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Hypothalamic damage in multiple sclerosis correlates with disease activity, disability, depression, and fatigue

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ABSTRACT

Objectives: Disturbances in the hypothalamo-pituitary axis are supposed to modulate activity of multiple sclerosis (MS). We hypothesised that the extent of HYP damage may determine severity of MS and may be associated with the disease evolution. We suggested fatigue and depression may depend on the degree of damage of the area.

Method: 33 MS patients with relapsing-remitting and secondary progressive disease, and 24 age and sex-related healthy individuals (CON) underwent 1H-MR spectroscopy (1H-MRS) of the hypothalamus. Concentrations of glutamate + glutamin (Glx), cholin (Cho), myoinositol (mIns), N-acetyl aspartate (NAA) expressed as ratio with creatine (Cr) and NAA were correlated with markers of disease activity (RIO score), Multiple Sclerosis Severity Scale (MSSS), Depressive-Severity Status Scale and Simple Numerical Fatigue Scale.

Results: Cho/Cr and NAA/Cr ratios were decreased and Glx/NAA ratio increased in MS patients vs CON. Glx/NAA, Glx/Cr, and mIns/NAA were significantly higher in active (RIO 1–2) vs non-active MS patients (RIO 0). Glx/NAA and Glx/Cr correlated with MSSS and fatigue score, and Glx/Cr with depressive score of MS patients. In CON, relationships between Glx/Cr and age, and Glx/NAA and fatigue score were inverse.

Conclusion: Our study provides the first evidence about significant hypothalamic alterations correlating with clinical outcomes of MS, using 1H-MRS. The combination of increased Glu or mIns with reduced NAA in HYP reflects whole-brain activity of MS. In addition, excess of Glu is linked to severe disease course, depressive mood and fatigue in MS patients, suggesting superiority of Glu over other metabolites in determining MS burden.

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Multiple sclerosis; hypothalamus; MR spectroscopy; glutamate toxicity; RIO score; disability; depression; fatigue

Introduction

The hypothalamus (HYP) is one of the most complex and essential regions of the brain due to its ability to sense both neural and physiological signals and to respond by releasing neurotransmitters and peptide neuromodulators into the brain. The hypothalamo-pituitary-adrenal (HPA) axis, via its projections to many brain regions, regulates reproduction, stress, circadian rhythms and immune function, as well as more complex behaviour including sleep, mood and cognition [1,2]. Several research lines in both animal and human studies implicate the role of the HPA axis in the management of multiple sclerosis (MS) [1–5]. However, only two non-conventional MRI studies have reported MS-related structural changes in the hypothalamus *in vivo* (one using T1 relaxation time and the other diffusion tensor imaging), both showing correlations with hypothalamic abnormalities [6,7].

Chronic activations of the HPA axis have also been described in mood disorders [8,9], increased fatigue [10] and in memory dysfunction [11].

We hypothesised that the extent of HYP damage may determine severity of MS and may be associated with the disease evolution. We were also hoping to find out whether fatigue and depression depend on the degree of damage of the area. The primary goal of the study was to compare metabolic characteristics of HYP between MS patients and healthy controls (CON). Secondly, we tested a possible relationship between metabolic changes and (i) disease activity and disability markers of MS, (ii) and fatigue and depression scales.

In this cross-sectional case-control study we focused on evaluation of hypothalamic metabolism via proton magnetic resonance spectroscopy (1H-MRS) as this method allows noninvasive characterisation of metabolic abnormalities in the central nervous system [12].

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Methods

Patients and control participants

Patients: 33 adult patients with RRMS or SPMS and 24 age- and sex-matched control participants (CON) were included to the study after giving their written consent. The protocol was approved by the ethics committee of Jessenius Faculty of Medicine, Comenius University. The reason for inclusion of both RRMS and SPMS patients is that there are no clear criteria to determine when RRMS converts to SPMS as the transition is usually gradual [13]. Therefore, patients of both categories can show similar phenotype and metabolic changes for some time.

Basic data of the patients and healthy controls are included in Table 1.

Patients with clinically definite MS, according to McDonald 2005 criteria [14], were selected from the Multiple Sclerosis Centre, University Hospital in Martin, Slovakia. Four patients, in whom neuromyelitis optica was not excluded by radiological/clinical examinations, were tested for the presence of anti-aquaporin-4 antibodies with negative results. All patients were treated with immunomodulatory agents (interferon beta, glatiramer acetate, natalizumab, fingolimod or intravenous methylprednisolone) depending on their disease activity (DA). The treatments were in accordance with national guidelines (www.health.gov.sk). Within the last 12 months before 1H-MRS imaging, the patients remained on the same treatment. In addition to immunomodulatory agents, 14 of the patients were treated with antidepressants (escitalopram, citalopram, mirtazapine, venlafaxine or trazodone). Clinical disability was evaluated by neurologists specialised in MS by Expanded Disability Status Scale (EDSS) and additionally adjusted for disease duration by Multiple Sclerosis Severity Scale (MSSS). DA was assessed according to 'RIO score' (RIO) [15]. The patients were considered as good responders to treatment when they were free of DA, RIO score was 0. They had no relapses and no substantial new T2 activity in their MRI examinations during the previous 12 months, and increase in EDSS of <1point. The patients with sustained DA were RIO ≥ 1. RIO 1 group included patients with the history of ≥1 relapse or >2 new active T2 lesions or increase in EDSS score of ≥ 1 point, sustained over at least 6 months. Patients scoring RIO 2 experienced ≥ 1 relapse together with either >2 new T2 lesions or increase in EDSS score of ≥1 point, sustained over at least 6 months [15]. No patients were evaluated as RIO 3.

Table 1. The demographic and clinical data of multiple sclerosis patients and controls.

	MS	CON	
Number of subjects	33	24	
Age (years, mean + SD)	36.2 (±11.3)	35.1 (±11.7)	*$p = 0.7$
Sex (M/F)	10/23	10/14	$p = 0.6$
Disease duration (years, mean + SD)	9.1 (±6.15)	NA	
Diagnosis (RRMS/SPMS)	27/6 (82/18%)	NA	
EDSS (mean + SD)	3.5 (±2.1)	NA	
MSSS (mean + SD)	4.9 (±2.7)	NA	
Simple Numerical Fatigue Scale (mean + SD)	6.2 (±2.3)	4.3 (±1.1)	*$p = 0.001$
Simple Depressive-Severity Status Scale (mean + SD)	5.7 (±1.8)	7.7 (±1.3)	*$p = 0.00007$

Notes: MS = patients with multiple sclerosis, CON = healthy controls, M = male, F = female, RRMS = relapsing-remitting MS; SPMS = secondary progressive MS; EDSS = Expanded Disability Status Scale, MSSS = Multiple Sclerosis Severity Scale, NA = not applicable, SD = standard deviation.
*Mann–Whitney test, 2-tailed *T*-test (with no mark), statistical significance $p = 0.05$.

Each subject underwent structural MRI scanning few minutes before MRI spectroscopy. Images were acquired using a 1.5 T Siemens Symphony scanner in the MRI unit of Radiology Clinic at the University Hospital in Martin, Slovakia. Structural MRI included T1-weighted MPRAGE, T2-weighted and FLAIR sequences.

Due to well-known issues of magnetic field (B_0) inhomogeneity especially in marginal brain regions located close to sinuses, phase maps of B_0 were obtained prior to 1H-MRS acquisition in each subject. The volume of interest for B_0 homogeneity adjustments (shimming volume) was manually placed at the hypothalamic region outside of any B_0 distortions visible on the B_0 map. Maximum possible B_0 homogeneity was then achieved by iterative field mapping and calculation of appropriate shim currents of the first- and second-order corrections. Three-dimensional spectroscopic imaging sequence based on Point Resolved Spectroscopy was used to acquire localised data focused on hypothalamic region (TE/TR = 30/1500 ms, FOV was 10 × 10 × 8, interpolated to 16 × 16 × 8 cm^3 with a nominal voxel

CON were recruited from volunteers such as medical staff, students, partners of patients or patients with other than brain disease (moderate lower back pain, carpal tunnel syndrome). No endocrine, oncological or other serious disorders, possibly leading to hypothalamus impairment, were confirmed in either patients or controls.

Mood status was evaluated in both patients and controls using the Simple Depression-rating Scale Score (SDSS) questionnaires. In SDSS, the subjects expressed their average mood perceived in the period of at least one month before 1H-MRS imaging. Value 1 represented sad, depressed, the worst possible mood. 5 the feeling 'in the middle, not happy nor sad' and 10 high, happy, great mood.

Fatigue was assessed in both patients and controls using the Simple Numerical Fatigue Score (SNFS). The SNFS expressed their average fatigue perceived in the period of at least one month before 1H-MRS imaging. Value 1 represented minimal or no fatigue, 5 the feeling 'in the middle' and 10 the worst possible fatigue.

Imaging protocol

size of 10 × 10 × 12.5 mm^3 and volumes of interest 50 × 60 × 35 mm^3, elliptical weighting, 7 min acquisition.

We preferred to use multi-voxel over single-voxel 1H-MRS due to its advantages of shorter measurement time for two voxels and especially due to voxel shifting in post-processing. During data acquisition, the signal from magnetically heterogeneous area under the hypothalamus was suppressed by a suppression slab.

Evaluation of structural MR images and 1H-MRS spectra

Structural MRI images of all subjects were inspected for macroscopic hypothalamic lesions. 1H-MRS data were evaluated by a blinded physicist. Spectra of two voxels precisely centred to the central part of the right and left hypothalamus in jSIPRO software [16] were analysed using LCModel software. The voxel centre was placed between anterior commissure and mammillary body 1 mm anteriorly to fornix in sagittal and 2 mm laterally to the border of 3rd ventricle in trans-axial plane (Figure 1(A)). Five main metabolites: Cr (Creatine), N-acetyl aspartate (NAA) (calculated as sum of N-acetylaspartate and N-acetylaspartylglutamate), Cho (Choline, sum of glycerylphosphorylcholine and phosphatidylcholine), Glx (sum of Glutamate and Glutamine) and mIns (myo-Inositol) were quantified in each voxel spectra (Figure 1(B)). If the spectral signal-to-noise ratio values were very low (<5) (the peak amplitudes showed more than 300% asymmetry between the right and left hypothalamus, and LCModel manifested improper residuals or large artefacts), we searched for higher quality by shifting both voxels in anteroposterior direction (in 9 of 57 subjects). In 5 subjects, less than 5 mm voxel displacement solved the low quality issue and the new values were used in analysis. Remaining 3 subjects (1 patient and 2 controls) were excluded.

Metabolites were expressed in ratios: NAA/Cr, Cho/Cr and NAA/Cho, Glx/Cr, Glx/NAA and mIns/NAA. Since the values from both hypothalamus voxels were highly intercorrelated for all ratios in both patients and controls ($r > 0.5$, $p < 0.0001$), and no statistical trend to side difference was found ($p > 0.1$), whole-hypothalamus values, calculated as the means from left and right side spectra, were used for statistical analyses.

Statistical analyses

Differences between patients and controls and between basic patients' subgroups (divided according to DA) were evaluated using Student's *t*-test for normally or Mann–Whitney *U* test for non-normally distributed data. Associations between metabolite ratios and other variables (age, sex, disease duration, MSSS, SDSS, SDFS) were assessed using Spearman rank analysis. The significance level was 0.5. All statistical procedures were performed using NCSS software.

Results

The demographic and clinical data of 33 MS patients and 24 CON are summarised in Table 1. In 2 patients, small T1 hypo-intense hypothalamic lesions (less than 0.25 ml in total) were detected by structural MRI. In CON, no structural abnormality was identified.

We found significant differences between patients with MS and CON in the Cho/Cr, NAA/Cr, and Glx/NAA ratios. Both Cho/Cr and NAA/Cr showed significantly lower levels in MS patients than in CON. Glx/NAA and mIns/NAA ratios were higher in MS patients than in CON. The differences in mIns/NAA and NAA/Cho ratios between MS and controls were subtle, showing trends.

The data are presented in Table 2.

Correlations of metabolite ratios with MSSS and RIO score

Neither NAA/Cr nor Cho/Cr ratios correlated with MSSS and RIO. In contrast, both Glx/NAA and Glx/Cr ratios significantly correlated with MSSS and RIO score.

mIns/NAA ratio was higher in active vs non-active MS patients when activity was measured by RIO score, but there was no relationship with MSSS. The results are shown in Table 3.

Correlations of age, depression and fatigue scores with metabolite ratios

Age: MS patients' metabolite ratios were unrelated to age. In contrast, in CON both Glx/Cr and Glx/NAA inversely correlated with age.

Depression score: We found a relationship between depressed mood (lower SDSS) and increased Glx/Cr ratio in MS patients. We found trends between Glx/NAA ratio and depressed mood. In CON none of the metabolite ratios were associated with depression.

Fatigue score: SNFS in MS patients correlated with both Glx ratios, stronger with Glx/NAA than with Glx/Cr. In CON fatigue score was also associated with increased glutamate and glutamine concentrations but the relationship was inverse, e.g. fatigue was more pronounced when glutamate concentrations were low. The data are included in Table 4.

Discussion

Our results provide evidence of significant metabolic alterations of HYP that correlate with clinical scales of MS patients.

NAA alone is not a representative marker of axonal damage and clinical worsening

Highly significant reduction in NAA/Cr ratio in our MS patients compared to controls is in accordance with the majority of previous reports evaluating other brain

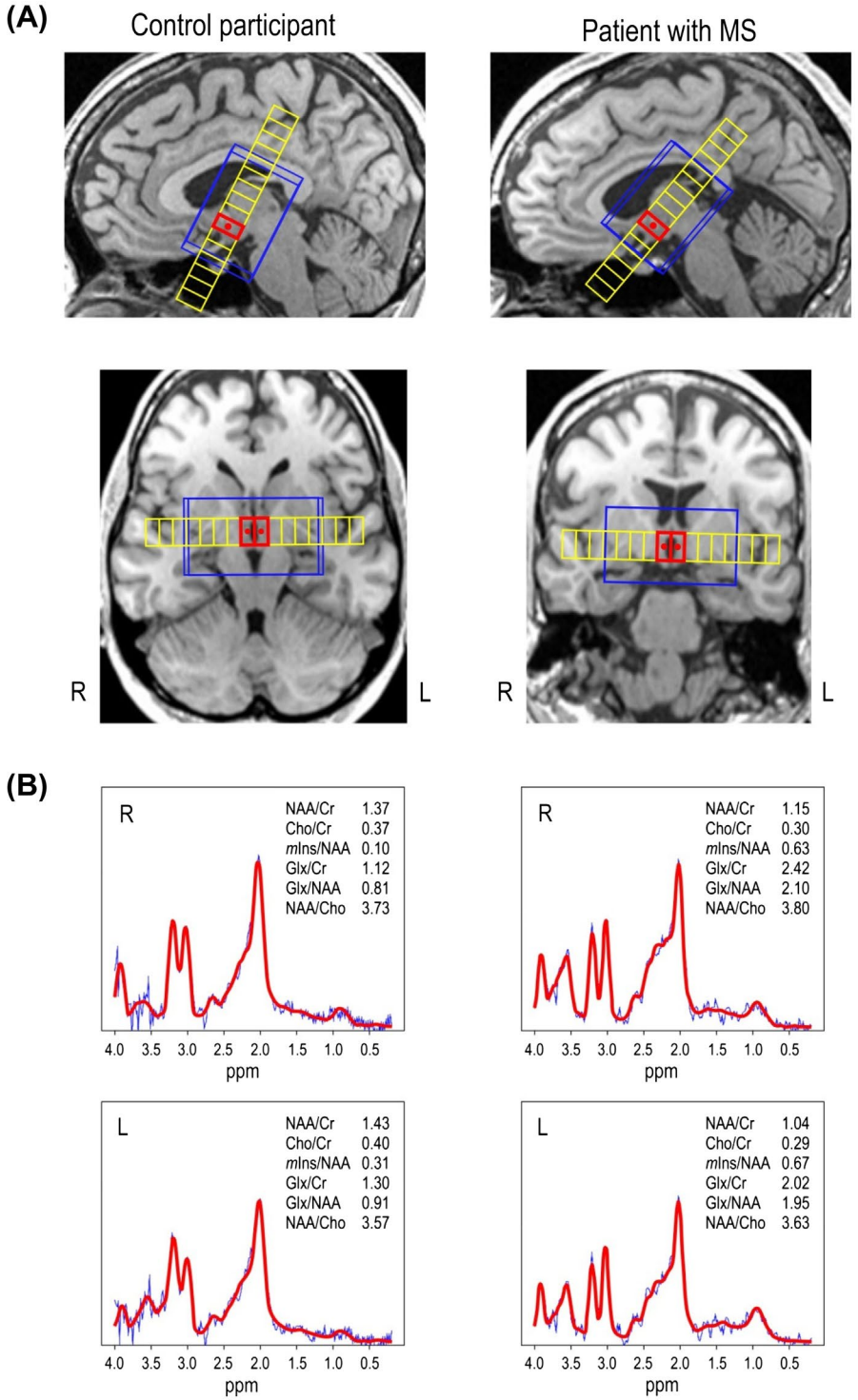

Figure 1. Morphological T_1-weighted reference images display the positioning of the targeting right (R) and left (L) hypothalamic ^{1}H-MRS voxels (red squares) in the representative patient with multiple sclerosis and the control participant (A). Acquired matrix size was $10 \times 10 \times 8$, interpolated to $16 \times 16 \times 8$ (yellow squares) with a nominal voxel size of $10 \times 10 \times 12.5$ mm^3 and volumes of interest (VOIs) $50 \times 60 \times 35$ mm^3 (blue squares). LCModel fitted spectra (red curves) with relative levels of metabolite ratios corresponding to selected voxels are shown below (B).

regions [17–21]. Although NAA is considered to be a reliable marker of axonal damage [12], the lack of correlations between hypothalamic NAA/Cr and other clinical markers in our study revealed low sensitivity of NAA itself to signalise clinically relevant damage of the whole brain. Other authors, aimed to subcortical GM assessment, also proved absence of correlation of NAA/Cr with EDSS [18,22], although association of NAA with neuroaxonal loss in GM was confirmed by histopathological evidence [19] or volumometry [20]. One of the reasons of low sensitivity of NAA/Cr to reflect disease burden can be fluctuation of NAA and Cr concentrations in deep GM (DGM), indicating variable degree of neuroaxonal disturbance in time, different in each patient [17]. Another explanation may be based on disease-related impaired cycling of NAA and glutamate (Glu) in MS [23].

Table 2. Summary of results of metabolite ratios in multiple sclerosis patients and healthy controls.

HYP	MS $n = 33$	CON $n = 24$	p Value
Cr	59.5 ± 20.3	62.3 ± 21.3	ns
Cho/Cr	0.36 ± 0.06	0.4 ± 0.05	0.04*
NAA/Cr	1.27 ± 0.24	1.5 ± 0.19	0.0004**
mIns/Cr	0.99 ± 0.31	1.09 ± 0.57	ns
mIns/NAA	0.85 ± 0.44	0.67 ± 0.29	0.059**
Glx/Cr	2.26 ± 0.8	2.23 ± 0.75	ns
Glx/NAA	1.8 ± 0.56	1.46 ± 0.42	0.014*
NAA/Cho	3.56 ± 0.69	3.88 ± 0.57	0.07**

Notes: HYP = hypothalamus, MS = patients with multiple sclerosis, CON = healthy controls, Cr = creatine concentration, Cho/Cr = cholin to creatine ratio, NAA/Cr = N-acetyl-aspartate to creatine ratio mIns/Cr = myoinositol to creatine ratio, mIns/NAA = myoinositol to N-acetyl-aspartate, Glx/Cr = glutamine and glutamate to creatine ratio, Glx/NAA = glutamine and glutamate to N-acetyl aspartate ratio, NAA/Cho = N-acetyl aspartate to choline ratio.
*t-test.; **Mann–Whitney test, statistical significance $p = 0.05$, ns = non-significant.

Table 3. Correlations of metabolites with MSSS and RIO score in multiple sclerosis patients.

Metabolites ratio	MSSS	RIO = 0 vs. RIO = 1–2
Cho/Cr	ns	$p = 0.2$
NAA/Cr	ns	$p = 0.4$
mIns/Cr	ns	$p = 0.3$
mIns/NAA	ns	$*p = 0.036$
Glx/Cr	$r = 0.4$, $p = 0.02$	$*p = 0.005$
Glx/NAA	$r = 0.56$, $p = 0.001$	$*p = 0.00018$

Notes: MSSS = Multiple Sclerosis Severity Scale, RIO = disease activity score, Cho/Cr = cholin to creatine ratio, NAA/Cr = N-acetyl-aspartate to creatine ratio, mIns/Cr = myoinositol to creatine ratio, mIns/NAA = myoinositol to N-acetyl-aspartate, Glx/Cr = glutamine and glutamate to creatine ratio, Glx/NAA = glutamine and glutamate to N-acetyl aspartate ratio, ns = non significant, Spearman correlation (with no mark).
*Mann–Whitney test, statistical significance $p = 0.05$.

Table 4. Relationships of Simple Depressive-Severity Status Scale and Simple Numerical Fatigue Scale with metabolite ratios – patients with multiple sclerosis vs. healthy controls.

	MS patients		CON	
Age	Cho/Cr	ns	Cho/Cr	ns
	NAA/Cr	ns	NAA/Cr	ns
	mIns/Cr	ns	mIns/Cr	ns
	mIns/NAA	ns	mIns/NAA	ns
	Glx/Cr	ns	Glx/Cr	$r = -0.42$, $p = 0.048$
	Glx/NAA	ns	Glx/NAA	$r = -0.41$, $p = 0.056$
SDSS	Cho/Cr	ns	Cho/Cr	ns
	NAA/Cr	ns	NAA/Cr	ns
	mIns/Cr	ns	mIns/Cr	ns
	mIns/NAA	ns	mIns/NAA	ns
	Glx/Cr	-0.33, $p = 0.028$	Glx/Cr	ns
	Glx/NAA	-0.33, $p = 0.08$	Glx/NAA	ns
SNFS	Cho/Cr	ns	Cho/Cr	ns
	NAA/Cr	ns	NAA/Cr	ns
	mIns/Cr	ns	mIns/Cr	ns
	mIns/NAA	ns	mIns/NAA	ns
	Glx/Cr	$r = 0.33$, $p = 0.054$	Glx/Cr	$r = -0.3$, $p = 0.12$
	Glx/NAA	$r = 0.39$, $p = 0.02$	Glx/NAA	$r = -0.4$, $p = 0.04$

Notes: MS patients = patients with multiple sclerosis, CON = healthy controls, SDSS = the Simple Depressive-Severity Status Scale, SNFS = The Simple Numerical Fatigue Scale, Cho/Cr = cholin to creatine ratio, NAA/Cr = N-acetyl-aspartate to creatine ratio, mIns/Cr = myoinositol to creatine ratio, mIns/NAA = myoinositol to N-acetyl-aspartate, Glx/Cr = glutamine and glutamate to creatine ratio, Glx/NAA = glutamine and glutamate to N-acetyl aspartate ratio, ns = non significant, Spearman correlation, statistical significance $p = 0.05$.

Glutamate correlates with activity and severity of multiple sclerosis

Considering the hypothalamic region, we found Glx/NAA ratio higher in MS patients than in CON. Firstly, Glx/Cr and Glx/NAA ratios were significantly increased in active vs non-active MS subgroups. Our results support the role of Glu in MS-related hypothalamic damage, pinpointing metabolic dysfunction as a crucial problem. Notably, increased Glu ratios in HYP were able to reflect whole-brain activity of MS, suggesting that this part of DGM plays a dominant role in disease evolution. Our results are supported by other recent histopathological [2–4] and neuroendocrinal studies [5].

Secondly, both Glx/Cr and Glx/NAA ratios of MS patients correlated with disease severity, measured by MSSS. The combined presence of increased Glu and reduced NAA sensitively indicating clinical worsening in our MS patients, may be explained by primary neuroaxonal loss that precedes inflammatory processes [24].

Our results, showing superiority of Glx/NAA ratio to reflect linked pathological processes of GM, are in accordance with other reports [11,25].

Although Glx was proved in normal appearing white matter (NAWM) [26] or in active white matter (WM) lesions [22], other authors, focusing on thalami and hippocampi, did not prove superiority of Glu over other metabolites in reflecting disease burden [18].

In one prospective longitudinal study, a combination of increased Glu and reduced NAA in NAWM was found to be predictive of brain tissue loss and clinical worsening of MS [25]. In another study, authors reported a link between concentrations of Glu and memory loss in MS patients [11]. However, both NAA and Glu were reduced in MS patients when compared to healthy individuals [11]. Other authors found a relationship of Glx with MSSS when testing WM [22,26]. So far, we have not found a study confirming our outcomes.

Interestingly, in our MS patients none of the metabolites was linked to age. In contrast, in CON we found age-related decline in Glu, that has already been reported [27,28].

Increased myoinositol reflects disease activity but not disease severity

In our study, mIns/NAA ratio was capable to separate MS patients from CON. Increased mIns could result from overactivity of glial cells that may have replaced damaged neurons [29] as well as from damage to brain cell membranes [29,30].

In accordance with our results, Geurts found increased mIns in thalami and hippocampi of MS patients [18].

In our study mIns was recognised as another marker of DA. We found mIns increased in patients with active MS when compared with non-active patients. Although other studies also found the parallel reduction in NAA and increase in mIns concentrations to reflect high DA, neuroaxonal damage and subsequent gliosis [27],

in contrast with our findings, they were aimed at WM, not GM.

Other research of WM showed elevation of mIns in WM positively correlating with disability [31], measured by the Multiple Sclerosis Functional Composite Scale (MSFC) [32], and EDSS [30].

Recent research of GM produced results partially comparable to our outcomes. They did not find a correlation of mIns in thalamus and hippocampus with disability, measured by EDSS and MSFC [18]. They studied lesion load in WM, showing activity of the disease, as our RIO score, and they also found its relationship with mIns/NAA in DGM [18].

Reduction in choline in the hypothalamus of multiple sclerosis patients

One of the unexpected findings in our study was a lower Cho/Cr ratio in MS patients than in controls. Cho is a compound of cell membrane, and increase in Cho is found in active demyelinated or re-myelinated WM [31]. One possible explanation of decreased hypothalamic Cho is that the pathogenesis of GM damage differs from that of WM. Extensive immune cell influx due to blood–brain barrier leakage is far less prominent in GM [33], where non-immune-related neurotoxicity is expected [33].

Increase in glutamate correlates with depression score in multiple sclerosis

Results of SDSS showed significant differences between MS patients and CON, while depressive mood was more prominent in patients with MS. Moreover, SDSS correlated with Glu/Cr ratio, but it was not the case of CON. These findings suggest that glutamate concentrations in HYP reflect processes that are linked with depressive mood in MS; these processes are unlikely to occur in the general population given that these correlations were not found in healthy controls.

Glu is involved in cortico-limbic, cortico-striatial and cortico-cortical pathways [27,34], where HYP is one of the control structures that mediate emotional expression [34,35]. A neural model, in which dysfunction within the circuits connecting limbic structures can account for the disturbances of emotional behaviour [27,35], can be applicable also in our MS patients. To our knowledge, there is no 1H-MRS-based research of MS-related depression. Our study is the first to report Glu increases in HYP correlating with depressive mood in MS.

Increased glutamate ratios in hypothalamus are linked with fatigue score

The differences in SNFS between MS patients and CON were considerable. Moreover, fatigue in our MS patients correlated with increased glutamate ratios, more in Glx/NAA than in Glx/Cr. The results indicate the combined effect of increased Glu and reduced NAA in HYP on fatigue evolution. Fatigue is known to be a hallmark of MS, however, its pathomechanism has not yet been properly described. Zellini et al. revealed an association of fatigue with structural abnormalities of HYP, measured by T1 relaxation time [6]. Other authors considered fatigue a multifactorial problem, which may overlap with depression or cognitive dysfunction [34]. In this context, is worth to note a connection between abnormalities of Glu and memory performance in MS [11]. Based on our results, we suggest that fatigue signalises active dysfunction of HYP and its connections with brain cortex [28], which is linked to excessive Glu in affected regions.

In CON, inverse relationship of fatigue with Glu ratios can be explained by age-related decline in glutamate in healthy individuals [28], which is usually associated with reduction in dopamin [27].

Conclusion

Our study provides the first evidence about significant hypothalamic alterations correlating with clinical outcomes of MS, using 1H-MRS. Considering our results, the combination of increased Glu or mIns with reduced NAA in HYP reflects whole-brain activity of MS. In addition, excess of Glu is linked to severe disease course, depressive mood and fatigue in MS patients, suggesting superiority of Glu over other metabolites in determining MS burden. The revealed link between Glu abnormalities and clinical scales may lead to new treatment approaches.

Contributors

Contributors – EKa, principal and corresponding author, conceived and supervised the study, drafted the manuscript, was involved in data analysis and interpretation and gave final approval for submission. HP was principally responsible for data analysis, helped revise the manuscript and gave final approval for submission. MB, EB, PH and DC helped revise the manuscript, were principally responsible for data collection, involved in data analysis. ŠS, VN and KZ helped revise the manuscript, assisted with data collection, were involved in data analysis. EKu helped revise the manuscript, was involved in accessing the validated tools for the study and gave final approval for submission.

Ethics approval

This study was approved by local Ethical committee of the Jessenius Faculty of Medicine in Martin, Comenius University (EK 1678/2015).

Disclosure statement

EKa, HP, MB, EB, PH, DC, ŠS, VN, EKu have nothing to disclose.

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