

## Sex differences in brain metabolite concentrations in healthy children – proton magnetic resonance spectroscopy study (<sup>1</sup>HMRS)

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### Abstract

**Purpose:** The aim of this <sup>1</sup>HMRS study was to define sex-related differences in metabolic spectrum between healthy children. Forty-nine girls and boys aged 6-15 years were examined.

**Material and methods:** Volume of interest was located in seven brain regions: frontal lobes, basal ganglia, hippocampi, and cerebellum.

**Results:** Statistical analysis of the results showed significantly higher ( $p < 0.05$ ) myo-inositol concentrations relative to the total concentrations in the boys than the girls, as well as higher absolute *N*-acetyl aspartate concentrations in the left frontal lobes in girls. No other significant differences were shown, except for trends in differences.

**Conclusions:** In clinical practice the diagnostic process first of all focuses on assessing concentrations of metabolites to relative cerebellum concentration. Thus, the findings of the present study allow the conclusion that when analysing the results of <sup>1</sup>HMRS studies in children it is not necessary to take into account the child's gender.

**Key words:** proton magnetic resonance spectroscopy (<sup>1</sup>HMRS), brain, children, sex differences.

### Introduction

Proton magnetic resonance spectroscopy (<sup>1</sup>HMRS) is a specialised method to obtain information about the composition, concentration, and spatial distribution of specific biochemical components produced during metabolic processes in brain tissues [1-6].

The <sup>1</sup>HMRS technique has been used primarily in diagnostic assessments of the brain [7-11]. To benefit from this method, we must have comprehensive data enabling determination of a reference spectrum appropriate for the subject's age. Some researchers also point to possible sex differences. Previous research into brain differences between the sexes was mainly limited to descriptions of such differences in the brain structures [12,13] assessed using morphometric measurements [14,15], and most importantly the differences between the volumes of male and female brains, based on magnetic resonance images. Many

papers have demonstrated significant differences in this regard [14,16]. When it comes to the biochemical composition of the brain, opinions are divided. Some research teams have found no sex differences [2,14] in metabolite concentrations in populations of healthy subjects, while other researchers have shown that such differences do indeed exist [17]. These studies, however, focused only on adults. The present study was designed to identify, with the use of <sup>1</sup>HMRS, and then to analyse, the possibly existing sex related differences in the metabolic profiles of the brain, in a group of children aged 6-15 years.

### Material and methods

The study involved 49 children aged 6-15 (mean age 11.6) years, including 21 girls aged 7-15 (mean age 12.1) years and 28 boys aged 6-15 (mean age 11.3) years. There was no significant difference in age between the girls and

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#### Authors' contribution:

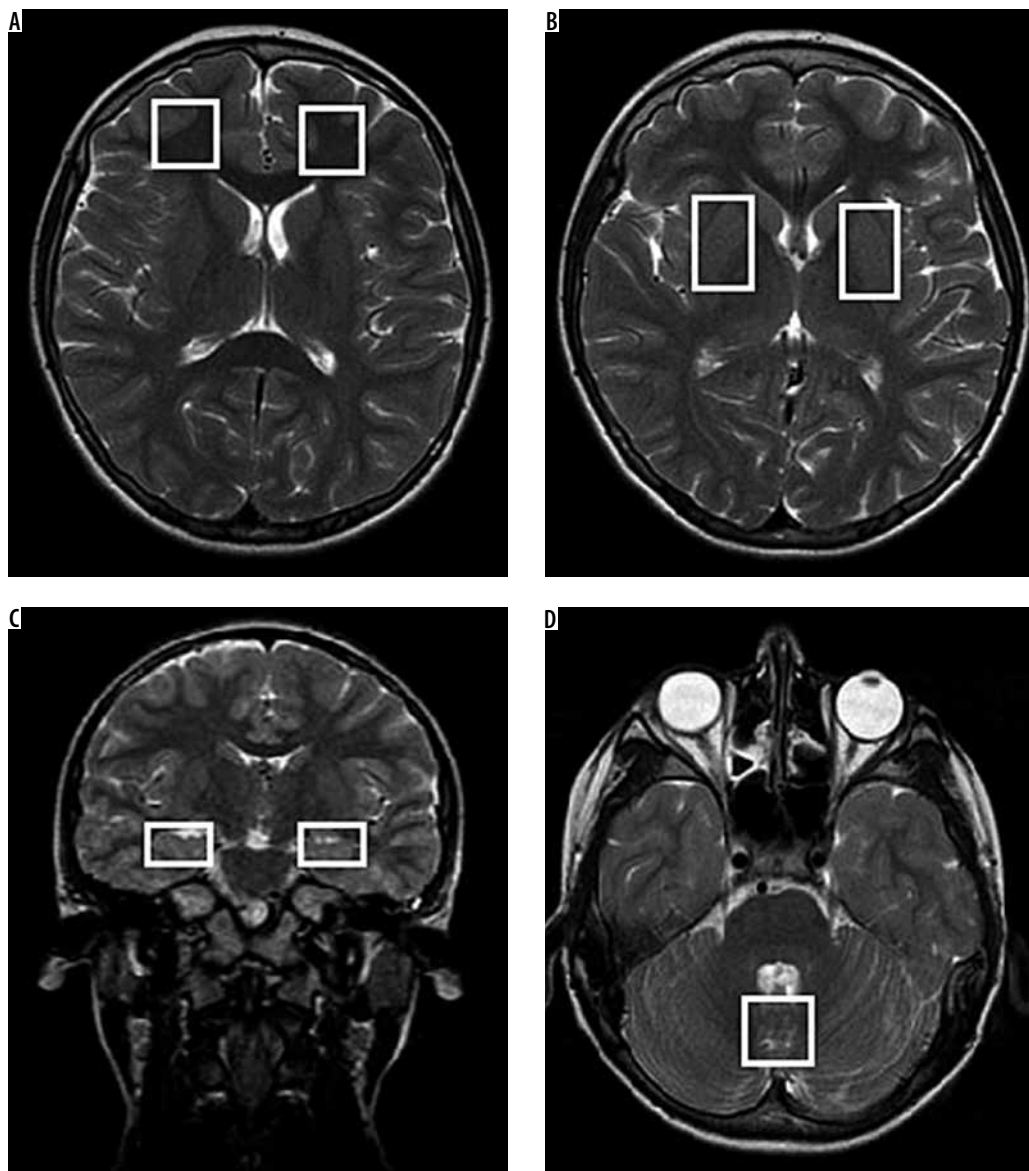
A Study design · B Data collection · C Statistical analysis · D Data interpretation · E Manuscript preparation · F Literature search · G Funds collection

boys ( $p < 0.05$ ). The qualified children had no history of nervous system disorders, craniocerebral injuries, or drug use. The study was approved by the local Bioethics Committee.

Magnetic resonance imaging (MRI) examinations were performed with a 1.5 T MRI system (Signa, GE). The main  $^1\text{H}$ MRS examination was preceded with T2-weighted imaging in order to visualise anatomical structures of the brain and to plan the relevant examination. The signal was obtained from the volume of interest (VOI) in seven locations, as presented in Figure 1. In both child groups (girls and boys) the right frontal lobe white matter (RFL), the left frontal lobe white matter (LFL), the right basal ganglia (RBG), the left basal ganglia (LBG), the right hippocampus (RH), the left hippocampus (LH), and the cerebellum (CR) were examined. The slice thicknesses of 20 mm and FOV = 2 cm x 2 cm were constant.  $^1\text{H}$ MRS examination was based on the PRESS technique (Point-Resolved Spectroscopy Sequence,

TE = 35 ms, TR = 1500 ms, NEX = 128). The duration of a single sequence was 3.43 minutes.

The quantitative analysis of the spectroscopic images was carried out using dedicated software – SAGE 7.0 (Spectroscopy Analysis, GE). The absolute levels were determined for the following metabolites: N-acetylaspartate (NAA), creatine (Cr), choline (Cho), myo-inositol (mI), lipids (Lip), and lactate (Lac) by calculating the areas under the relevant peaks in each spectrum. Then the concentration ratios of each metabolite were determined relative to the total concentration of all the metabolites visible in the spectrum (e.g.  $\text{NAA}/\Sigma$ ) and to the creatine concentration (e.g.  $\text{NAA}/\text{Cr}$ ). All the values were calculated for each VOI. Furthermore, for each metabolite the average result from all the seven locations was computed: for each child, metabolite concentrations in all the locations were summed and the total value was divided by seven (the number of locations).



**Figure 1.** Signal obtained from volume of interest in 7 locations: A) right and left frontal lobe, B) right and left basal ganglia, C) right and left hippocampus, D) cerebellum

**Table 1.** Each metabolite concentrations and metabolite concentrations ratios in the brain of girls and boys – average values of 7 regions (\* $p < 0.05$ )

	NAA	Cr	Cho	ml	Lip	Lac
Girls	0.12 ± 0.025	0.08 ± 0.020	0.07 ± 0.010	0.05 ± 0.013	0.02 ± 0.007	0.02 ± 0.006
Boys	0.11 ± 0.023	0.07 ± 0.012	0.07 ± 0.021	0.06 ± 0.013	0.01 ± 0.004	0.02 ± 0.005
<i>p</i> -value	0.38	0.18	0.99	0.87	0.12	0.95
	NAA/Σ	Cr/Σ	Cho/Σ	ml/Σ	Lip/Σ	Lac/Σ
Girls	0.34 ± 0.024	0.22 ± 0.040	0.19 ± 0.016	0.16 ± 0.018	0.05 ± 0.013	0.04 ± 0.010
Boys	0.34 ± 0.028	0.21 ± 0.026	0.20 ± 0.040	0.17 ± 0.018	0.04 ± 0.009	0.05 ± 0.011
<i>p</i> -value	0.80	0.42	0.46	0.11	0.16	0.26
	NAA/Cr		Cho/Cr	ml/Cr	Lip/Cr	Lac/Cr
Girls	1.62 ± 0.254	–	0.91 ± 0.136	0.75 ± 0.145	0.23 ± 0.071	0.21 ± 0.059
Boys	1.65 ± 0.175	–	0.97 ± 0.321	0.81 ± 0.124	0.21 ± 0.049	0.23 ± 0.064
<i>p</i> -value	0.68	–	0.32	0.13	0.40	0.19

Cho – choline, Cr – creatine, Lac – lactates, Lip – lipids, ml – myo-inositol, NAA – *N*-acetyl aspartate

The statistical analysis was performed for both the absolute and the relative concentrations of the metabolites. The results of the data analysis are presented as mean values and their standard deviations. Differences in the metabolite concentrations in various brain areas between girls and boys were verified using t-test for independent samples. It was assumed that the differences in results can be recognised as statistically significant when  $p < 0.05$ .

## Results

A comparative analysis was carried out taking into account concentrations of selected metabolites averaged for all the seven locations (the absolute concentration, and the metabolite concentration relative to Cr concentration and relative to the total of the concentrations of all the metabolites) in children of both sexes. The results are listed in Table 1. The findings showed no statistically significant sex differences, but there were some trends in the differences. The girls were found with slightly higher Cr, Lip, and Lip/Σ concentrations, and the boys with higher levels of ml/Σ, ml/Cr, and Lac/Cr.

Subsequently, the mean concentrations of selected metabolites were examined in the specific locations. The results are presented in the form of graphs and tables. Tables 2-4 show the identified metabolite concentrations and the significance levels for the differences. The following sex differences of statistical significance ( $p < 0.05$ ) were observed based on the results obtained during <sup>1</sup>HMRS examination of girls and boys:

- ml/Σ concentration is higher in boys in LFL (Fig. 2),
- NAA concentration is higher in girls in LFL (Fig. 3).

Some sex differences in the metabolite concentrations were just above the limits of statistical significance:

- Cho concentration is higher in girls in LFL ( $p < 0.060$ ),
- Lac/Cr concentration is higher in boys in LBG ( $p < 0.052$ ).

Additional analyses investigated the trends in the differences between the girls and the boys in terms of the metabo-

lite concentrations in the specific locations. The findings show the following trends in the differences:

- Cr concentration is higher in boys in RBG, and in girls in LFL and in LBG,
- ml concentration is higher in boys in RBG,
- Cho/Cr concentration is higher in boys in CR,
- ml/Cr concentration is higher in boys in LFL and RH,
- Lip/Cr concentration is higher in girls in RGB,
- Lac/Cr concentration is higher in boys in CR,
- NAA/Σ concentration is higher in girls in LFL,
- ml/Σ concentration is higher in boys in RH,
- Lip/Σ concentration is higher in girls in RBG,
- Lac/Σ concentration is higher in boys in LBG and CR.

The various brain locations were examined for the occurrence of sex differences. It was observed that in the basal ganglia in the left hemisphere relative concentrations of all the metabolites to Cr concentrations were insignificantly higher in the boys than in the girls. It was also found that in all VOIs, in the left hemisphere there was an insignificantly higher Lac concentration in the boys and a higher Cho concentration in the girls. In the right hemisphere there was insignificantly higher relative Cho/Cr concentration in boys. In most locations, however, no permanent trends in the differences were identified with regard to all the metabolites in a given location.

## Discussion

Research conducted so far has demonstrated that the metabolism in the brain changes with age [16,18,19]. Nevertheless, differences in metabolites occurring in female and male brains have previously been mainly investigated in adult subjects. Consequently, there are few reports in the literature focusing on the relevant sex differences in children [2,16], so the present study was designed to make such an assessment. Therefore, when comparing the metabolite concentrations identified in the present study with the results reported by other research teams, it

**Table 2.** Absolute concentration ratios of each metabolite in the brains of girls and boys in different regions (\**p* < 0.05)

	Lip	Lac	NAA	Cr	Cho	ml
Right frontal lobe						
Girls	0.02 ± 0.009	0.02 ± 0.009	0.13 ± 0.020	0.08 ± 0.011	0.08 ± 0.010	0.06 ± 0.016
Boys	0.02 ± 0.009	0.02 ± 0.006	0.13 ± 0.031	0.08 ± 0.040	0.07 ± 0.018	0.06 ± 0.021
<i>p</i> -value	0.22	0.69	0.62	0.84	0.54	0.28
Left frontal lobe						
Girls	0.02 ± 0.010	0.02 ± 0.007	0.14 ± 0.027	0.07 ± 0.012	0.07 ± 0.011	0.06 ± 0.022
Boys	0.01 ± 0.007	0.02 ± 0.007	0.12 ± 0.028	0.07 ± 0.015	0.07 ± 0.017	0.06 ± 0.020
<i>p</i> -value	0.44	0.95	0.04*	0.10	0.06	0.32
Right basal ganglia						
Girls	0.02 ± 0.015	0.02 ± 0.012	0.15 ± 0.046	0.09 ± 0.023	0.07 ± 0.016	0.05 ± 0.019
Boys	0.02 ± 0.009	0.02 ± 0.010	0.16 ± 0.042	0.10 ± 0.022	0.10 ± 0.130	0.06 ± 0.028
<i>p</i> -value	0.43	0.64	0.27	0.16	0.27	0.10
Left basal ganglia						
Girls	0.02 ± 0.008	0.02 ± 0.011	0.15 ± 0.034	0.12 ± 0.011	0.08 ± 0.019	0.06 ± 0.021
Boys	0.02 ± 0.011	0.02 ± 0.008	0.14 ± 0.048	0.08 ± 0.022	0.07 ± 0.020	0.06 ± 0.020
<i>p</i> -value	0.74	0.80	0.31	0.19	0.26	0.52
Right hippocampus						
Girls	0.01 ± 0.010	0.01 ± 0.004	0.07 ± 0.016	0.04 ± 0.008	0.04 ± 0.010	0.04 ± 0.018
Boys	0.01 ± 0.004	0.01 ± 0.004	0.06 ± 0.018	0.04 ± 0.011	0.04 ± 0.011	0.04 ± 0.013
<i>p</i> -value	0.38	0.70	0.67	0.81	0.92	0.42
Left hippocampus						
Girls	0.01 ± 0.006	0.01 ± 0.005	0.07 ± 0.014	0.04 ± 0.016	0.04 ± 0.012	0.04 ± 0.013
Boys	0.01 ± 0.006	0.01 ± 0.017	0.06 ± 0.017	0.04 ± 0.012	0.04 ± 0.011	0.04 ± 0.017
<i>p</i> -value	0.66	0.48	0.30	0.47	0.20	0.82
Cerebellum						
Girls	0.02 ± 0.013	0.01 ± 0.008	0.10 ± 0.032	0.08 ± 0.019	0.07 ± 0.021	0.07 ± 0.025
Boys	0.01 ± 0.006	0.02 ± 0.010	0.10 ± 0.032	0.08 ± 0.024	0.07 ± 0.021	0.06 ± 0.026
<i>p</i> -value	0.33	0.47	0.91	0.79	0.77	0.79

Cho – choline, Cr – creatine, Lac – lactates, Lip – lipids, ml – myo-inositol, NAA – *N*-acetyl aspartate

is necessary to keep in mind the age differences between the study groups.

For each metabolite the average concentration was computed, based on all the seven locations, which enabled assessment of the entire brain volume from this point of view. The findings showed no statistically significant sex differences, but there were some trends of differences. The boys were found with slightly higher levels of mI/Σ, mI/Cr, and Lac/Cr, and the girls with higher concentrations of Cr, Lip, and Lip/Σ. Likewise, a number of studies performed by other researchers found no statistically significant differences in the entire brain volume [3], or such differences were only identified in selected regions [16].

Analysis of the results obtained in this study showed that in the majority of the examined metabolites and locations there were no statistically significant sex-related

differences between the girls and the boys. It was only observed that in the frontal lobe of the left hemisphere ml/Σ concentrations were considerably higher in the male subjects and NAA concentrations were significantly higher in the female subjects. Similar results were obtained worldwide by other research teams; in most 'HMRS examinations of the brain, sex differences in the concentrations of NAA, mI, Cho, Cr, Lip, and Lac were negligible or completely invisible [2,8,18,20,21].

The present study identified statistically significant sex differences in mI/Σ concentrations in the left frontal lobe; the relevant values were significantly higher in the case of the boys. On the other hand, no significant differences were identified in absolute mI concentrations, which is consistent with reports by Schweinburg [17]. However, slightly higher mI concentrations were observed in the boys, in the right basal ganglia. The findings report-

**Table 3.** Relative concentrations ratios of each metabolite calculated for creatine concentration in the brains of girls and boys in different regions (\* $p < 0.05$ )

	Lip/Cr	Lac/Cr	NAA/Cr	Cho/Cr	ml/Cr
Right frontal lobe					
Girls	0.26 ± 0.105	0.24 ± 0.108	1.80 ± 0.258	1.02 ± 0.152	0.86 ± 0.216
Boys	0.24 ± 0.131	0.24 ± 0.108	1.86 ± 0.409	1.03 ± 0.217	0.84 ± 0.298
<i>p</i> -value	0.82	0.66	0.56	0.84	0.78
Left frontal lobe					
Girls	0.23 ± 0.110	0.25 ± 0.091	1.98 ± 0.277	1.06 ± 0.177	0.79 ± 0.401
Boys	0.22 ± 0.085	0.28 ± 0.093	1.93 ± 0.319	1.04 ± 0.198	0.94 ± 0.242
<i>p</i> -value	0.85	0.31	0.57	0.66	0.12
Right basal ganglia					
Girls	0.24 ± 0.129	0.23 ± 0.120	1.69 ± 0.261	0.86 ± 0.167	0.61 ± 0.188
Boys	0.19 ± 0.087	0.20 ± 0.092	1.66 ± 0.159	1.05 ± 1.241	0.64 ± 0.188
<i>p</i> -value	0.16	0.29	0.69	0.45	0.60
Left basal ganglia					
Girls	0.20 ± 0.087	0.19 ± 0.096	1.61 ± 0.405	0.81 ± 0.273	0.62 ± 0.228
Boys	0.22 ± 0.109	0.25 ± 0.099	1.69 ± 0.336	0.85 ± 0.093	0.67 ± 0.156
<i>p</i> -value	0.47	0.05	0.43	0.56	0.38
Right hippocampus					
Girls	0.29 ± 0.228	0.20 ± 0.081	1.62 ± 0.207	1.06 ± 0.219	0.96 ± 0.344
Boys	0.24 ± 0.082	0.23 ± 0.093	1.61 ± 0.271	1.09 ± 0.201	1.11 ± 0.297
<i>p</i> -value	0.42	0.36	0.80	0.61	0.13
Left hippocampus					
Girls	0.26 ± 0.220	0.23 ± 0.118	1.91 ± 0.403	1.12 ± 0.390	1.12 ± 0.656
Boys	0.22 ± 0.107	0.29 ± 0.370	1.65 ± 0.431	1.03 ± 0.262	1.08 ± 0.308
<i>p</i> -value	0.44	0.46	0.44	0.38	0.80
Cerebellum					
Girls	0.23 ± 0.204	0.17 ± 0.071	1.31 ± 0.236	0.86 ± 0.138	0.84 ± 0.211
Boys	0.18 ± 0.071	0.20 ± 0.101	1.36 ± 0.156	0.92 ± 0.150	0.83 ± 0.155
<i>p</i> -value	0.38	0.20	0.44	0.15	0.77

Cho – choline, Cr – creatine, Lac – lactates, Lip – lipids, ml – myo-inositol, NAA – *N*-acetyl aspartate

ed by Tayoshi *et al.* [9] and Pouwels *et al.* [18] showed considerable sex differences in mI concentrations. These authors observed elevated mI levels in women in the left basal ganglia [9] and in the occipital lobe [18]. Myo-inositol is involved in many biological processes. Based on studies focusing on cell-type specific culture, researchers suggest that mI is a marker for glia, and it also plays an important role in the metabolism of cell membranes and in the neuronal signalling system. Various mI concentrations essentially depend on the density of glial cells and cell membranes [16,18].

Another metabolite whose concentration shows significant sex-related differences is NAA. NAA is involved in synaptic processes, and it is produced by mitochondria of nerve cells, therefore in <sup>1</sup>HMRS it is used as a marker for neurons [2,16,17]. In the study performed by our team,

it was shown that the concentration of this metabolite was significantly higher in the left frontal lobe in the girls than in the boys. In contrast, Schweinsburg *et al.* [17] and Chang *et al.* [22] found that female subjects had decreased NAA levels in the white matter in the frontal lobe. Moreover, in the present study, in other locations there were no significant differences in NAA. This may suggest there are no differences in the density of nerve cells between girls and boys in most regions of the brain suggested by Wilkinson [16]. Sijens and Hadel demonstrated there were no sex-related differences in NAA concentrations in all the examined locations [15, 20], and Chang *et al.* [22] reported only slightly higher NAA concentrations in basal ganglia in men compared to women. Conversely, Tayoshi observed higher levels of NAA in women in the left basal ganglia [9].

**Table 4.** Relative concentration ratios of each metabolite calculated for the total amount of concentrations of all metabolites in the brains of girls and boys in different regions (\* $p < 0.05$ )

	Lip/ $\Sigma$	Lac/ $\Sigma$	NAA/ $\Sigma$	Cr/ $\Sigma$	Cho/ $\Sigma$	mI/ $\Sigma$
Right frontal lobe						
Girls	0.05 $\pm$ 0.022	0.05 $\pm$ 0.020	0.35 $\pm$ 0.023	0.20 $\pm$ 0.019	0.20 $\pm$ 0.020	0.17 $\pm$ 0.035
Boys	0.05 $\pm$ 0.023	0.05 $\pm$ 0.015	0.35 $\pm$ 0.047	0.20 $\pm$ 0.081	0.20 $\pm$ 0.034	0.16 $\pm$ 0.043
<i>p</i> -value	0.53	0.87	0.73	0.66	0.96	0.42
Left frontal lobe						
Girls	0.04 $\pm$ 0.020	0.05 $\pm$ 0.016	0.37 $\pm$ 0.038	0.19 $\pm$ 0.023	0.20 $\pm$ 0.025	0.14 $\pm$ 0.047
Boys	0.04 $\pm$ 0.015	0.05 $\pm$ 0.016	0.36 $\pm$ 0.037	0.19 $\pm$ 0.020	0.19 $\pm$ 0.026	0.17 $\pm$ 0.037
<i>p</i> -value	0.74	0.40	0.10	0.47	0.21	0.02*
Right basal ganglia						
Girls	0.05 $\pm$ 0.024	0.05 $\pm$ 0.024	0.37 $\pm$ 0.031	0.22 $\pm$ 0.026	0.18 $\pm$ 0.023	0.13 $\pm$ 0.036
Boys	0.04 $\pm$ 0.019	0.04 $\pm$ 0.019	0.36 $\pm$ 0.051	0.22 $\pm$ 0.031	0.20 $\pm$ 0.098	0.14 $\pm$ 0.036
<i>p</i> -value	0.16	0.24	0.79	0.95	0.55	0.51
Left basal ganglia						
Girls	0.04 $\pm$ 0.016	0.04 $\pm$ 0.020	0.36 $\pm$ 0.063	0.24 $\pm$ 0.109	0.18 $\pm$ 0.043	0.14 $\pm$ 0.039
Boys	0.05 $\pm$ 0.021	0.05 $\pm$ 0.020	0.36 $\pm$ 0.062	0.22 $\pm$ 0.029	0.18 $\pm$ 0.030	0.14 $\pm$ 0.028
<i>p</i> -value	0.65	0.09	0.88	0.26	0.62	0.47
Right hippocampus						
Girls	0.05 $\pm$ 0.036	0.04 $\pm$ 0.016	0.32 $\pm$ 0.036	0.20 $\pm$ 0.026	0.21 $\pm$ 0.033	0.18 $\pm$ 0.048
Boys	0.05 $\pm$ 0.014	0.04 $\pm$ 0.016	0.31 $\pm$ 0.044	0.19 $\pm$ 0.028	0.21 $\pm$ 0.026	0.21 $\pm$ 0.046
<i>p</i> -value	0.31	0.69	0.33	0.56	0.93	0.08
Left hippocampus						
Girls	0.04 $\pm$ 0.024	0.04 $\pm$ 0.018	0.32 $\pm$ 0.051	0.20 $\pm$ 0.047	0.20 $\pm$ 0.022	0.19 $\pm$ 0.030
Boys	0.04 $\pm$ 0.021	0.05 $\pm$ 0.036	0.31 $\pm$ 0.056	0.20 $\pm$ 0.037	0.19 $\pm$ 0.039	0.20 $\pm$ 0.045
<i>p</i> -value	0.78	0.37	0.54	0.96	0.35	0.34
Cerebellum						
Girls	0.05 $\pm$ 0.040	0.04 $\pm$ 0.014	0.30 $\pm$ 0.041	0.23 $\pm$ 0.026	0.20 $\pm$ 0.030	0.19 $\pm$ 0.033
Boys	0.04 $\pm$ 0.016	0.04 $\pm$ 0.021	0.30 $\pm$ 0.020	0.22 $\pm$ 0.018	0.20 $\pm$ 0.023	0.18 $\pm$ 0.029
<i>p</i> -value	0.37	0.18	0.64	0.42	0.32	0.53

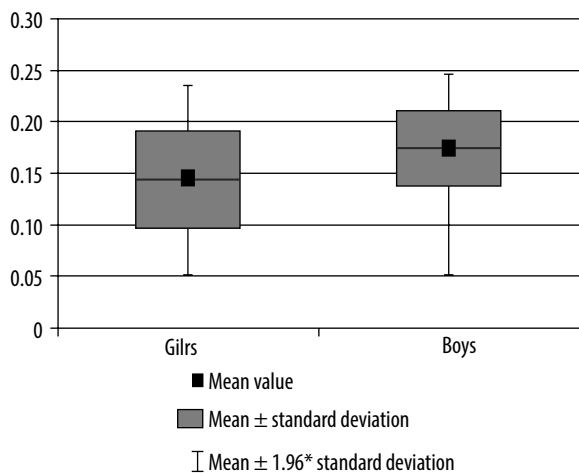
Cho – choline, Cr – creatine, Lac – lactates, Lip – lipids, mI – myoinositol, NAA – *N*-acetyl aspartate,  $\Sigma$  – total concentration of all the metabolites

In addition to the scarce statistically significant gender differences, the present study identified a number of trends in differences between the girls and the boys related to metabolite concentrations in the specific locations. These differences relate to some of the metabolites and their ratios in the various locations. Such tendencies indicating differences in metabolite concentrations were found in the case of the Cho/Cr ratio in the cerebellum and mI in the left frontal lobe as well as the right hippocampus. In the left frontal lobe NAA/Cr and Cho/Cr ratios are higher in women [8]. Komoroski *et al.* [14] analysed the spectra collected from the left and the right hippocampus, the left frontal lobe, and the left basal ganglia in adults and then calculated NAA/Cr, Cho/Cr, and mI/Cr concentration ratios. As in the present study, they did not identify any significant sex differences, but only statistically

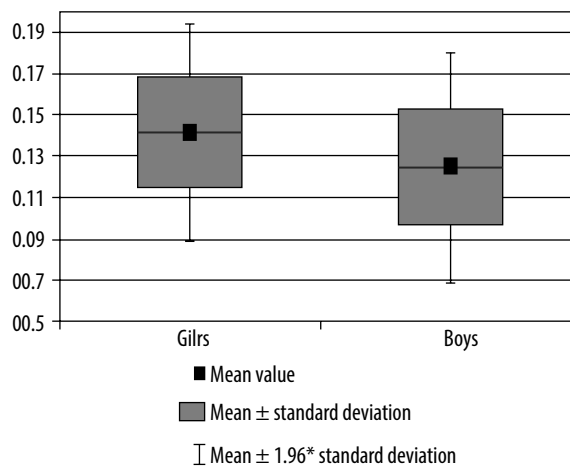
insignificant trends. They found that male subjects had higher concentration ratios of Cho/Cr and mI/Cr in the left basal ganglia and Cho/Cr in the right hippocampus, while NAA/Cr in the right hippocampus was higher in the female subjects [14]. Moreover, the findings of the present study show higher absolute Cr concentrations in the boys in the right basal ganglia and in the girls in the left basal ganglia and in the left frontal lobe. These findings are consistent with other reports [22]. Hadel [20] observed slightly higher Cr concentrations in the hippocampus in women. Furthermore, Sailasuta [7] found female subjects with higher Cho/Cr concentrations in the white matter.

Our team also analysed Lip and Lac concentrations, and trends indicating differences have been identified in the basal ganglia in the right (Lip/ $\Sigma$ ) and in the left hemisphere (Lac/ $\Sigma$ ) as well as in the cerebellum (Lac/ $\Sigma$ ). Due





**Figure 2.** Comparison of the relative ml concentrations calculated for the total amount of concentrations of all metabolites in left frontal lobe in girls and boys



**Figure 3.** Comparison of the absolute *N*-acetyl aspartate concentrations in left frontal lobe in girls and boys

to their low levels, these metabolites are rarely examined, and that is why no reports on gender differences related to these metabolites were found in the literature.

Investigations focusing on the role of sex in brain functioning have presented evidence showing that the sex hormones may affect growth factors, neurotransmitters, and neuropeptides, consequently regulating the formation of synapses and the growth and differentiation of nerve cells [16,18]. Possible gender differences may be linked with the process of neuronal maturation, i.e. an increase in the number of axons, dendrites, and synapses. These processes are not complete in children, and therefore the concentrations of certain metabolites may be different in children, depending on their age, and they may be linked with the individual's rate of maturation more than with his/her gender [16,19]. Differences in metabolite concentrations between women and men may be caused by biological factors, but this has not been fully explored – our understanding of the specific gender- and age-related role of neurotransmitters is still limited [9,18]. The greatest biological difference in the metabolite concentrations between girls and boys is due to the occurrence of the menstrual cycle in females, which is associated with changes in the concentration of the steroid hormones, oestrogens, and progesterone [7,20,23,24]. These hormones can regulate growth factors and neuropeptides and thereby influence neural differentiation and formation of synaptic connections [16]. In the examined group of children, gender differences are small because some of the girls have not entered puberty yet, so the metabolic composition of their brains does not depend on the relevant hormones [19].

Most studies investigating sex differences visualised by <sup>1</sup>H MRS images have focused on adults, but, as mentioned above, such differences may change with age, so they may vary between children and adults [4,15,19]. Gender-related concentrations of metabolites in children were only investigated by Kułak *et al.* [2,16] and Dickstein *et al.* [25].

Kułak *et al.* published findings related to the basal ganglia, and acquired from healthy controls, in a study of children with cerebral palsy. They only reported higher NAA/Cr concentrations in the girls and Cho/Cr concentrations in the boys in the basal ganglia [16]. Also, they failed to identify other sex differences in metabolite concentrations in children [2]. The present study found that boys had higher Cho/Cr concentrations in the cerebellum, but the difference was not statistically significant and only showed a certain tendency. Conversely, no trends in differences were identified in NAA/Cr concentrations in any locations. Dickstein observed significantly higher mI/Cr concentrations in girls' temporal lobes [25]. Weber *et al.* [26] found no sex-related differences in children's prefrontal white matter in either hemisphere, but this was not the main purpose of his study. Conversely, the present findings show slightly higher mI/Cr levels in the boys' left frontal lobes and the right hippocampi.

Notably, the results obtained by various research teams are not fully compatible and, in fact, in some cases they are contradictory. These discrepancies may result not only from different ages of the subjects. In a number of experiments, the studied populations were relatively small (< 30 subjects), and this may significantly affect the obtained results [17]. Moreover, in some studies the percentage of women among the examined subjects was low [9]. Comparisons of examination results obtained by various research teams are difficult because the spectroscopic signatures were collected from different locations [24,27] and then the results were presented in various forms [15,12]. In this paper the results are presented in three ways: as the absolute values of the specific metabolite concentrations, and as the ratios of metabolite concentrations to Cr concentrations and to the total concentrations of all the metabolites. Such presentation of the results in the form of the relative values has a number of advantages: they are easy to compute and are not affected by the VOI. However, the disadvantage of this data presentation method

is the fact that the concentration ratios are sensitive to any changes in concentrations of both metabolites and not only of one of them [14].

## Conclusions

In clinical practice the diagnostic process first of all focuses on assessing relative concentrations of metabolites to

Cr concentration. Thus, the findings of the present study allow the conclusion that when analysing the results of <sup>1</sup>H MRS studies in children it is not necessary to take into account the child's gender.

## Conflict of interest

The authors report no conflict of interest.

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