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FULL PAPER

A quantitative analysis of ^1H -MR spectroscopy at 3.0 T of three brain regions from childhood to middle age

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Objective: To study age-related metabolic changes in different brain regions.

Methods: Point-resolved spectroscopy (repetition time/echo time = 2000 ms/30 ms) was performed in the left and right hippocampus, the left thalamus and the left centrum semiovale of 80 healthy subjects (37 females and 43 males aged 7–64 years). Analysis of covariance and linear regression were used for statistical analysis. Both metabolite concentration ratios with respect to total creatine (tCr) and absolute metabolite concentrations were included for analysis.

Results: Ins (myo-inositol)/tCr ($p < 0.001$) and absolute Ins concentration ($p = 0.031$) were significantly increased with age after adolescence. NAA (*N*-acetylaspartic acid)/tCr ($p < 0.001$) and absolute NAA concentration ($p = 0.010$) significantly declined with age after adolescence.

Conclusion: Age-related increase of Ins and decline of NAA are found in all three regions, especially at the hippocampus, indicating possible gliosis in the ageing brain.

Advances in knowledge: We could use NAA/tCr and Ins/tCr as an indicator to estimate the neurons-to-glia cells ratio at the thalamus. This may be an index to distinguish normal tissues from gliosis.

INTRODUCTION

Development and ageing are two issues of common concern that scientists have studied for many years. As summarized in Table 1, these two issues have been investigated in several studies using proton MR spectroscopy (^1H -MRS).

Previous studies of brain development have found that the increase in the number of neuronal cells reaches its peak by 6 months after birth, and then the centrum semiovale attains maturity with a significant increase in the number and size of axons and dendrites.²¹ Although major events in the maturation of the cerebrum take place during the first few years of life,²² the myelination, which is most closely related to the full functional capacity of neuronal interconnections, is not completed before adolescence.²¹ In previous ^1H -MRS studies, the age range of the studied subjects rarely included childhood. Girard et al²³ have studied the growth of the brain in 22- to 39-week foetuses and reported reductions in Ins (myo-inositol) and Cho (choline) and increases in NAA (*N*-acetylaspartic acid) in

the foetal brains. Kadota et al¹¹ found that NAA/Cho reduced at the centrum semiovale with progressive age from 4 to 88 years. Kreis et al¹⁴ scanned 21 infants from 23 to 43 weeks and found that absolute NAA concentration significantly increases with age in the white matter and grey matter. Horská et al¹⁵ have employed MRS imaging (MRSI) and studied 15 subjects from 3 to 19 years old and found that NAA/Cho increased to a maximum at 10 years old in grey matter regions, while NAA/Cho increased linearly with age in the white matter.

The normal ageing of the brain has been associated with loss of both neurons and brain volume,²⁴ and some age-related diseases such as mild cognitive impairment and Alzheimer's disease also have these symptoms. ^1H -MRS has the potential to observe these changes in the brain by the non-invasive measurement of brain metabolites. A large variability in observations was found in previous studies, partly due to the different inclusion criteria (size, age range), regions of interest, data acquisition [field strength,

Table 1. MR spectroscopy (MRS) findings of brain development and ageing in the present study and published data

Study	Number of subjects (male/female ratio)	Age range (years)	Regions of interest	Field strength (T)	MRS (I)	Quantification analysis	Trends seen with increasing age
Chang <i>et al</i> ¹	36	19–78	Frontal WM	1.5	MRS	A + R	tCr↑, Cho↑, Ins↑, NAA/tCr↓
Lim <i>et al</i> ²	10 (10/0)	20–28, 65–75	Centrum semiovale	1.5	MRSI	R	NAA (GM)/NAA (WM)↓
Fukuzako <i>et al</i> ³	36 (17/19)	24–79	Left, medial, temporal	2	MRS	R	NAA/tCr↓
Pfefferbaum <i>et al</i> ⁴	34 (24/10)	25.3 ± 2.9, 73.3 ± 4.1	Centrum semiovale	1.5	MRSI	A	tCr↑, Cho↑
Saunders <i>et al</i> ⁵	30 (17/13)	24–89	Parietal, WM, occipital, GM	1.5	MRS	A	tCr↑
Lundbom <i>et al</i> ⁶	12 (0/12)	35 ± 6, 74 ± 7	Frontal, parietal, temporal, WM, GM	1.5	MRSI	R	NAA/Cho↓, NAA/tCr↓
Leary <i>et al</i> ⁷	44 (22/22)	22–62	Parietal, WM	1.5	MRS	A	tCr↑, Cho↑
Angelie <i>et al</i> ⁸	32 (14/18)	21–61	Cortical GM, centrum semiovale, temporal	1.5	MRSI	R	NAA/tCr↓, NAA/Cho↓
Brooks <i>et al</i> ⁹	50 (50/0)	20–70	Frontal	1.5	MRS	A + R	NAA↓, NAA/tCr↓
Grachev and Apkarian ¹⁰	35 (23/12)	19–31, 40–52	Cingulate, thalamus, insula, DPFC, orbit frontal cortex, sensorimotor cortex	1.5	MRS	R	NAA/tCr↓, glutamate/tCr↓, Gln/tCr↓, Glc/tCr↓, Lac/tCr↓, GABA/tCr↓
Harada <i>et al</i> ²	21	20–70	Frontal, lentiform nucleus	1.5	MRS	A	NAA↓
Kadota <i>et al</i> ¹¹	90 (45/45)	4–88	Centrum semiovale	1.5	MRSI	R	NAA/Cho↓
Pouwels <i>et al</i> ¹²	97	1–39	Cortical parietal GM, parieto-occipital WM, cerebellum, thalamus, basal ganglia	2	MRS	A	NAA↑, NAAG↑, Tau↓
Driscoll <i>et al</i> ¹³	32 (16/16)	20–39, 60–85	Hippocampus, frontal WM	1.5	MRSI	R	NAA/tCr↓
Kreis <i>et al</i> ¹⁴	21	1–3	Centrum semiovale, thalamus, occipital grey matter	1.5	MRS	A	NAA↑
Horská <i>et al</i> ¹⁵	15	3–19	Putamen, thalamus, posterior pre-frontal cortex, deep pre-motor/ motor/parietal WM, dorsal parietal cortex	1.5	MRSI	A + R	NAA/Cho↑

(Continued)

Table 1. (Continued)

Study	Number of subjects (male/female ratio)	Age range (years)	Regions of interest	Field strength (T)	MRS (I)	Quantification analysis	Trends seen with increasing age
Szentkuti et al. ¹⁶	35 (16/19)	22–27, 60–75	Hippocampus, extra hippocampus	1.5	MRS	R	NAA/(tCr + Cho)↓
Sailasuta et al. ¹⁷	50	21–71	Frontal GM/WM, parietal, GM, basal ganglia	3	MRS	NAA↓, glutamate↓	
Gruber et al. ¹⁸	63 (24/39)	18–65	DPFC, frontal, parietal, thalamus, caudate nucleus, lentiform nucleus	3	MRSI	A + R	NAA↓, tCr↑, Cho↑, Ins↑, NAA/tCr↓, Cho/tCr↑, Ins/tCr↑
Raininko and Mattson ¹⁹	57 (32/25)	13–72	Supraventricular WM	1.5	MRS	A	NAA↓, Ins↑
Reyngoudt et al. ²⁰	90 (48/42)	18–76	Posterior cingulate, hippocampus	3	MRS	A + R	Ins/tCr↑, Ins↑, tCr↑
Present study	80 (43/37)	7–64	Centrum semiovale, hippocampus, thalamus	3	MRS	A + R	NAA↓, Ins↑, NAA/tCr↓, Ins/tCr↑, NAA/Ins↓, glutamate plus glutamine/tCr↓

A, absolute; Cho, choline; DPFC, dorsolateral pre-frontal cortex; GM, grey matter; Ins, myo-inositol; MRSI, MRS imaging; NAA, *N*-acetylaspartic acid; R, relative; tCr, total creatine; WM, white matter.

single-voxel *vs* multiple-voxel studies, spectroscopy sequence, repetition time (TR)/echo time (TE)] and data processing (relative *vs* absolute quantification). Some infant studies found the increase of NAA from 22 weeks to 10 years in most grey and white matters.^{14,15,23} On the contrary, a decreased amount of NAA with increasing age was observed in frontal,^{6,7,11,17,18} parietal^{17,18,25} and temporal cortices.^{3,6,8} And some of these studies did not find age-related NAA changes either in the frontal cortex⁴ or in the parietal cortex.^{5,7} The results of NAA in these studies seem unclear and contradictory sometimes. In several studies, an age-related increase in total creatine (tCr) was found in the frontal white matter,^{1,4,5,7,18} the parietal white matter,^{5,7} the temporal lobe⁸ and the posterior cingulate cortex.²⁰ The age-related increase in Cho was also found in the frontal white matter^{1,4,7,18} and the parietal white matter.^{7,18} In a small number of studies, an increased amount of Ins was found in the frontal white matter,^{1,18,19} the parietal white matter,^{18,19} the hippocampus²⁰ and the posterior cingulate cortex.²⁰

LCModel (Stephen Provencher, Oakville, ON, Canada) is widely used and accepted because it performs spectral fitting based on prior knowledge. Our study used LCModel software (<http://s-provencher.com/pages/lcmodel.shtml>) to analyse the MRS data from three different brain regions (centrum semiovale, hippocampus and thalamus) of volunteers between 7 and 64 years old. We chose a relatively short TE (30 ms) to reduce the influences of *T*₂ relaxation time to absolute metabolite signals.^{20,26} We chose the centrum semiovale (partly represents the white matter) and the thalamus (partly represents the grey matter) to observe the development and ageing processes in different

regions of the human brain. The hippocampus is important for development because it contains neural progenitor cells²⁷ for neuron meiosis, and it has also been reported to be connected with memory functions and degenerated by ageing and other diseases.^{13,16,28–30} Hence, the hippocampus was also chosen as a region of interest in this study. Our study uses absolute metabolite concentration of these three brain regions to analyse the trends in chemical changes in these three different brain regions from childhood to middle age.

METHODS AND MATERIALS

Subjects

We studied 80 healthy volunteers (43 males and 37 females) with a mean age of 31 years (range, 7–64 years). None had any history of disorders affecting the central nervous system or demonstrated any abnormal signs on MRI. All subjects were right-handed, non-smokers and non- or light drinkers. This study was approved by the Ethics Committee of Wuhan University, Wuhan, China. Written informed consent was obtained from all the subjects or (for subjects under 18 years) their guardians.

MR examination

MR examination was performed on a Siemens MAGNETOM® Trio 3.0-T whole-body MR system (Siemens Healthcare, Erlangen, Germany) with a 32-channel head coil. First, high-resolution *T*₂ weighted images in three orthogonal planes (sagittal, coronal and transversal with continuous slicing) were performed to permit localization of the voxels of interest (VOIs), including the left and right hippocampus, the left thalamus and the left

centrum semiovale (Figure 1). Then single-voxel ^1H -MRS was performed using point-resolved spectroscopy (PRESS) with TR/TE = 2000 ms/30 ms, spectral bandwidth = 1200 Hz, 1024 data points, 128 signals averaged and sampling interval = 0.83 ms. The normal size of the VOI was 2000 mm^3 (normal of regions sizes are: hippocampus, $8 \times 8 \times 30\text{ mm}^3$; centrum semiovale, $10 \times 10 \times 20\text{ mm}^3$; thalamus, $10 \times 10 \times 20\text{ mm}^3$), and the normal sizes of the voxels in all three regions are nearly the same, while in some subjects the VOI was adapted to fit the hippocampal region in order to avoid cerebrospinal fluid (CSF) contamination. A similar PRESS sequence without water suppression was scanned at the same voxels to acquire a water signal as the internal reference for absolute quantification. Field shimming and water suppression were automatically accomplished by the spectral software package. Pre-saturation bands for outer volume suppression were placed around the VOI to avoid contamination from neighbouring CSF or scalp fat. For the volunteers' comfort, no more than three spectra were acquired from each subject, and only two spectra from the seven subjects under 18 or over 55 years old so that total scanning time did not exceed 30 min.

Post-processing of MR spectroscopy data

All spectra were taken for analysis with LCModel v. 6.2 using the water-scaling option. Raw data were selected in two subsequent steps: first, spectra were rejected if full width at half maximum (FWHM) of NAA was $>0.1\text{ ppm}$ (13 Hz) or if the signal-to-noise ratio (SNR) of the LCModel output file was <6 for the NAA peak; second, metabolites could be accepted for further statistical analyses only if the estimated standard deviation (SD) provided by LCModel was $<20\%$. LCModel fits a linear combination of the full spectral pattern for each metabolite included in the basis set. In the LCModel fitting result, the metabolite concentration ratio (/tCr) and absolute concentration by water scaling (mmol kg^{-1} of wet matter, $\text{mmol kg}_{\text{ww}}^{-1}$) of NAA, tCr, Cho, Ins, glutamate plus glutamine (Glx), macromolecules at 0.9 ppm (MM09), macromolecules at 1.2 ppm (MM12) and macromolecules at 1.4 ppm (MM14), referenced at 2.01, 3.03, 3.19, 3.52, 2.12–2.46, 0.9, 1.2 and 1.4, respectively (Figure 2), were chosen for statistical analysis. At the hippocampus, since CSF is unavoidable, a correction for CSF was needed. To correct the concentrations for the amount of CSF contained in the VOIs of the hippocampus, segmentation of the high-resolution transversal T_2 weighted images was performed as we described previously.³¹ The volume of CSF in each

slice was calculated by multiplying their areas by the slice thickness, and their summation gave the total volume of CSF parts within the voxel. We assumed that CSF parts contributed to tissue water rather than to metabolite, and the corrected metabolite concentration was calculated as follows: corrected metabolite concentration at the hippocampus = metabolite concentration $\times$ VOI volume/(VOI volume – CSF volume).

Statistical analysis

Statistical analysis was performed using the SPSS 16.0 software package (SPSS® 16.0 for Windows; SPSS Inc., Chicago, IL). Subjects younger than 19 years were classified as “Children”; from 19 to 39 years as “Adults”; and older than 40 years as “Middle-aged”. An unpaired Student's *t*-test was applied to compare metabolite concentration ratios and absolute metabolite concentrations between males and females and between the left and right hippocampus. Analysis of covariance (ANCOVA) was used to test the effects of region and age on metabolite concentration ratios (/tCr) and absolute metabolite concentrations. Subsequent multiple comparisons (using the Bonferroni correction method) of the three age-dependent subgroups in the same regions (the centrum semiovale in Children, Adults and Middle-aged groups; the hippocampus in Children, Adults and Middle-aged groups; the thalamus in Children, Adults and Middle-aged groups) were taken to exclude interactions of regions and to carry out the region-dependent analysis. When there were significant differences in metabolite concentration ratios and absolute metabolite concentrations at different age levels, a Pearson correlation analysis was used to analyse the correlation between age and metabolite concentration ratios or absolute metabolite concentrations. $p < 0.05$ was taken as statistically significant.

RESULTS

A total of 196 spectral data were acquired from 80 volunteers. Data with poor spectral quality [low SNR <5 , large FWHM $>0.1\text{ ppm}$ (13 Hz)] were excluded, and the remaining 153 data (details in Table 2) were included for further analysis. The SNR of the data from the centrum semiovale, the hippocampus and the thalamus was 19.1, 7.74 and 9.89, respectively. The FWHM (mean $\pm$ SD) of NAA was $0.057 \pm 0.017\text{ ppm}$ ($7.41 \pm 0.22\text{ Hz}$).

Table 3 and Table 4 show the results of ANCOVA of the metabolite concentration ratios and the absolute metabolite concentrations,

Figure 1. An example of the volume of interest (VOI). The VOIs (indicated by the white rectangles) are placed at the hippocampus (a), the centrum semiovale (b) and the thalamus (c).

Figure 2. An example of an LCModel result of spectroscopy. *N*-acetylaspartic acid (NAA), total creatine (tCr), choline (Cho), myo-inositol (Ins), glutamate plus glutamine (Glx), macromolecules at 0.9 ppm (MM09), macromolecules at 1.2 ppm (MM12) and macromolecules at 1.4 ppm (MM14) were chosen for statistical analysis.

respectively, with *p*-values for age, region and the region-by-age interaction effect. Table 5 shows the quantitative analysis result of all data in different age groups and region groups.

In the first-round analysis, there was no significant difference between males and females (*p* = 0.09) and between the left and right hippocampus (*p* = 0.70); therefore, the male/female and the hippocampus data were combined for further analysis.

Metabolite concentration ratios

Age difference

ANCOVA demonstrated that NAA/tCr in the Middle-aged group was significantly lower than in the Children group and

Adults group (Tables 3 and 5). The Ins/tCr in the Middle-aged group was significantly higher than in the Children group and Adults group (Tables 3 and 5). The Glx/tCr in the Children group was significantly higher than in the Middle-aged group and Adults group (Tables 3 and 5).

Region difference

ANCOVA demonstrated that NAA/tCr, MM09/tCr, MM12/tCr and MM14/tCr in the centrum semiovale and thalamus were higher than in the hippocampus (Tables 3 and 5). The Cho/tCr in the centrum semiovale was the highest of the three regions (Tables 3 and 5). The Glx/tCr in the centrum semiovale was the lowest in the three regions (Tables 3 and 5).

Table 2. The distribution of MR spectroscopy data acquired and analysed

Group	Acquired	Analysed
Female/male	90/106	71/82
Children/Adults/Middle-aged	40/90/66	25/76/52
Centrum semiovale/hippocampus/thalamus	67/74/55	59/53/41

The total acquired spectral data was 196, and the total analysed spectral data was 153.

Table 3. *p*-values of analysis of covariance of metabolite concentration ratios [tCr (total creatine)] vs age and region

/tCr	Age (at the same region)	Region (at the same age level)
Macromolecules at 0.9 ppm	0.606	0.001 ^a
Macromolecules at 1.2 ppm	0.845	0.010 ^a
Macromolecules at 1.4 ppm	0.721	0.012 ^a
<i>N</i> -acetylaspartic acid	0.001 ^a	0.008 ^a
Glutamate plus glutamine	0.011 ^a	0.001 ^a
Choline	0.428	0.001 ^a
Myo-inositol	0.001 ^a	0.001 ^a

^aSignificant difference ($p < 0.05$).

Post hoc analysis

Myo-inositol/total creatine

The *post hoc* multiple comparisons (using the Bonferroni correction method) of Ins/tCr shows (Table 5): in the centrum semiovale, the Adults group was significantly lower than the Children ($p_{\text{Adults vs Children}} < 0.001$) and Middle-aged groups ($p_{\text{Adults vs Middle-aged}} < 0.001$); in the hippocampus, the Middle-aged group was significantly higher than the Children ($p_{\text{Children vs Middle-aged}} < 0.001$) and Adults groups ($p_{\text{Adults vs Middle-aged}} < 0.001$); in the thalamus, the Children group was significantly lower than the Adults ($p_{\text{Adults vs Children}} < 0.001$) and Middle-aged groups ($p_{\text{Children vs Middle-aged}} < 0.001$).

N-acetylaspartic acid/total creatine

The *post hoc* multiple comparisons (using the Bonferroni correction method) of NAA/tCr shows (Table 5): in the centrum semiovale, the Adults group was significantly higher than the Middle-aged groups ($p_{\text{Adults vs Middle-aged}} = 0.002$); in the hippocampus, the Middle-aged group was significantly lower than the Adults group ($p_{\text{Adults vs Middle-aged}} = 0.005$); in the thalamus, the Middle-aged group was significantly lower than the Children ($p_{\text{Children vs Middle-aged}} < 0.001$) and Adults groups ($p_{\text{Adults vs Middle-aged}} = 0.002$).

Glutamate plus glutamine/total creatine

The *post hoc* multiple comparisons (using the Bonferroni correction method) of Glx/tCr found: in the centrum semiovale and hippocampus, the Glx/tCr have no significant difference among the three age levels, whereas in the thalamus the Middle-aged

group was significantly lower than the Children ($p_{\text{Children vs Middle-aged}} = 0.003$) and Adults groups ($p_{\text{Adults vs Middle-aged}} = 0.005$).

Absolute metabolite concentrations

Age difference

ANCOVA demonstrated that the absolute NAA concentration in the Adults group was significantly higher than in the Children group and Middle-aged group (Tables 4 and 5). Absolute Ins concentration in the Middle-aged group was significantly higher than in the Children group and Adults group (Tables 4 and 5).

Region difference

ANCOVA demonstrated that the absolute NAA, MM09 and MM12 concentrations in the thalamus were the highest of the three regions (Tables 4 and 5). Absolute Glx concentration in the hippocampus was the highest of the three regions and in the thalamus was higher than in the centrum semiovale (Tables 4 and 5). Absolute Ins concentration in the centrum semiovale was higher than in the hippocampus and in the thalamus in the Children group. In the Adults and Middle-aged groups, absolute Ins concentration in the hippocampus was higher than in the centrum semiovale and in the thalamus.

Post hoc analysis

Myo-inositol

Table 5 shows: in the centrum semiovale, the Ins absolute concentration in the Adults group was significantly lower than in the Children ($p_{\text{Adults vs Children}} < 0.001$) and Middle-aged groups

Table 4. *p*-values of analysis of covariance of absolute concentrations vs age and region

Water scaling	Age (at the same region)	Region (at the same age level)
Macromolecules at 0.9 ppm	0.544	0.002 ^a
Macromolecules at 1.2 ppm	0.606	0.027 ^a
Macromolecules at 1.4 ppm	0.786	0.004 ^a
<i>N</i> -acetylaspartic acid	0.010 ^a	0.001 ^a
Myo-inositol	0.031 ^a	0.001 ^a
Choline	0.389	0.269
Glutamate plus glutamine	0.251	0.001 ^a
Total creatine	0.522	0.001 ^a

^aSignificant difference ($p < 0.05$).

Table 5. The metabolite concentration ratios [$\mu\text{mol/kg}$ (total creatine)] and absolute concentrations ($\text{mmol kg}_{\text{ww}}^{-1}$, expressed as mean $\pm$ standard deviation) in different ages and regions (the first-line data are metabolite concentration ratios and the second-line data are absolute concentrations)

Metabolites	Age groups	Centrum semiovale	Hippocampus	Thalamus
N-acetylaspartic acid ^a	Children	1.21 $\pm$ 0.39 6.62 $\pm$ 0.37	1.14 $\pm$ 0.20 6.18 $\pm$ 0.73	1.14 $\pm$ 0.18 7.71 $\pm$ 1.13
	Adults	1.57 $\pm$ 0.44 7.50 $\pm$ 0.67	1.12 $\pm$ 0.21 6.19 $\pm$ 1.01	1.12 $\pm$ 0.22 7.73 $\pm$ 0.91
	Middle-aged	1.05 $\pm$ 0.26 6.98 $\pm$ 0.92	1.01 $\pm$ 0.33 5.58 $\pm$ 0.65	1.03 $\pm$ 0.20 7.08 $\pm$ 1.02
Choline	Children	0.35 $\pm$ 0.08 2.05 $\pm$ 1.45	0.30 $\pm$ 0.03 2.06 $\pm$ 0.29	0.28 $\pm$ 0.04 1.72 $\pm$ 0.23
	Adults	0.34 $\pm$ 0.06 1.78 $\pm$ 0.25	0.29 $\pm$ 0.04 1.87 $\pm$ 0.22	0.25 $\pm$ 0.03 1.61 $\pm$ 0.15
	Middle-aged	0.36 $\pm$ 0.04 1.79 $\pm$ 0.20	0.29 $\pm$ 0.27 1.79 $\pm$ 0.31	0.26 $\pm$ 0.05 1.58 $\pm$ 0.30
Myo-inositol ^a	Children	0.71 $\pm$ 0.15 4.00 $\pm$ 0.57	0.30 $\pm$ 0.03 2.05 $\pm$ 0.43	0.28 $\pm$ 0.04 1.72 $\pm$ 0.33
	Adults	0.51 $\pm$ 0.10 3.60 $\pm$ 0.57	0.40 $\pm$ 0.09 4.86 $\pm$ 0.97	0.60 $\pm$ 0.15 4.32 $\pm$ 0.82
	Middle-aged	0.82 $\pm$ 0.17 4.12 $\pm$ 0.76	0.99 $\pm$ 0.22 6.86 $\pm$ 1.26	0.71 $\pm$ 0.16 4.75 $\pm$ 0.96
Glutamate plus glutamine	Children	1.24 $\pm$ 0.15 5.40 $\pm$ 1.05	1.45 $\pm$ 0.13 9.90 $\pm$ 1.82	1.54 $\pm$ 0.16 9.55 $\pm$ 1.77
	Adults	1.16 $\pm$ 0.16 5.31 $\pm$ 0.89	1.30 $\pm$ 0.25 9.07 $\pm$ 1.76	1.33 $\pm$ 0.30 8.24 $\pm$ 1.65
	Middle-aged	1.08 $\pm$ 0.27 5.04 $\pm$ 1.18	1.41 $\pm$ 0.24 8.77 $\pm$ 1.52	1.14 $\pm$ 0.30 6.83 $\pm$ 1.21
Macromolecules at 0.9 ppm	Children	1.21 $\pm$ 0.15 5.32 $\pm$ 1.06	0.81 $\pm$ 0.12 5.57 $\pm$ 1.00	1.09 $\pm$ 0.09 6.75 $\pm$ 0.83
	Adults	1.05 $\pm$ 0.15 5.21 $\pm$ 0.65	0.98 $\pm$ 0.19 6.31 $\pm$ 1.02	0.98 $\pm$ 0.16 6.12 $\pm$ 0.79
	Middle-aged	1.02 $\pm$ 0.15 4.98 $\pm$ 1.19	0.91 $\pm$ 0.39 5.34 $\pm$ 2.13	1.03 $\pm$ 0.20 6.43 $\pm$ 0.99
Macromolecules at 1.2 ppm	Children	0.34 $\pm$ 0.05 1.52 $\pm$ 0.33	0.24 $\pm$ 0.06 1.65 $\pm$ 0.40	0.37 $\pm$ 0.03 2.31 $\pm$ 0.47
	Adults	0.36 $\pm$ 0.06 1.79 $\pm$ 0.54	0.31 $\pm$ 0.05 1.85 $\pm$ 0.56	0.32 $\pm$ 0.04 2.08 $\pm$ 0.45
	Middle-aged	0.32 $\pm$ 0.07 1.51 $\pm$ 0.24	0.30 $\pm$ 0.06 1.79 $\pm$ 0.36	0.33 $\pm$ 0.09 2.07 $\pm$ 0.42
Macromolecules at 1.4 ppm	Children	1.13 $\pm$ 0.48 4.51 $\pm$ 1.43	0.65 $\pm$ 0.17 4.50 $\pm$ 1.21	0.99 $\pm$ 0.20 6.17 $\pm$ 1.14
	Adults	0.94 $\pm$ 0.23 4.39 $\pm$ 1.10	0.85 $\pm$ 0.29 5.83 $\pm$ 1.33	0.85 $\pm$ 0.21 5.27 $\pm$ 1.28
	Middle-aged	0.86 $\pm$ 0.12 3.96 $\pm$ 0.90	0.88 $\pm$ 0.13 5.38 $\pm$ 0.91	0.89 $\pm$ 0.11 5.76 $\pm$ 0.98
tCr	Children	4.53 $\pm$ 0.45	6.89 $\pm$ 0.93	6.21 $\pm$ 0.36
	Adults	4.57 $\pm$ 0.85	6.88 $\pm$ 0.97	6.22 $\pm$ 0.57
	Middle-aged	4.91 $\pm$ 0.37	6.10 $\pm$ 0.94	6.25 $\pm$ 0.49

^aSignificant difference ($p < 0.05$).

Figure 3. Negative correlations between NAA (N-acetylaspartic acid)/tCr (total creatine) and age in the thalamus.

($p_{\text{Adults vs Middle-aged}} < 0.001$); in the hippocampus, the Ins absolute concentration in the Children group was significantly lower than in the Adults ($p_{\text{Adults vs Children}} < 0.001$), and the Adults group was significantly lower than the Middle-aged groups ($p_{\text{Adults vs Middle-aged}} < 0.001$); in the thalamus, the Ins absolute concentration in the Children group was also significantly lower than in the Adults ($p_{\text{Adults vs Children}} < 0.001$) and Middle-aged groups ($p_{\text{Children vs Middle-aged}} < 0.001$).

N-acetylaspartic acid

The *post hoc* multiple comparisons (using the Bonferroni correction method) of NAA suggests that (Table 5): in the centrum semiovale, the absolute concentration of NAA in the Adults group was significantly higher than in the Children ($p_{\text{Adults vs Children}} = 0.005$) and Middle-aged groups ($p_{\text{Adults vs Middle-aged}} < 0.001$); in the hippocampus, the absolute concentration of NAA in the Middle-aged group was significantly lower than in the Children ($p_{\text{Children vs Middle-aged}} = 0.004$) and Adults groups ($p_{\text{Adults vs Middle-aged}} = 0.005$); in the thalamus, the absolute concentration of NAA in the Middle-aged group was also significantly lower than in the Children ($p_{\text{Children vs Middle-aged}} < 0.001$) and Adults groups ($p_{\text{Adults vs Middle-aged}} < 0.001$).

Correlations

Linear regression analysis confirmed that there is a negative correlation between NAA/tCr and age in the thalamus (Figure 3; $r_{\text{thalamus}} = -0.451$; $p = 0.003$) and a positive correlation between Ins/tCr and age in the thalamus (Figure 4; $r_{\text{thalamus}} = 0.522$; $p < 0.001$).

DISCUSSION

We found that the concentrations of NAA and MM09, MM12 in the thalamus are higher than in the hippocampus and in the centrum semiovale. This may be proof of a previous study²¹ that showed the grey matter may have a higher density in neurons than in the white matter.

NAA, a prominent peak in the proton spectrum, is an amino acid thought to be present in neurons and their dendritic and axonal extensions. It is localized primarily in the central and peripheral nervous system. Brain NAA is commonly believed to provide a marker of neuronal density. Sometimes, the decreased NAA levels may reflect neuronal dysfunction.³² In our study, the absolute concentration of NAA is significantly lower in the Middle-aged group than in the Children or Adults groups in the hippocampus and thalamus, and the absolute concentration of NAA in the Adults group was significantly higher than in the Children or Middle-aged groups in the centrum semiovale. These trends were nearly the same with the NAA/tCr ratio in all three regions. This may show a possibility that, in the white matter, the neural cells mature in adults, but in the grey matter, they might mature earlier than in the white matter. Furthermore, neural cells in both white matter and grey matter will decrease in middle age. Horská et al¹⁵ studied 15 subjects from 3 to 19 years old and found NAA/Cho increased to a maximum at 10 years old in the grey matter regions, but NAA/Cho increased linearly with age in the white matter. Both studies show the maximum of NAA/tCr or NAA/Cho came much earlier in the grey matter (at about 6–15 years old) than in the white matter (after 19 years old). These findings may correspond with myelination processes: the full development period of neurons was always before 7 years old, and the dendritic and axonal extensions finish and neural networks mature usually by 19 years old, which has been proved by previous anatomy and diffusion tensor imaging studies.^{22,33–35} And another observation in our research is that the increased concentration value of NAA from child to adult in the centrum semiovale (Table 5; $7.50 - 6.62 = 0.88 \text{ mmol kg}_{\text{ww}}^{-1}$) is higher than in the hippocampus (Table 5; $6.19 - 6.18 = 0.01 \text{ mmol kg}_{\text{ww}}^{-1}$) and in the thalamus (Table 5; $7.73 - 7.71 = 0.02 \text{ mmol kg}_{\text{ww}}^{-1}$). The maturation of the white matter may lead to a higher increase in NAA concentration in the centrum semiovale. Our finding may correspond with axonal development processes in the white matter elucidated in an anatomic study²² that the white matter contains more axons. The

Figure 4. Positive correlations between Ins (myo-inositol)/tCr (total creatine) and age in the thalamus.

declining trend in NAA concentration from the Adults group to the Middle-aged group is also the same as the declining trend of cognitive function. This decline in NAA concentration may be a consequence of neuronal dysfunction.³²

Ins is believed to be involved in messaging and to act as an osmolyte in glial cells. Its altered levels have been associated with Alzheimer's disease and ageing.³² In our study, we found that the absolute Ins concentration in the white matter (centrum semi-ovale), the grey matter (thalamus) and the hippocampus increases from the Adults group to the Middle-aged group. Our finding of the high level of Ins concentration in the Middle-aged group corresponds to a high density of glial cells in the ageing brain tissues.^{24,32}

We also found that there is a negative correlation between NAA/tCr and age. With increasing age and brain ageing, the neurons may become dysfunctional and gradually turn into glial cells. Our findings indicated a reduced neuronal density and an increased glial cell density with age. The ageing process has an overall effect on the whole brain, which may be the reason that the neuron density declined in different brain tissues. Especially in the thalamus, NAA/tCr has an obvious negative correlation with age (Figure 3; $r = -0.451$; $p = 0.003$), and neuronal cell density declines the most in the grey matter. The Ins/tCr has a positive correlation with age in the thalamus (Figure 4), demonstrating that the glial cell density increases with age, indicating that gliosis is possibly related to brain ageing. This finding agrees with several previous ¹H-MRS studies.^{19,20} We could use NAA/tCr and Ins/tCr as an indicator to estimate the neurons-to-glial cells ratio in the thalamus. This may be an index to distinguish the normal tissues from gliosis, and it may also be an important marker for differentiating normal brain from the age-related pathologies such as mild cognitive impairment and Alzheimer's disease.

In our study, we found that the absolute concentrations of Cho, Glx and tCr do not change with age. Although we found that Glx/tCr significantly declines with increasing age we could not find the same declining trend in absolute concentration of Glx.

Our study has several limitations. First, although we studied a wide range of subjects from 7 to 64 years old, we did not include subjects below 6 years old. The first six years of life is a critical period of development in the brain.^{21,22} We did not include these subjects because it is too hard to obtain reliable and stable spectra from them without anaesthesia. Second, MRSI has been widely used to analyse the metabolic changes in the brain with age and region.^{2,4,6,8,11,13,18} In order to obtain a high reliability of absolute quantitative metabolite levels, we stopped using MRSI to acquire the data but chose single-voxel PRESS to guarantee the high quality of spectrum. We are going to use MRSI in our further studies of the brain to obtain a map of the whole brain showing changing metabolite levels clearly. Third, the water scaling method compares the unsuppressed water signal intensity in the basis set and the acquired water signal intensity with an assumed tissue water concentration.³⁷ This water scaling method in LCModel has its limitations. This method may involve potential uncertainty since the water relaxation time may change owing to different tissue compositions.

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