the increased free water content in the frontal brain and helps in connecting ammonia as a major toxin responsible for the increased brain water through the detoxification process.

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Figure 1.

Brain regions with significantly higher T_2 values in patients compared to controls displayed in glass brain view with projections across the 3D volume onto 2D axial, coronal, and sagittal views ($p < 0.001$, uncorrected). Gray and dark regions show significantly increased T_2 values (with increasing significance, from gray to dark) in those brain sites in HE, compared to control subjects, whereas white regions show no significant differences in T_2 values between the two groups.

Figure 2.

2D L-COSY spectra recorded from the left occipital lobe of A) a 40-year-old MHE patient and B) a 45-year-old healthy subject, using a voxel size of 27 mL. Projected one-dimensional spectra are also shown on top of each figure. Cr_d, diagonal creatine; Ch_d, diagonal choline; NAA_d, diagonal N-acetyl aspartate; mICh, overlapping cross peak of myo-inositol and choline; Asp, aspartate; Glx, glutamate/glutamine; mI, myo-inositol; Tau, taurine; PCh, phosphocholine; GABA, gamma-aminobutyric acid; PE, phosphoethanolamine; ThrLac, overlapping cross peaks of threonine and lactate, MM, macromolecules.

Figure 3. Correlation of T₂ values and selected metabolite ratios recorded from the dorsolateral prefrontal region of MHE patients: A) Glx/Cr_d ($r = 0.81$, $p = 0.014$) and B) Ch_d/Cr_d ($r = -0.69$, $p = 0.059$).

Table 1

Position of 2D L-COSY metabolite peaks

Metabolites	Symbols used	Peak locations (ppm)	
		F ₂	F ₁
N-acetyl aspartate	NAA_d	2.0	2.0
	NAA	4.3	2.6
Creatine	Cr_d	3.0	3.0
	Cr	3.9	3.0
Choline	Ch_d	3.2	3.2
Glutamine/Glutamate	Glx	3.7	2.0
Myo-inositol	mI	3.5	3.1
Myo-inositol/Choline	mICh	4.0	3.5
Phosphoethanolamine	PE	4.0	3.2
Phosphocholine	PCh	4.3	3.6
Aspartate	Asp	3.9	2.7
GABA	GABA	2.9	1.9
Threonine/Lactate	ThrLac	4.1	1.3
Taurine	Tau	3.4	3.2

Table 2

T₂ values of HE and Controls.

Brain regions	T ₂ value (Mean $\pm$ SD, ms)		p values
	MHE (n=8)	Controls (n=13)	
Right frontal lobe	130.93 $\pm$ 16.22	116.40 $\pm$ 6.88	0.005
Left occipital lobe	120.67 $\pm$ 8.64	115.14 $\pm$ 4.56	0.068

SD = Standard deviation; MHE = Minimal hepatic encephalopathy

Table 3

Metabolite ratios of MHE and control subjects.

Metabolite ratios	MHE		Controls		P values	
	Frontal (Mean $\pm$ SD) [A]	Occipital (Mean $\pm$ SD) [B]	Frontal (Mean $\pm$ SD) [C]	Occipital (Mean $\pm$ SD) [D]	[A] vs [C]	[B] vs [D]
Ch_d/Cr_d	0.786 $\pm$ 0.142	0.697 $\pm$ 0.168	1.001 $\pm$ 0.082	0.954 $\pm$ 0.092	0.002	0.002
NAA_d/Cr_d	1.612 $\pm$ 0.119	1.445 $\pm$ 0.141	1.716 $\pm$ 0.157	1.624 $\pm$ 0.100	0.152	0.009
mICh/Cr_d	0.064 $\pm$ 0.019	0.058 $\pm$ 0.024	0.112 $\pm$ 0.011	0.117 $\pm$ 0.022	0.000	0.000
Asp/Cr_d	0.023 $\pm$ 0.007	0.028 $\pm$ 0.006	0.025 $\pm$ 0.004	0.025 $\pm$ 0.005	0.603	0.294
Cr/Cr_d	0.012 $\pm$ 0.006	0.017 $\pm$ 0.006	0.014 $\pm$ 0.002	0.014 $\pm$ 0.004	0.360	0.289
Glx/Cr_d	0.148 $\pm$ 0.034	0.178 $\pm$ 0.062	0.120 $\pm$ 0.016	0.116 $\pm$ 0.014	0.029	0.011
mI/Cr_d	0.011 $\pm$ 0.005	0.013 $\pm$ 0.005	0.022 $\pm$ 0.004	0.020 $\pm$ 0.005	0.000	0.017
NAA/Cr_d	0.308 $\pm$ 0.081	0.271 $\pm$ 0.076	0.321 $\pm$ 0.028	0.304 $\pm$ 0.052	0.653	0.314
Tau/Cr_d	0.007 $\pm$ 0.002	0.007 $\pm$ 0.003	0.011 $\pm$ 0.002	0.009 $\pm$ 0.003	0.016	0.347
PCh/Cr_d	0.007 $\pm$ 0.003	0.008 $\pm$ 0.003	0.007 $\pm$ 0.002	0.007 $\pm$ 0.002	0.817	0.639
GABA/Cr_d	0.007 $\pm$ 0.002	0.007 $\pm$ 0.004	0.006 $\pm$ 0.003	0.007 $\pm$ 0.003	0.757	0.829
PE/Cr_d	0.005 $\pm$ 0.002	0.005 $\pm$ 0.003	0.007 $\pm$ 0.002	0.005 $\pm$ 0.001	0.095	0.638
ThrLac/Cr_d	0.012 $\pm$ 0.005	0.011 $\pm$ 0.003	0.011 $\pm$ 0.002	0.015 $\pm$ 0.009	0.456	0.259

Cr_d, diagonal creatine; Ch_d, diagonal choline; NAA_d, diagonal N-acetyl aspartate; mICh, overlapping cross peak of myo-inositol and choline; Asp, aspartate; Glx, glutamate/glutamine; mI, myo-inositol; Tau, taurine; PCh, phosphocholine; GABA, gamma-aminobutyric acid; PE, phosphoethanolamine; ThrLac, overlapping cross peaks of threonine and lactate.