〔Chiba Medical J. 88E：19 ～ 25, 2012〕

〔Original Paper〕

Age and gender differences in metabolites of normal human occipital cortex measured by proton magnetic resonance spectroscopy（^{1}H-MRS）

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（Received October 28, 2011, Accepted December 22, 2011）

SUMMARY

To determine the concentrations of N-acetylaspartate（NAA）, creatine（Cr）, and choline（Cho）metabolites in the occipital cortex of normal subjects by proton magnetic resonance spectroscopy（^{1}H-MRS）, and to investigate the effect of aging and gender on the concentrations.

Sixty-nine normal subjects（mean age, 44.0 ± 18.1 years; range, 20 to 83 years）were studied. Thirty-seven men and 32 women were examined by 1H-MRS with a whole-body 1.5-T magnetic resonance system. The relative metabolic concentrations of NAA, Cr, and Cho were determined.

The significant lower concentration of NAA within the population of men and women in the ≥ 60-years-old group compare with the 20-39-years-old group（$P < 0.001$）and the 40-59-years-old group（$P = 0.001$）was found. Women had significantly higher concentrations for all three metabolites（NAA, 57.01 ± 6.48; Cr, 33.77 ± 4.45; and Cho, 16.33 ± 3.24）than men（NAA, 47.44 ± 6.19, $P < 0.001$; Cr, 27.55 ± 5.24, $P < 0.001$; and Cho 12.99 ± 3.38, $P < 0.001$）. No significant effects of age and gender were seen for any metabolite ratios.

Our results show the spectroscopic detectable NAA, Cr, and Cho metabolites in the visual cortex and the effects of age and gender in this method.

Key words: **proton magnetic resonance spectroscopy, aging, visual occipital brain, ^{1}H-MRS, metabolite concentrations**

Ⅰ. Introduction

The visual capabilities of normal humans decline with age even without any pathological problems, and this decline is related to changes in the morphology and physiology of the visual pathways[1-3]. Aging is associated with neuronal dysfunction and a decrease of neuronal and synaptic volume in the brain, and the reduction of neuronal volume is accompanied by an

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Abbreviations: proton magnetic resonance spectroscopy（^{1}H-MRS）, N-acetylaspartate（NAA）, creatine（Cr）, choline（Cho）, volume of interest（VOI）, visually evoked potentials（VEPs）, cerebrospinal fluid（CSF）, intracranial volume（ICV）

increase in the number of glial cells[4]. The decrease in synaptic proteins observed is associated with the plasticity of axons and dendrites that contribute to cognitive dysfunctions[4]. However, the strength of the correlation of the visual pathway changes with normal aging has still not been determined.

Recent improvements in magnetic resonance (MR) imaging techniques have made it possible to obtain MR images of the functional organization and metabolic activity of the brain. These techniques include functional magnetic imaging (fMRI) and magnetic resonance spectroscopy (MRS). The MRS technique measures the activity of metabolites quantitatively in different areas of the brain[5-10]. As such, the MRS technique can detect metabolic abnormalities which in some cases may be present without any structural abnormalities in the MR images. The MRS technique is a good method to detect not only wide-spread metabolic disorders but also focal tissue-specific diseases such as tumors and inflammatory or ischemic disorders[10]. It can be used for diagnostic and monitoring purposes in different systematic and psychiatric diseases[5-13].

Proton magnetic resonance spectroscopy (^{1}H-MRS) is especially suitable for evaluating focal damage in the visual pathway. Automation of data acquisition has allowed this to be easily done in the clinic using conventional MRI scanners[10]. The standard ^{1}H-MRS method can measure several major brain metabolites, e.g., N-acetylaspartate (NAA), creatine (Cr), and choline (Cho)[5-11]. Immunohistochemical studies have shown that NAA is exclusively expressed in neurons and is a marker of neuronal integrity[7-10]. A reduction in the relative concentration of NAA corresponds to neuronal damage or loss[5-9,11].

Cr acts as a marker of the reservoir of energy and is therefore a marker of cellular metabolism. The level of Cr is used as an internal reference because it is relatively resistant to brain changes[5-9,11]. Cho is present in the plasma membrane and is a marker of cell turnover. A reduction in the concentration of Cho is linked to reduced cell division and pathological conditions, such as demyelination and malignancies[5-9,11].

Earlier ^{1}H-MRS studies reported a significant decrease in the concentrations of metabolites due to the normal aging process but in only certain brain areas[14-18]. With improvements in the signal-to-noise ratio by the use of single-voxel point resolved spectroscopy sequence (PRESS) and long TE, it is now possible to measure metabolites in different brain areas[14,19]. These ^{1}H-MRS method can measure the concentration of NAA, Cr, and Cho, in the occipital cortex (The striate visual cortex (Brodmann area 17) and some extra striate cortical areas, e.g., Brodmann area 18), where is activated by light stimuli of different shape, color, and intensity[11]. The purpose of this study was to investigate the metabolite concentrations of NAA, Cr and Cho in the visual cortical areas of normal subjects measured by ^{1}H-MRS, and how the relative concentrations are affected by aging and gender.

Ⅱ. Subjects and Methods

Subjects

Sixty-nine normal subjects (mean age 44.0 ± 18.1 years; range 20 to 83 years) were recruited from the Department of Ophthalmology of the National Rehabilitation Center for the Disabled. The best-corrective visual acuity was ≥ 1.0 decimal units in all eyes and none of the eyes has any ophthalmic diseases. None of the subjects had any systemic diseases. There were 37 men (mean age ± SD, 43.1 ± 18.6 years; range, 22 to 83 years) and 32 women (mean age, 44.2 ± 17.8 years; range, 20 to 80 years).

The subjects were divided into three age groups: the 20- to 39-years-old group included 20 men and 14 women; the 40- to 59-years-old group included 10 men and 11 women; and the ≥ 60-years-old group included 7 men and 7 women.

The procedures used conformed to the tenets of the Declaration of Helsinki, and an informed consent was obtained from all subjects after the nature and possible consequences of the study were explained. This study protocol was approved by the IRB of the National Rehabilitation Center for the Disabled.

Methods

^{1}H-MRS data acquisition

Magnetic resonance (MR) images were acquired with a 1.5-Tesla (T) magnetic resonance system (MAGNETOM, Vision Plus, SIEMENS, Germany) using a head coil suited for magnetic resonance imaging (MRI) and ^{1}H-MRS. The spectroscopic volume of interest (VOI) was localized in the midsagittal area of the visual cortex and included both hemispheres (figure 1) based on T_1 weighted sagittal, axial and coronal images (scanning parameters were: TE = 135 millisecond (ms), TR = 1.5 s, and 100 signal averages) with use of the spin-echo sequence. The size of the VOI was 20 × 20 × 20 mm, and the total scan time was 150 sec / voxel and the ^{1}H-MRS spectra were acquired with PRESS, and chemical shift selective saturation pulse with a Gaussian function with a half-width of 5 or 8 Hz. The integral of each peak was determined by curve-fitting software provided manufacturer. The metabolite peaks on the ^{1}H-MR spectrum NAA resonated at 2.01 parts per million (p.p.m), Cr resonated at 3.03 p.p.m, and Cho resonated at 3.20 p.p.m (Figure 1). Individual signal intensity was used to calculate metabolite ratios (NAA / Cho, NAA / Cr, and Cho / Cr). The MR images, typical spectrum, and VOI position in a 22-year-old normal man are shown in Figure 1.

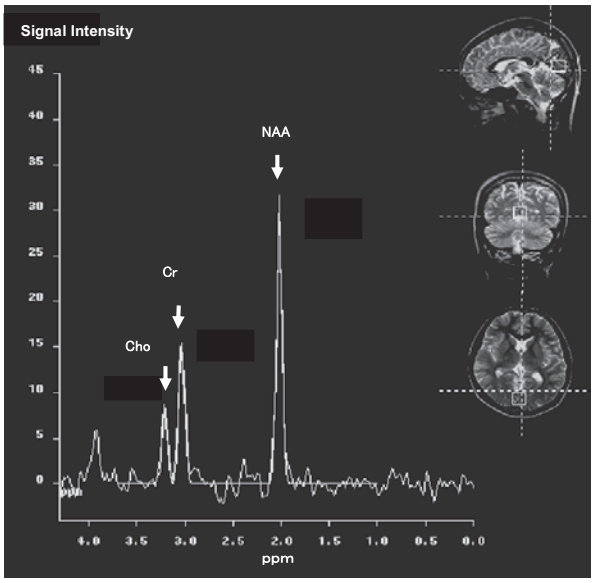

Fig. 1 Position of the volume of interest and example of a magnetic resonance sprectrum (MRS) of a 22-year-old normal man. MR images guided localization of the spectroscopic volume of interest in the occipital lobe are shown in transversal, coronal, and sagittal slices. The results of MRS are indicated as a spectrum of resonance (peaks) distributed along the X-axis labeled in parts per million (ppm). The amplitude of the resonance is measured on the y-axis using signal intensity in an arbitrary scale. The resonances of interest were N-acetylaspartate (NAA), creatine (Cr), and choline (Cho).

Statistical analyses

A one-way analysis of variance (ANOVA) was used to determine any significant differences in the concentration of the three metabolites in men and women, and among the 20-to-39-years-old group, the 40-to59-years-old group, and the ≥ 60-years-old group, and following by a post hoc test (Bonferroni's correction). All statistical analyses were performed with the SPSS 11.0 software package (SPSS Inc., Chicago, Illinois, USA). A $P < 0.05$ was considered statistically significant.

Ⅲ. Results

Results

Changes of metabolite concentrations with aging

The reductions in the relative concentrations of NAA, Cr, and Cho with increasing age are shown in Table 1. The mean metabolite concentrations of NAA (A), Cr (B) and Cho (C) in the VOI for the three groups separated by gender were shown in Figure 2. No significant age-related reduction in the concentrations of Cr and Cho was found, but in the case of NAA, there were significant lower concentration within the population of men and women in the ≥ 60-years-old group compare with the 20-39-years-old group ($P < 0.001$) and the 40-59-years-old group ($P = 0.001$). No significant effects of age were seen for any metabolite ratios (Table 2).

Gender differences

The means and standard deviations of the three metabolite concentrations for each age-group and gender are shown in Table 1. The concentrations of NAA ($P < 0.001$), Cr ($P < 0.001$), and Cho ($P < 0.001$) were significantly higher in women than in men for all age groups. Significant differences in the concentration of

Table 1 Signal intensity of metabolites

	Men	Women
N-acetylaspartate (NAA)		
All	47.44 ± 6.19	57.01 ± 6.48
20-39	38.60 ± 5.14	60.47 ± 3.51
40-59	49.01 ± 4.75	58.92 ± 5.81
≥ 60	41.86 ± 8.22	48.53 ± 4.68
Creatine (Cr)		
All	27.55 ± 5.24	33.77 ± 4.45
20-39	28.30 ± 3.56	34.22 ± 3.83
40-59	28.94 ± 4.61	34.23 ± 4.28
≥ 60	23.40 ± 8.21	32.61 ± 6.23
Choline (Cho)		
All	12.99 ± 3.38	16.33 ± 3.24
20-39	13.08 ± 3.15	15.33 ± 3.29
40-59	13.63 ± 3.91	17.14 ± 3.12
≥ 60	11.80 ± 3.42	17.19 ± 3.37

Numbers represent mean ± standard deviation (SD).

Table 2 The means and standard deviations of the metabolite ratios

age group	gender	Metabolite ratio		
		NAA / Cho	NAA / Cr	Cho / Cr
20-39	men	3.68 ± 0.80	1.64 ± 0.22	0.46 ± 0.10
	women	4.11 ± 0.88	1.79 ± 0.22	0.45 ± 0.07
	all	4.01 ± 0.69	1.76 ± 0.23	0.46 ± 0.09
40-59	men	3.99 ± 1.10	1.73 ± 0.32	0.45 ± 0.09
	women	3.25 ± 0.98	1.58 ± 0.46	0.47 ± 0.16
	all	3.63 ± 0.97	1.68 ± 0.35	0.47 ± 0.12
≥ 60	men	3.69 ± 0.72	1.88 ± 0.39	0.51 ± 0.88
	women	2.98 ± 1.12	1.47 ± 0.39	0.49 ± 0.15
	all	3.47 ± 1.00	1.67 ± 0.38	0.49 ± 0.12

Numbers represent mean ± standard deviation (SD).

NAA between men and women was similarly found in all age groups (20-to-39-years-old group, $P < 0.001$; 40-to-59-years-old group, $P = 0.001$; and ≥ 60-years-old group, $P = 0.02$). A significantly higher concentration of Cr was also found in women than in men in all three age groups ($P < 0.01$). No significant differences in the concentration of Cho were found between women and men in 20-to-39-years-old group and 40-to-59-years-old group, but there was a significant difference in ≥ 60-years-old group ($P < 0.01$). No significant effects of age and gender were seen for any metabolite ratios (Table 2).

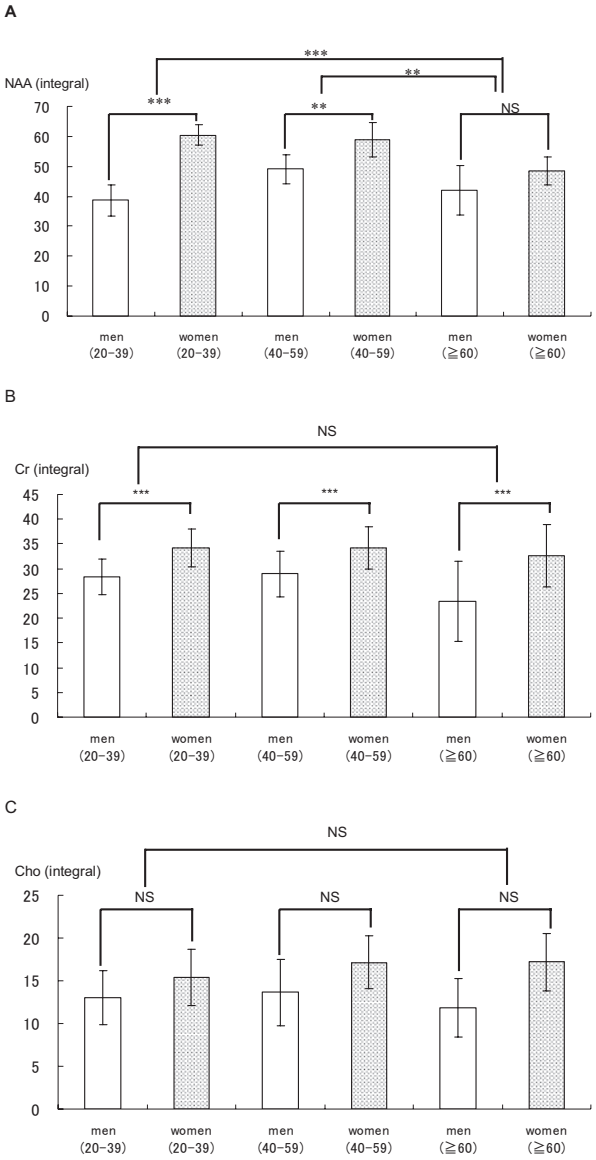

Fig. 2 The mean metabolite concentration in the visual brain area

The concentrations of NAA (A), Cr (B), Cho (C) between men (white) and women (black dots) in the visual brain area with relation to age was shown.

Note – significant correlation coefficients: $^{**}P = 0.001$, $^{***}P < 0.001$

Ⅳ. Discussion

Our major findings were that the relative concentration of NAA in the visual cortex decreased in ≥ 60-years-old group, and significantly higher concentrations of NAA, Cr, and Cho metabolites were found in women than in men for all ages.

Our findings show that a significant reduction of the concentration of NAA in ≥ 60-years-old group; however, no statistic reduction in the concentration of Cr and Cho were found (Figure 2). Cr is fairly stable and commonly used as internal standard[5-9,11]. Cho is partially MR visible because its visible form is released under

pathological conditions, such as acute myelin break down and increased cellular density[5-9,11]. Therefore, it can be argued that the decrease in the relative concentration of NAA was due to a decrease in the number of cells in the visual cortex, but this did not explain the lack of significant changes in Cr and Cho with age. Our NAA findings support earlier anatomical, histological, and radiological studies that demonstrated a significant correlation between age and neuronal dysfunction, and a link between aging and neuronal or synaptic brain volume decreases[4].

It has been reported that there is a significant age-related decrease in the NAA concentration in the semioval and temporal area of the brain[4]. In addition, a significant difference in the degree of decrease of the absolute levels of NAA in the occipital cortex between younger and older age groups has been shown, while other metabolites did not show any significant age-related changes[15]. Comparing these findings to our results, a significant decrease in the NAA concentration in the occipital cortex with increasing age as opposed to no change in the other metabolites were found as previously reported.

The comparison of genders showed significantly higher concentrations of NAA, Cr and Cho in women than in men (Table 1). Previous ^{1}H-MRS studies that investigated metabolite differences in different brain regions with relation to the gender in normal subjects obtained different findings[14,20]. A set of normal values in various brain regions was provided, but no gender difference in metabolic ratios was found[14]. Gender differences in the CSF, intracranial volume (ICV), and metabolite concentrations of NAA, Cr and Cho in the parieto-occipital region were investigated by single-voxel ^{1}H-MRS[20]. The mean brain volume, CSF, and ICV were significantly higher in men than women, but the NAA / Cr ratio was not significant different in men and women[20]. There are morphological and biochemical differences related to gender which would suggest that women have higher axonal density than men [20]. Earlier studies showed that the brain of men is larger than that of women; however, women have more neurons[21,22]. In addition, it is known that there are gender-specific differences in the concentrations of some of the neurotransmitters in different parts of the brain as well as significant differences in neuronal numbers, brain structure, and function[21,22]. These gender differences support our findings that the concentrations of NAA, Cr and Cho metabolites in the visual cortex are significantly higher in women than in men for all ages.

Our findings show that no significant effects of age and gender were seen for any metabolite ratios. Earlier clinical applications of ^{1}H-MRS mainly focused on the ratios of various metabolites which were used to set limits between normal and pathological processes. In case of malignancy, a linear relationship between Cho and NAA and ratio of Cho / NAA is increased [10]. The NAA / Cr ratio enable differentiation of Alzheimer's disease from other dementia with a relatively high specifity and sensitivity[16]. The variability in Cho / Cr could be biological in natural[14]. In this study, different results between the relative metabolite concentrations and the metabolite ratios due to normal variation of metabolites which occurs by aging or gender, must be more susceptible to fractional change than pathological changes. In addition, the ratio of metabolites can be misleading, because the concentrations of both metabolites can change.

There are some limitations in our ^{1}H-MRS study. First, single-voxel MRS measurements display the metabolite distribution, but its performance is limited by the sensitivity of the technique. The sensitivity of this technique can be increased by using a stronger magnetic field[10]. Otherwise, compared with PRESS, stimulated echo acquisition mode (STEAM) is benefit of its shorter echo times can be used to detect small metabolites with shorter T2 time. PRESS technique was used in this study, because it took advantage of improved signal intensity-to-noise ratio and simpler spectrum with less peak interference from lipid signals and metabolite with short T2 value, which helped in the interpretation of the spectrum. Longer echo times, such as 270 ms can be used, but they can get in signal intensity loss due to T2 signal decay[4]. Kreis et al obtained five different echo times (30, 60, 90, 135, 270 ms) spectra with constant recovery time, the myo-inositol (together with

Cho）peaks dominates at the shortest echo time. The metabolite peak areas do not decreased exponentially with increasing echo time, it is only at the longest echo time that the baseline must be considerable[23]. A TE of 135 ms was used in this study, because it can detect under the less influence of J-modulation and T2 relaxation[4,23]. Second, the voxel was located at the striate visual cortex（Brodmann area 17）and included some extra striate cortical areas, e.g. Brodmann area 18. Therefore, the examination was not limited to the visual cortex due to technical limitations. Thus, the results do not measure the exact visual areas but contains other areas. Third, we applied the same size of voxel for all subjects, even though women have smaller brains and would have a relatively larger VOI value than men. The voxel is composed of different amounts of white and gray matter and cerebrospinal fluid（CSF）for each subject. The concentrations of metabolites of interest are lower in CSF or extracellular space[14,17]. It is not possible to precisely determine the amount of gray and white matter that is contained in each VOI. The difference in distribution of metabolite concentrations in gray and white matter must be determined by more advanced MR techniques with improved spatial resolution.

Despite these limitations, our data were acquired by a 1.5-T MR system so that this could be a simple and valuable procedure that can be used under clinical conditions. Consequently, our results show the spectroscopic detectable NAA, Cr, and Cho metabolites in the visual cortex and the effects of age and gender in this method.

Acknowledgments

Our deepest thanks go to Christine C. Boucard for providing advice and encouragement, and Duco Hamasaki, PhD for editing the manuscript.

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