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ORIGINAL ARTICLE

Does B12 deficiency lead to change in brain metabolites in pediatric population? A MR spectroscopy study

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Abstract

Objectives The aim of this study is to examine metabolite changes in different brain regions of the children with vitamin B12 deficiency disease using MR spectroscopy.

Methods Eighteen children with serum vit. B12 deficiency and 12 healthy volunteer children were included in the study. All children were examined with single-voxel spectroscopy examination via 1.5-Tesla MRI. The spectra were obtained from the left frontal periventricular white matter, left lentiform nucleus and left cerebellar hemisphere. The comparisons between patient group and control group were made with ratios calculated as NAA/Cr, Cho/Cr, mI/Cr, and Glx/Cr. All brain images were also examined in terms of brain atrophy, abnormal brain parenchyma intensity changes, or myelination status.

Results The children were between 3 months and 16 years old in the patient group, and between 3 months and 15 years old in the control group. There were no statistical differences in terms of metabolite ratios in the three different brain regions between the patients and control group. In two patients, periventricular white matter hyperintensities were observed. In four patients, brain atrophy was detected.

Discussion MR spectroscopy examination demonstrated that there were no statistical differences in terms of all metabolite ratios in left frontal periventricular white matter, left lentiform nucleus and left cerebellar hemisphere.

Keywords Vitamin B12 · MR spectroscopy · Brain metabolites · Pediatric population · Brain MRI

Introduction

Vitamin B12 (vit. B12) deficiency is the main cause of megaloblastic anemia in childhood. In developing countries, B12 deficiency usually occurs due to a defect in dietary intake, and it is important to diagnose and to treat this pathology early in childhood. Vit. B12 is a crucial factor for development of the central and peripheral nervous system in childhood. Despite the low cost of treatment, delayed treatment may cause serious and sometimes irreversible neurological problems [1, 2]. Lethargy, hypotonia, seizures, mental and psychomotor retardation and behavior disturbance are among the neurologic

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symptoms that can be seen in infancy and childhood [3]. It is well known that vit. B12 deficiency leads to demyelination in the brain and spinal cord which is usually reversible with appropriate treatment [4].

Some brain MRI findings that can be seen in vit. B12 have been described in the literature. White matter hyperintensities, basal ganglia lesions, and cerebellar hyperintensities were reported in adults and children [5–7].

Although there are many studies on this issue, the pathologic mechanisms of vit. B12 deficiency in the central nervous system are not fully understood yet [8, 9]. The objective of this study is to investigate the brain metabolite differences in children with vit. B12 deficiency by magnetic resonance spectroscopy (MRS) examination.

Methods

Patients and controls

This prospective case-controlled study was conducted between January 2015 and August 2017. Eighteen patients (14 male, 4 female) with vit. B12 deficiency and 14 healthy volunteer children (11 male, 3 female) were included in the study. The most common complaints of patients were weakness, hypotonia, and cognitive impairments such as memory weakness and concentration difficulty. The diagnosis of vit. B12 deficiency was made with low levels of serum B12 (< 200 pg/ml). Folate levels were normal in all children (> 5.38 ng/ml). All patients were evaluated with complete blood count. Anemia was present in all patients with hemoglobin levels under 12 g/dl and normocytosis (MCV 80–95 fL) or macrocytosis (MCV > 95 fL). Patients with iron deficiency anemia and/or with microcytosis were not included in the study. Patients who had undergone any operation or patients with metabolic, congenital or any chronic disease were also not included in the study. There was no pathology in the biochemical laboratory tests of patients. The control group consisted of age-matched healthy children. They were also examined with complete blood count and serum B12 and folate levels, all of which were in normal limits. The study was approved by the local ethics committee (no 74059997.050.01.04/99) and a written informed consent was obtained from parents of all children. MRI and MRS studies were performed before being treated after the diagnosis.

MRI and MR spectroscopy and analysis

Patients and healthy children were imaged with a 1.5-Tesla MRI scanner (Magnetom Symphony; Siemens, Erlangen, Germany) using a standard head coil. All children under 6 years old were left sleepless during the night before the examination. One parent stayed with these children in the scanner during the examination. Sedation was not necessary for any of the patients or control group. Imaging parameters were as follows: T2-weighted images—repetition time (TR) 3300 ms, echo time (TE) 88 ms; fluid-attenuated inversion recovery (FLAIR) sequence—TR 7000 ms, TI 2214 ms, TE 93 ms; T1-weighted images—TR 530 ms, TE 8.70 ms. The slice thickness was 4 mm, the matrix was 256 × 256 pixels, and the field of view was 200 × 230. All MR images were examined for parenchymal pathology and myelination status according to the steps determined by Barkovich [10].

After brain MRI imaging, single-voxel MR spectroscopy examination was performed from three distinct brain parenchyma regions, left periventricular frontal white matter region, left lentiform nucleus, and left cerebellar region, using sagittal plane T1-weighted images, axial plane T2-weighted images, and coronal plane FLAIR images to guide volume selection (Fig. 1a–c). All the children were right-handed and we selected the dominant hemisphere for sampling. The three brain regions selected for MRS were brain parenchyma which had been shown to be affected by vit. B12 deficiency in various studies [4, 6, 7]. The spectra were obtained with point-resolved spectroscopy (PRESS) technique. The spectral parameters were TR, 1500 ms; echo time, 30 ms; flip angle (FA), 90°–180°–180°. Signal average was 256 and acquisition time was approximately 6:24. The sizes of the voxels were 20 × 20 × 20 mm for each single-voxel spectroscopy examination. After water signal suppression with chemical shift-selective techniques, spectroscopic data were obtained. Total examination time was 25 min per child.

The data were analyzed with a workstation (Leonardo, Siemens, Forcheim, Germany) which was equipped with manufacturer-supplied software package. The data were Fourier transformed and baseline fitted. Metabolite signals included NAA (2.04 ppm), Cho (3.24 ppm), Cr (3.05 ppm), Glx (glutamate+glutamine) (3.80 ppm), mI (3.58 ppm), and lactate (1.33 ppm). We used only short TE to shorten the examination time because all of our patients and healthy volunteers were children and it was hard to lie immobile in a MRI scanner for more than half an hour. To determine the quality, the metabolite peaks were assessed for full width at half maximum peak height which was < 0.1 ppm for all [11]. Four MRS examinations were repeated because of insufficient quality. All metabolite ratios to Cr (Cho/Cr, NAA/Cr, Glx/Cr, and mI/Cr) were calculated. We have used Cr as the inner standard proven by the arbitrary units in Table 1. After MRI and MRS examinations, all patients were treated with parenteral cyanocobalamin therapy 100 mcg daily for the first week, 100 mcg one in 2 days for 2 weeks, and one a week for 1 week and one a month for 3 months. We could obtain control spectra only from 2 patients 3 months after the treatment. Before the control MRS, serum B12 levels were tested which were higher than 200 pg/ml for these 2 patients.

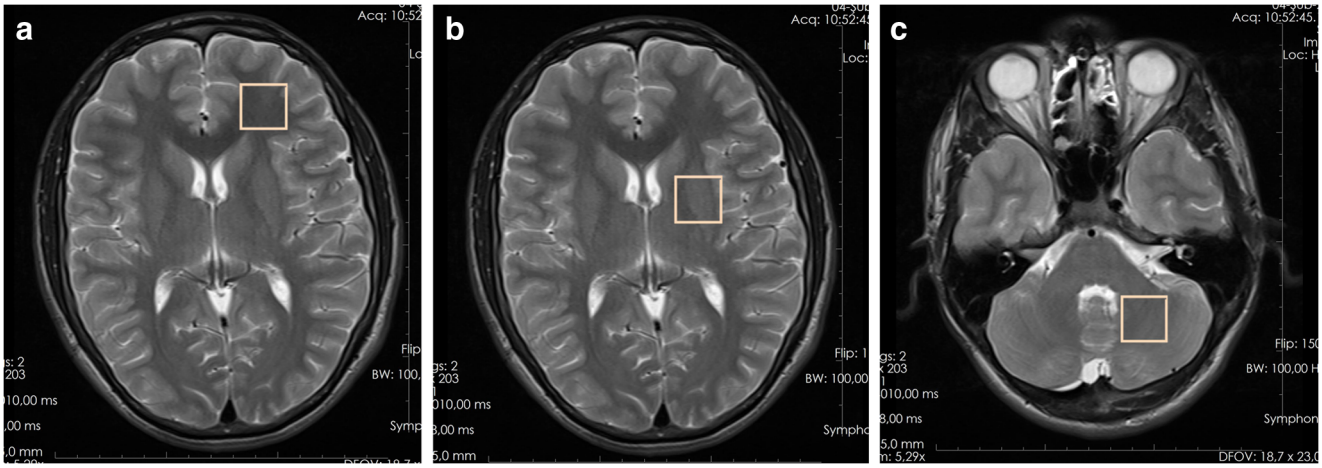

Fig. 1 Single-voxel MR spectroscopy examination was performed from three distinct brain parenchyma regions: left frontal subcortical region (**a**), left lentiform nucleus (**b**), and left cerebellar dentate nucleus (**c**)

Statistical analysis

For statistical data analysis, SPSS for Windows version 20.0 software package (SPSS Inc., Chicago, USA) was used. Metabolite ratios were used as quantitative data and they were expressed as mean ± standard deviation (SD). Quantitative data were normally distributed due to One-Sample Kolmogorov-Smirnov test results. Student *t* test was used for analyzing data. Comparison of the data was performed using Spearman's correlation. Pearson correlation test was used for analyzing correlation between B12 levels and metabolite ratios. Bonferroni correction is also done due to the multiple testing. A value of $p < 0.05$ was considered statistically significant.

Results

The mean age of the patients was 7.05 ± 6.41 (3 months–16 years) and the mean age of the control group was 6.92 ± 6.18 (3 months–15 years). There was no statistically significant difference in age between the patients and control group ($p > 0.05$). In the patient group, mean serum vit. B12 level was 83.44 ± 46.967 pg/ml with a minimum level of 27 pg/ml and a maximum level of 189 pg/ml. There was no delay in myelination on the patients' MR images. In two patients, there were periventricular white matter hyperintensities on FLAIR sequence (Fig. 2). These hyperintensities were several focal foci with dimensions less than 5 mm. Brain atrophy was present in four patients (Fig. 3). In the remaining 11 patients, the brain parenchyma was normal. Brain MR images of the control group were also normal.

There were no significant differences in terms of NAA/Cr, Cho/Cr, Glx/Cr, and mI/Cr in the left periventricular frontal white matter region, left lentiform nucleus, and left cerebellar dentate nucleus ($p > 0.05$ for all) (Table 1). There was no correlation between patients' serum vit. B12 levels and metabolite ratios ($p > 0.05$ for all). Lactate doublet was not seen in any of the spectra. After treatment, we could get control MRS examination results only from 2 patients. We determined elevated NAA/Cr and mI/Cr ratios in three brain regions of these 2 patients (Table 2). The Cho/Cr ratios were raised in frontal periventricular white matter and lentiform nucleus after treatment. Reduced ratios of Glx/Cr were also determined in all three regions after treatment.

Discussion

Vitamin B12 is a crucial factor for the development of hematopoietic and nervous systems. Vitamin B12 deficiency causes impaired DNA methylation in the hematopoietic system and demyelination in the central nervous system. However, the effective pathophysiological mechanisms of vitamin B12 deficiency cannot be fully described. To our knowledge, this is the first prospective study which examines metabolite abnormalities in vit. B12–deficient children with MRS. Ekici et al. retrospectively studied the results of MRS of 14 children with B12 deficiency performed with long TE (TE = 144) and found the mean values of NAA/Cr and Cho/Cr in basal ganglia as 1.31 ± 0.17 and 1.04 ± 0.27, respectively. They could not compare their results with the control group because their study was retrospective [9]. In our study, the mean values of lentiform nucleus in children with B12 deficiency were 1.64 ± 0.46 as NAA/Cr and 0.72 ± 0.11 as Cho/Cr. The most noticeable difference between the two studies is that children were diagnosed with developmental delay in Ekici et al.'s study. None of the children had developmental delay in our study. The difference between these values may also be related to the age group of children taken into the studies. Ekici et al. have evaluated infants in their study; we have included older children. Difference in TE values used in MRS examinations

Table 1 Mean metabolite ratios in the three different brain regions in the patient group and control group

		Patient group (mean ± SD)	Control group (mean ± SD)	*p*
NAA/Cr	Frontal	2.05 ± 0.62	2.44 ± 0.29	> 0.05
	Lentiform n.	1.64 ± 0.46	1.63 ± 0.21	> 0.05
	Cerebellum	2.07 ± 0.88	2.14 ± 0.23	> 0.05
Cho/Cr	Frontal	0.99 ± 0.22	0.99 ± 0.17	> 0.05
	Lentiform n.	0.72 ± 0.11	0.75 ± 0.13	> 0.05
	Cerebellum	0.78 ± 0.47	0.63 ± 0.08	> 0.05
mI/Cr	Frontal	0.31 ± 0.10	0.39 ± 0.13	> 0.05
	Lentiform n.	0.24 ± 0.09	0.26 ± 0.05	> 0.05
	Cerebellum	0.22 ± 0.07	0.21 ± 0.05	> 0.05
Glx/Cr	Frontal	0.16 ± 0.07	0.14 ± 0.09	> 0.05
	Lentiform n.	0.17 ± 0.06	0.15 ± 0.05	> 0.05
	Cerebellum	0.07 ± 0.03	0.05 ± 0.01	> 0.05
Cr (integral)	Frontal	225.11 ± 41.28	209.49 ± 27.28	> 0.05
	Lentiform n.	262.31 ± 28.82	269.91 ± 32.85	> 0.05
	Cerebellum	260.26 ± 50.12	274.47 ± 9.46	> 0.05

$p < 0.05$ was considered statistically significant

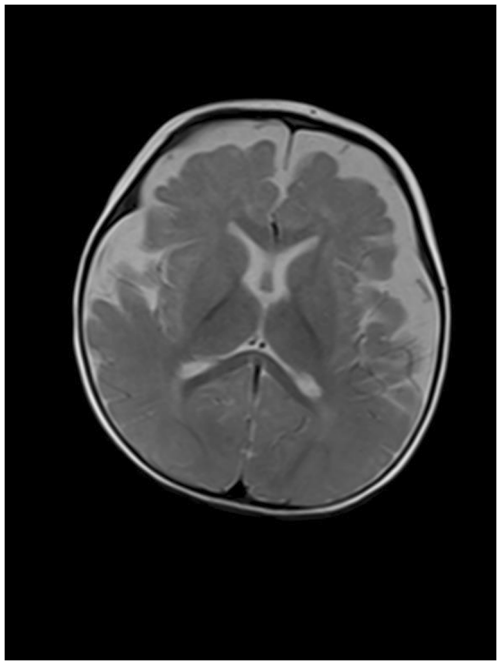

Fig. 3 A 9-month-old boy with vit. B12 deficiency. In the axial T2-weighted image, brain atrophy can be seen

of these two studies may be another reason. Studies have shown that NAA/Cr ratio increases and Cho/Cr ratio decreases in normal brain maturation process [12]. After treatment, they examined one patient's results of control MRS and found increase in NAA/Cr and Cho/Cr values. In our study, we could obtain two patients' control MRS data after treatment and we found elevated NAA/Cr ratios and Cho/Cr ratios in three brain regions in concordance with Ekici et al. Increase in the NAA/Cr and Cho/Cr ratios may be due to the stimulated neural growth and increased myelination promoted by B12 therapy. We did not find a statistical difference in terms of NAA/Cr, Cho/Cr, Glx/Cr, and mI/Cr ratios in the left frontal periventricular white matter, left lentiform nucleus, and left cerebellar dentate nucleus between the patients and control group. Ford et al. investigated the effect of 6-month high-dose B-group vitamin supplementation on neural metabolites (NAA, Cho, Cr, mI, and Glx) and found no significant differences in the posterior cingulate cortex, between the vitamin-supplemented group and placebo group, in concordance with our study [13]. In a comprehensive study examining brain metabolites (S-adenosylmethionine, S-adenosylhomocysteine, methionine, cystathionine, choline, and betaine) in various brain regions in rats fed a B12-poor diet, no difference was detected in betaine and choline values between the rats fed B12-poor diet and fed normal diet [14]. Although we did not find a significant relationship between B12 deficiency and the metabolites we examined, some metabolite changes in the two patients' MRS results before and after treatment might be presumed as interesting. One of these interesting points was elevated mI/Cr ratios in the three brain regions after treatment. There were studies showing increased mI levels in various brain regions in subacute and chronic periods of neurotrauma and improved depression [15, 16]. In these studies, it is hypothesized that glial activation had increased during recovery. Our study supports this hypothesis. The other interesting point was the decrease in the ratio of Glx/Cr in these three brain regions. The Glx term is expressed both glutamine and glutamate metabolites. Glutamate is the metabolite of

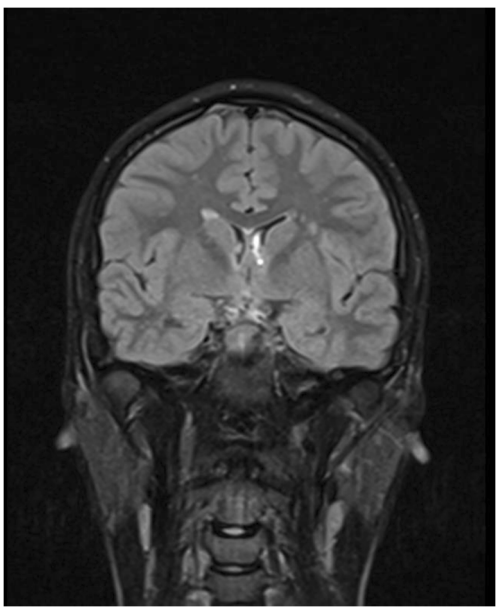

Fig. 2 A 15-year-old girl with vit. B12 deficiency. Bilateral parietal periventricular white matter hyperintensities can be seen in the coronal FLAIR image

Table 2 Metabolite ratios in the three different brain regions of two patients before and after treatment

		Patient 1		Patient 2	
		Before therapy	After therapy	Before therapy	After therapy
NAA/Cr	Frontal	1.37	2.23	1.37	1.57
	Lentiform n.	1.56	1.59	1.03	1.61
	Cerebellum	1.96	2.24	1.81	1.93
Cho/Cr	Frontal	0.75	0.87	0.75	1.19
	Lentiform n.	0.70	0.72	0.62	0.78
	Cerebellum	0.67	0.69	0.77	0.77
mI/Cr	Frontal	0.24	0.41	0.24	0.37
	Lentiform n.	0.23	0.25	0.10	0.19
	Cerebellum	0.26	0.33	0.28	0.38
Glx/Cr	Frontal	0.18	0.13	0.18	0.14
	Lentiform n.	0.15	0.14	0.15	0.13
	Cerebellum	0.06	0.04	0.09	0.07

glutamatergic system in the brain and spinal cord which is associated with excitation [17]. It is thought that, in some diseases such as ischemia, Alzheimer, or amyotrophic lateral sclerosis, glutamate-induced toxicity is an effective mechanism in the pathophysiology [18, 19]. It is shown that vit. B12 protects rat cerebellar granule cells from glutamate toxicity [20]. In another report, the ability of cyanocobalamin to inhibit glutamate release from nerve terminals was demonstrated in rats [21]. Further studies with a larger sample are needed to determine the complex mechanisms of vit. B12 and probable interactions between vit. B12 and neural metabolites in humans.

In a study that evaluated 15 infants' cranial MRI findings with B12 deficiency, MRI findings were defined as thinning of the corpus callosum (40%), cortical atrophy (33.3%), large Sylvian fissure (33.3%), ventricular dilatation (20%), asymmetric large lateral ventricle (13.3%), and delay in myelination (13.3%) [8]. Brain atrophy was present in 22% and periventricular white matter hyperintensities were present in 11% of our patients. Although the mean serum vitamin B12 level was lower in our study, ratio of atrophy and variety of findings were also low. This is probably because of the fact that we examined infants and children in the older age group. The neurological damage due to vit. B12 deficiency presumably might have revealed more MRI findings in infancy, in which period development, myelination, and maturation are very rapid. In a study by Gupta et al., brain networks related to cognition control were found to be lower in all cerebrums using resting state functional MRI in 13 adult patients with B12 deficits and cognition control studies to evaluate brain networks [22]. The lower part of these networks was the prefrontal cortex. Roy et al. found a decrease in all fractional anisotropy (FA) values measured by diffusion tensor imaging in patients with vit. B12 deficiency [23]. This data points to the structural damage in brain parenchyma. After appropriate treatment, they reported recovered FA values as we found elevated NAA/Cr ratios in the three different brain regions of two patients.

There are some limitations of our study. Firstly, the number of patient group and control group is small. After therapy, we could obtain only two patients' control MRS results and we could not compare these results statistically and adequately. Secondly, we could only get Glx levels that mean glutamate + glutamine together, but not the level of the metabolites separately. Third, in the lentiform nucleus, the voxel size was bigger than the anatomical region so partial volume effect may have affected the results in the lentiform nucleus.

In conclusion, MRS examination demonstrated no significant difference in terms of NAA/Cr, Cho/Cr, mI/Cr, and Glx/Cr ratios in the left frontal periventricular white matter, left lentiform nucleus, and left cerebellar hemisphere in vit. B12–deficient children.

References

1. Brocadello F, Levedianos G, Piccione F, Manara R, Pesenti FF (2007) Irreversible subacute sclerotic combined degeneration of the spinal cord in a vegan subject. Nutrition 23:622–624
2. Honzik T, Adamovicova M, Smolka V, Magner M, Hruba E, Zeman J (2010) Clinical presentation and metabolic consequences in 40 breastfed infants with nutritional vitamin B12 deficiency-what have we learned? Eur J Paediatr Neurol 14:488–495
3. Reynolds E (2006) Vitamin B12, folic acid, and the nervous system. Lancet Neurol 5(11):949–960
4. Lövblad K, Ramelli G, Remonda L, Nirkko AC, Ozdoba C, Schroth G (1997) Retardation of myelination due to dietary vitamin B12 deficiency: cranial MRI findings. Pediatr Radiol 27(2):155–158
5. De van der Schueren MA, Lonterman-Monasch S, van der Flier WM, Kramer MH, Maier AB, Muller M (2016) Malnutrition and

risk of structural brain changes seen on magnetic resonance imaging in older adults. J Am Geriatr Soc 64(12):2457–2463
6. Kitamura T, Gotoh S, Takaki H, Kiyuna F, Yoshimura S, Fujii K (2016) A case of vitamin B12 deficiency with involuntary movements and bilateral basal ganglia lesions. Rinsho Shinkeigaku 56(7):499–503
7. Chakrabarty B, Dubey R, Gulati S, Yoganathan S, Kumar A, Kumar A (2014) Isolated cerebellar involvement in vitamin B12 deficiency: a case report. J Child Neurol 29(11):NP161–NP163
8. Taskesen M, Yaramis A, Pirinccioglu AG, Ekici F (2012) Cranial magnetic resonance imaging findings of nutritional vitamin B12 deficiency in 15 hypotonic infants. Eur J Paediatr Neurol 16(3): 266–270
9. Ekici F, Tekbas G, Hattapoğlu S, Yaramış A, Önder H, Bilici A (2016) Brain MRI and MR spectroscopy findings in children with nutritional vitamin B12 deficiency. Clin Neuroradiol 26(2):215–220
10. Barkovich AJ (2005) Normal development of the neonatal and infant brain, skull and spine. In: Barkovich AJ (ed) Pediatric neuroimaging, 4th edn. Lippincott Williams and Wilkins, Philadelphia, pp 17–75
11. Kreis R (2004) Issues of spectral quality in clinical H1-magnetic resonance spectroscopy and a gallery of artifacts. NMR Biomed 17: 361–381
12. Ghosh N, Holshouser B, Oyoyo U, Barnes S, Tong K, Ashwal S (2017) Combined diffusion tensor and magnetic resonance spectroscopic imaging methodology for automated regional brain analysis: application in a normal pediatric population. Dev Neurosci 39(5): 413–429
13. Ford TC, Downey LA, Simpson T, McPhee G, Oliver C, Stough C (2018) The effect of a high-dose vitamin B multivitamin supplement on the relationship between brain metabolism and blood biomarkers of oxidative stress: a randomized control trial. Nutrients 10(12):1860
14. Arora K, Sequeira JM, Alarcon JM, Wasek B, Arning E, Bottiglieri T, Quadros EV (2018) Neuropathology of vitamin B(12) deficiency in the Cd320(−/−) mouse. FASEB J 33:2563–2573. https://doi.org/10.1096/fj.201800754RR
15. Taylor MJ, Selvaraj S, Norbury R, Jezzard P, Cowen PJ (2009) Normal glutamate but elevated myo-inositol in anterior cingulate cortex in recovered depressed patients. J Affect Disord 119(1–3): 186–189
16. Kierans AS, Kirov II, Gonen O, Haemer G, Nisenbaum E, Babb JS, Grossman RI, Lui YW (2014) Myoinositol and glutamate complex neurometabolite abnormality after mild traumatic brain injury. Neurology. 82(6):521–528
17. Curtis DR, Watkins JC (1960) The excitation and depression of spinal neurones by structurally related amino acids. J Neurochem 6:117–141
18. Meldrum BS (2000) Glutamate as a neurotransmitter in the brain: review of physiology and pathology. J Nutr 130(4S Suppl):1007–1015
19. Blasco H, Mavel S, Corcia P, Gordon PH (2014) The glutamate hypothesis in ALS: pathophysiology and drug development. Curr Med Chem 21(31):3551–3575
20. Lin Y, Desbois A, Jiang S, Hou ST (2004) Group B vitamins protect murine cerebellar granule cells from glutamate/NMDA toxicity. Neuroreport. 15(14):2241–2244
21. Hung KL, Wang CC, Huang CY, Wang SJ (2009) Cyanocobalamin, vitamin B12, depresses glutamate release through inhibition of voltage-dependent Ca2+ influx in rat cerebrocortical nerve terminals (synaptosomes). Eur J Pharmacol 602(2–3):230–237
22. Gupta L, Gupta RK, Gupta PK, Malhotra HS, Saha I, Garg RK (2016) Assessment of brain cognitive functions in patients with vitamin B12 deficiency using resting state functional MRI: a longitudinal study. Magn Reson Imaging 34(2):191–196
23. Roy B, Trivedi R, Garg RK, Gupta PK, Tyagi R, Gupta RK (2015) Assessment of functional and structural damage in brain parenchyma in patients with vitamin B12 deficiency: a longitudinal perfusion and diffusion tensor imaging study. Magn Reson Imaging 33(5):537–543