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Regional effects of age and sex in magnetic resonance spectroscopy

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Age;
Sex;
Reproducibility of results

Abstract
Objective: To determine the regional effects of age and sex on the metabolic ratios obtained in the medial temporal lobe, the posteromedial region, and the frontal lobe at 1.5 T single-voxel magnetic resonance spectroscopy.
Material and methods: We used single-voxel magnetic resonance spectroscopy to study the areas of the brain most affected in neurodegenerative disease (the left frontal lobe, the left medial temporal lobe, and the posteromedial region) in 31 healthy subjects older than 55 years of age (group 1) and in 20 healthy subjects under 30 years of age (group 2). We calculated the following ratios for each voxel: N-acetyl-aspartate/creatine-phosphocreatine (NAA/Cr), N-acetyl-aspartate/choline (NAA/Cho), N-acetyl-aspartate /myoinositol (NAA/mI), choline/creatine-phosphocreatine (Cho/Cr), and myoinositol (mI/Cr). We compared the metabolic ratios in each region in each group and the correlation between age and the ratios within age ranges. Finally, we analyzed the differences in the metabolic ratios between groups and between sexes.
Results: In group 1, we found negative correlations between age and Cho/Cr in the frontal region and NAA/mI in the temporal region. In group 2, we found negative correlations between age and mI/Cr and NAA/Cho in the temporal region as well as a positive correlation between age and NAA/mI in the temporal region. In the frontal lobe and the posteromedial region, NAA/Cr, NAA/Cho, and NAA/mI were lower in group 1 ($P \leq 0.003$). No differences between groups were seen in Cho/Cr or mI/Cr. The values of the ratios differed regionally in all cases ($P < 0.001$). In the temporal lobe, NAA/Cr and Cho/Cr were higher in women ($P \leq 0.034$).

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Conclusions: When using single-voxel magnetic resonance spectroscopy, especially in patients with neurodegenerative disease, variations due to region, age, and sex should always be taken into account.

PALABRAS CLAVE
Espectroscopia;
Resonancia magnética;
Cerebro;
Lóbulo temporal;
Lóbulo frontal;
Giro cingular;
Precuña;
Edad;
Sexo;
Reproducibilidad de resultados

Efecto regional, de la edad y el sexo en la espectroscopia por resonancia magnética cerebral

Resumen
Objetivo: Estudiar con espectroscopia univóxel por resonancia magnética (1,5 T) el efecto regional, de la edad y el sexo en las ratios metabólicas del lóbulo temporal medial, la región cerebral posteromedial (RPM) y el lóbulo frontal.
Material y métodos: Estudiamos 31 personas sanas mayores de 55 años (grupo 1) y 20 menores de 30 años (grupo 2) con espectroscopia univóxel en el lóbulo frontal izquierdo, el lóbulo temporal medial izquierdo y la RPM, especialmente afectadas por las enfermedades neurodegenerativas. Se calcularon las ratios NAA/Cr, NAA/Cho, NAA/mI, Cho/Cr, y mI/Cr, de cada vóxel. En cada grupo se compararon regionalmente las ratios metabólicas, y se estudió la correlación ratio-edad dentro de sus rangos de edad. Finalmente, se analizaron las diferencias de las ratios metabólicas entre grupos y entre sexos.
Resultados: En el grupo 1, las ratios Cho/Cr frontal y NAA/mI temporal se correlacionaron negativamente con la edad. En el grupo 2, las correlaciones con la edad fueron negativas para las ratios temporales mI/Cr y NAA/Cho, y positiva para la NAA/mI temporal. En el lóbulo frontal y la RPM, las ratios NAA/Cr, NAA/Cho y NAA/mI fueron menores en el grupo 1 ($P \leq 0{,}003$). Las ratios Cho/Cr y mI/Cr nunca mostraron diferencias entre grupos. Los valores de las ratios difirieron regionalmente en todos los casos ($P < 0{,}001$). NAA/Cr y Cho/Cr en el lóbulo temporal medial fueron mayores en las mujeres ($P \leq 0{,}034$).
Conclusiones: Cuando se utilice la espectroscopia univóxel por resonancia magnética, en particular en las enfermedades neurodegenerativas, siempre deben considerarse las variaciones inducidas por la región, la edad y el sexo.

Introduction

Magnetic resonance spectroscopy (MRS) is a technique that has been shown to be sensitive to changes in the absolute (concentrations) and relative (ratios) concentrations of some brain metabolites in neurodegenerative diseases and psychiatric disorders.[1,2] These diseases can affect patients over a wide range of ages and include changes in the connections between different brain areas including the hippocampus, posterior cingulate and precuneus.[3,4] However, patient age and brain location also influence physiological metabolic differences, and they are still under analysis.[5-8]These studies are usually separated from common clinical conditions in which magnetic resonance imaging systems and conventional software provide, in general, relative metabolic values represented by the ratios between their spectral peaks. In a previous study, we investigated the variation of water diffusion due to age and sex in a conventional magnetic resonance system.[9] It is important to perform a similar investigation with MRS as a basis for the interpretation of metabolic changes in the clinical field of neuropsychiatric diseases.

The main purpose of this MRS study is to analyze the influence of age and sex on metabolic quantification and to study the differences between brain areas affected by neurodegenerative diseases and psychiatric disorders. All this will be done using conventional magnetic resonance imaging equipment in a standard technical environment.

Material and methods

Subjects

The sample consisted of 51 individuals who were selected to be a part of two groups of healthy subjects clearly differentiated by age. The study was approved by the Local Ethics Committee, and all participants gave their informed consent. To form the first of the two groups (group 1), we took advantage of a sample of healthy volunteers who were > 55 years and already enrolled in a regional study regarding the cognitive state of asymptomatic hypertensive individuals, using convenience sequential sampling. The sequence is outlined in figure 1. As described in a previous publication,[10] 54 hypertensive patients were selected; patient were without neurological symptoms or signs and did not describe (nor did their families) the presence of symptoms of cognitive impairment. In addition, 20 normotensive relatives

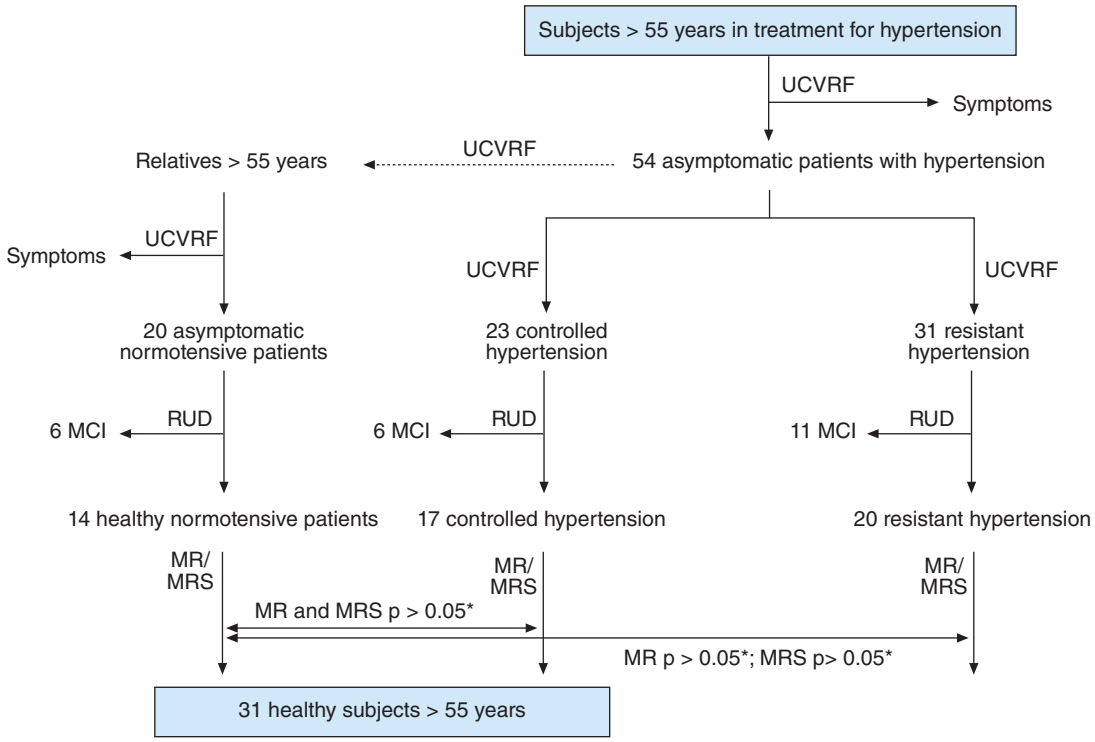


Figure 1 Flowchart of the selection process of patients in the sample. UCVRF: Unit of Control of Vascular Risk Factors, HT: Hypertension; RUD: Regional Unit for Dementia, MCI: Mild cognitive impairment, MRI: Magnetic Resonance Imaging, MRS: magnetic resonance spectroscopy. Patients with resistant hypertension were excluded from the sample because they showed metabolic differences (*) compared to the rest of subjects over 55 years old in a previous study.[10]

who fulfilled the same criteria were selected to be part of a control group of healthy individuals. None of the 74 participants met criteria for dementia according to the DSM IV (Diagnostic and Statistical Manual of Mental Disorders, fourth edition).[11] However, after studying their cognitive status through the validated Spanish version of the Mini-Mental State Examination, adjusting for age and education level (a-MMSE)[12,13] and performing a full battery of neuropsychological tests, 23 subjects were excluded due to meeting criteria for mild cognitive impairment,[14] which could affect the values of the ratios.[15] For the same reason, another 20 patients presenting with resistant hypertension were excluded because in a previous study in the same sample such patients showed metabolic differences when compared to normotensive or controlled hypertensive patients.[10] The final sample was composed of 31 subjects (19 women and 12 men). Seventeen of these patients were hypertensive but were shown not to differ from the 14 normotensive patients in demographics, clinical profile, biochemically or morphologically (structural brain imaging).[10] The mean age and standard deviation of the members of this group were 67.61 ± 4.83 years, with a range of 17 years (59-76 years). All patients were subjected to thorough clinical and laboratory studies in order to rule out metabolic and psychiatric diseases. The degrees of global cortical atrophy and cerebral ischemic involvement were analyzed using the structural MRI images, as described below.

The second study group (group 2) consisted of 20 subjects < 30 years of age (10 women and 10 men) recruited from the medical students, radiology technicians, nurses and resident doctors of the hospital. They had no remarkable family or personal history of disease, and they underwent the same structural imaging and MRS study protocols but not the clinical and laboratory studies that the first group underwent. The mean age and standard deviation for this group was 24 ± 3.11 years, with a range of 10 years (19-29 years).

Imaging study protocol

Each participant was studied on conventional MRI equipment, MRI LX.1, 5 T GE Medical Systems (Milwaukee, WI) with a standard quadrature coil. Structural images consisted of a 3D spoiled gradient-recalled echo T1-weighted sequence (TR 30 ms, TE 6 ms, flip angle 45°, slice thickness 1.3 mm) in the sagittal plane, an axial plane Fast Spin Echo double echo sequence (TR 3000 ms, TE 25/90 ms, slice thickness 5 mm) and a coronal plane Fast Spin Echo T2-weighted sequence (TR 2000 ms, TE 20/120 ms, slice thickness 5 mm). The structural images allowed the determination of both the atrophy and the total ischemic burden through visual scales by a single observer (JMGS) who had 20 years of MRI experience with agreement indices rated between good and very good.[10] The total ischemic burden scores and global cortical atrophy in each patient, previously published[10], were established by simple scales.[16,17]

MRS data were obtained with the commercial software PROBE / SV, GE Medical Systems (Milwaukee, WI) using a PRESS sequence (Point-Resolved Spectroscopy) (TR / TE 1.500/35 ms, 2048 data, 128 averages) and a voxel of 2 × 2 × 2cm (8 cc) in three anatomic areas: the posteromedial

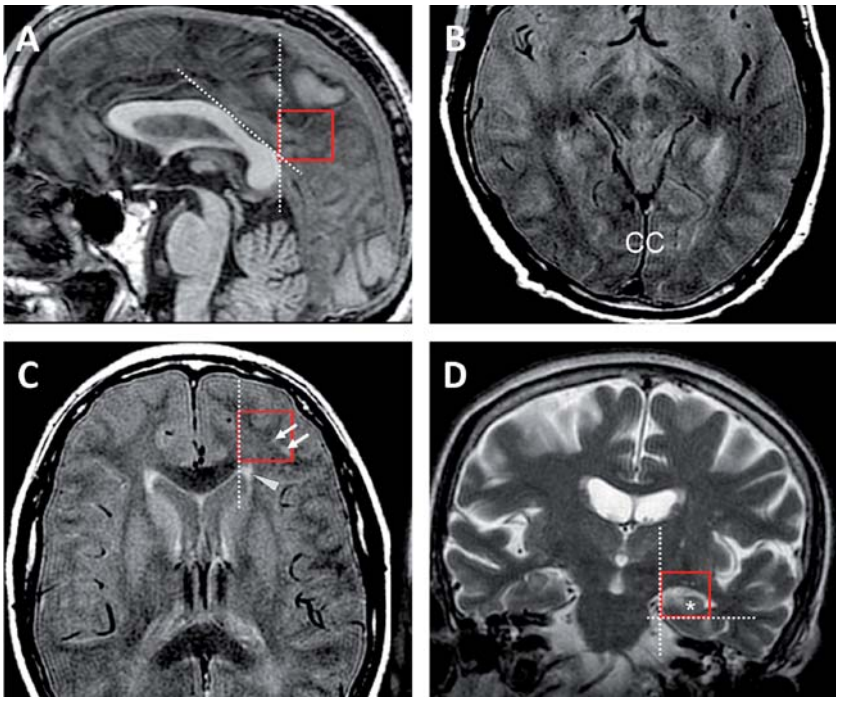


Figure 2 A) Sagittal medial T1-weighted SPGR image. Lower anterior corner of the voxel at the confluence of the dorsal- splenic line and the vertical tangent of the caudal edge of splenium. B) Axial FSE proton-density-weighted image (CC: calcarine cortex). This section serves as a reference for selecting the frontal voxel (two levels above this slice). C) Axial FSE proton density-weighted image. The medial border of the voxel coincides with the vertical line passing through the apex of the frontal horn, trying to avoid the ependymitis granularis area or the anterior periventricular hyperintensity (arrowhead), even if focal lesions in the white matter remain included (arrows). D) Coronal T2-weighted FSE image. The section is the second behind to the last coronal section showing the amygdala. The medial border of the voxel is the vertical line tangent to the medial temporal lobe margin and the bottom horizontal line tangent to the lower margin of the hippocampus (*).

region of the brain (PMB), the left frontal lobe and the left hippocampus (medial temporal lobe) (fig. 2). The choice of these brain regions was because these regions are part of the so-called "Default Mode Network"[18] that is thought to be involved in regular brain activity when the brain is not involved in any specific activity and thought to be affected in neurodegenerative diseases like Alzheimer's disease and in psychiatric disorders such as schizophrenia.[19] The algorithm of PROBE / SV adjusts the transmission and reception gains, the center frequency and the magnetic field before the PRESS sequence, while suppressing the signal from water using chemical shift (CHESS — Chemical Shift Water Suppression). The peaks of the spectrum are integrated automatically without operator intervention, and their values are assigned to N-acetyl-aspartate (NAA), choline compounds (Cho), creatine-phosphocreatine (Cr), and myo-inositol (mI) as a function of chemical shift. Using the values provided by the algorithm, the NAA/Cr, Cho/Cr and mI/Cr ratios were calculated automatically, whereas the NAA/mI and NAA/Cho ratios were calculated manually. PROBE does not allow for manually measuring of the value of the metabolic peaks over the spectra. Thus, when the algorithm failed to provide a value, the value of the corresponding ratio could not be obtained either automatically or manually, despite being visually recognizable. All spectra were evaluated by one of the authors (JMGS) who had 10 years of clinical experience in MRS, and values were considered valid when the signal to noise ratio of creatine was above the group average minus 1.5 standard deviations. Spectra were also included in the study if this ratio was not lower than two standard deviations and the peaks of NAA, creatine and choline were clearly recognizable and, at the same time, the algorithm PROBE automatically calculated their values and ratios. Spectra were excluded if the signal to noise ratio of creatine was lower than two standard deviations below the mean or if the algorithm PROBE did not simultaneously provide individual measures for the three metabolites previously mentioned.

Statistical analysis of data

All variables were expressed as percentages or mean and standard deviation, depending on their qualitative or quantitative nature. After checking the normality of the distribution of the variables with the Kolmogorov-Smirnov test, two types of analysis were run:

1. Intra-group. In each group, the correlation of metabolic ratios with age, within the range of the age of the group, was studied with the Pearson correlation coefficient. The differences in the values of metabolic ratios between different voxels were analyzed in the two groups separately with a one-way ANOVA for dependent samples and Bonferroni correction for multiple comparisons.

2. Inter-group. The differences between the two groups with respect to gender distribution, age, cortical atrophy and overall ischemic burden, and measures of metabolic ratios and differences in metabolic ratios between sexes (grouping the two groups) were analyzed with the Pearson χ^2 test and a 2-tailed Student t-test, as appropriate for each case.

In all cases, the level of statistical significance was set at $p \leq 0.05$. The analysis was performed using the SPSS 15.0 for Windows software (Chicago, IL).

Results

The spectra that were considered valid for the study varied according to the location of the voxel. Data from the PMB were adequate in the 51 participants. In the left frontal lobe, we could not obtain a valid spectrum for one of the subjects in group 1 and one in group 2. In another four cases (two subjects in group 1 and two in group 2), the value of myo-inositol was not obtained. Therefore, the left frontal sample consisted of 30 subjects in group 1 and 19 in group 2 for the NAA/Cr, Cho/Cr, NAA/Cho ratios, and 28 in group 1 and 17 in group 2 for the mI/Cr and NAA/mI ratios. The degradation of the spectrum was greater in the left medial temporal lobe. While the number of spectra valid for the NAA/Cr, Cho/Cr, NAA/Cho ratios were 30 in group 1 and 17 in group 2, the distortion of the measurement of myo-inositol reduced the size of the left medial temporal sample mI/Cr and NAA/mI ratios to 24 individuals in group 1 and 11 subjects in group 2.

Table 1 Comparison of the values of the metabolic ratios between the voxels located in different anatomical areas*

	PMB	LFL	LMTL	P ANOVA
NAA/Cr				
G1	1.52 ± 0.11	1.58 ± 0.16	1.48 ± 0.18	0.039
G2	1.63 ± 0.10	1.97 ± 0.20	1.54 ± 0.23	< 0.001
Cho/Cr				
G1	0.63 ± 0.05	0.94 ± 0.15	1.03 ± 0.15	< 0.001
G2	0.62 ± 0.08	0.91 ± 0.13	1.03 ± 0.20	< 0.001
mI/Cr				
G1	0.65 ± 0.06	0.80 ± 0.21	0.87 ± 0.16	< 0.001
G2	0.62 ± 0.07	0.69 ± 0.18	0.91 ± 0.22	< 0.001
NAA/Mi				
G1	2.37 ± 0.29	2.10 ± 0.61	1.74 ± 0.24	< 0.001
G2	2.66 ± 0.31	3.00 ± 0.68	1.75 ± 0.37	< 0.001
NAA/Cho				
G1	2.42 ± 0.27	1.71 ± 0.27	1.47 ± 0.23	< 0.001
G2	2.70 ± 0.38	2.19 ± 0.35	1.53 ± 0.29	< 0.001

G1: group 1, subjects over 55; G2: group 2, subjects younger than 30 years; LFL: left frontal lobe; LMTL: left medial temporal lobe; PMB: posteromedial region of the brain.
*All variables are presented as mean ± standard deviation.

1. Intra-group analysis. In the PMB, none of the two groups showed a significant ratio-age correlation within their respective age range. In group 1, only the Cho/Cr ratio in the left frontal lobe (r —0.375, p = 0.041) and NAA/mI in the medial temporal lobe left (r —0.485, p = 0.016) had a relationship (negative) with age. In contrast, in the left medial temporal lobe, subjects in group 2 showed a negative correlation of age with the mI/Cr (r —0.661, p = 0.027) and NAA/Cho (r —0.545, p = 0.024) ratios and a positive correlation of age with the NAA/mI (r 0.605, p = 0.049) ratio.
   In the two groups of subjects, differences in the ratios between voxels were significant in all cases (table 1). When performing multiple comparisons, 78% of the regional comparisons were significantly different. However, the NAA/Cr ratio in group 1, the mI/Cr ratio in group 2 and the NAA/mI ratio in both groups were not different in the PMB with respect to the left frontal lobe. In addition, the NAA/Cr ratios in the 2 groups were not different between the PMB and the left medial temporal lobe. Finally, the NAA/Cr and mI/Cr ratios in group 1 were not different between the left frontal lobe and the left medial temporal lobe, although the difference in the NAA/Cr ratio (p = 0.051) was virtually significant (table 2).
2. Inter-group analysis. The two groups of this study showed equal gender distribution (table 3). Similarly, although the scores on the scales of global cortical atrophy and total ischemic burden for patients in group 1 were low,

Table 2 P values for the Bonferroni correction for multiple comparisons of the values of metabolic ratios between the voxels located in different anatomical areas

	Voxels	NAA/Cr	Cho/cr	mI/Cr	NAA/mI	NAA/Cho
G1	PMB-LFL	0.543	< 0.001	< 0.001	0.095	< 0.001
G2	PMB-LFL	< 0.001	< 0.001	0.782	0.181	< 0.001
G1	PMB-LMTI	1.000	< 0.001	< 0.001	< 0.001	< 0.001
G2	PMB-LMTL	0.816	< 0.001	< 0.001	< 0.001	< 0.001
G1	LFL-LMTL	0.051	0.024	0.507	0.018	0.012
G2	LFL-LMTL	< 0.001	0.041	< 0.001	< 0.001	< 0.001

G1: group 1, subjects over 55; G2: group 2, subjects younger than 30 years; LFL: left frontal lobe; LMTL: left medial temporal lobe; PMB: posteromedial region of the brain. The differences were not significant on 22% of all multiple comparisons.

Table 3 Differences in age, gender, atrophy and ischemic burden between groups*

	Group 1	Group 2	*P*
Age	67.61 ± 4.83	24.00 ± 3.11	< 0.001
Gender			
Women	19 (61.3)	10 (50.0)	0.427
GCA**			
Score 0	14 (45.2)	20 (100)	
Score 1	11 (35.5)	0 (0)	< 0.001
Score 2	6 (19.3)	0 (0)	
Score 3	0 (0)	0 (0)	
TIB***			
Score 0-3	19 (61.3)	20 (100)	0.001
Score > 3	12 (38.7)	0 (0)	

GCAGCGCA: global cortical atrophy; TIB: total ischemic burden; group 1: subjects over 55 years, Group 2: subjects younger than 30 years.

*Variables are presented as mean ± standard deviation or number (percentage).

**The maximum score for the scale is 3 points (reference 10).

***The maximum score for the scale is 27 points (reference 10).

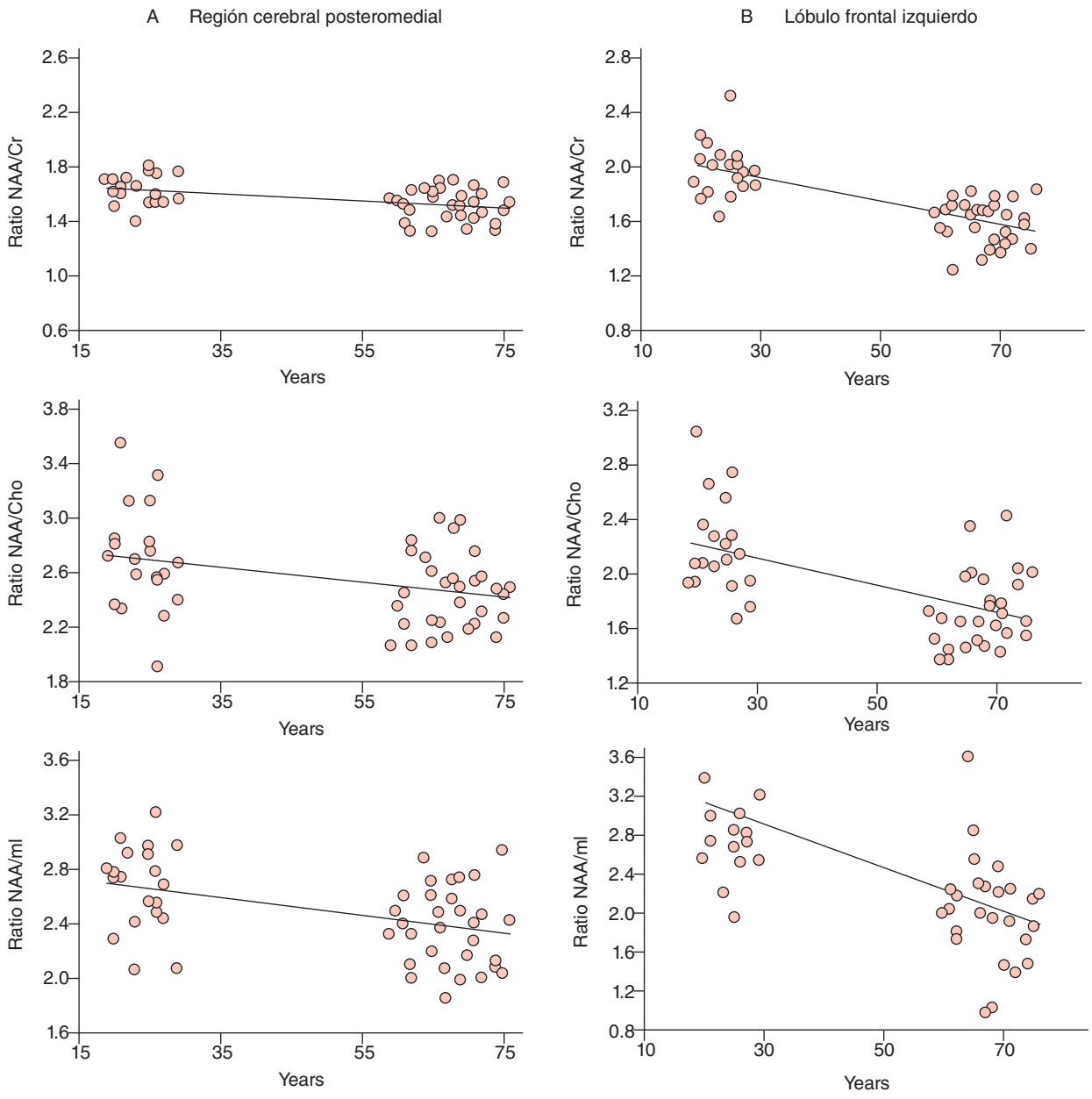
they were significantly different from those of group 2 (table 3). With respect to the comparison of the values of the metabolic ratios between the two groups, the differences between subjects from both groups were significant for all the ratios of NAA in the PMB (variation of 6.7%, 10.9% and 10 4% for the NAA/Cr, NAA/mI and NAA/Cho ratios, respectively) and in the left frontal lobe (variation of 19.8%, 28.7% and 21.9% for the NAA/Cr, NAA/mI and NAA/Cho ratios, respectively) (fig. 3). Neither the NAA ratios in the medial temporal lobe nor the Cho/Cr and mI/Cr ratios in all voxels were significantly different between the two groups. In addition, there were no differences in the values of the ratios between males and females in the PMB or the left frontal lobe. Similarly, the NAA/Cr (1.56 ± 0.22 vs. 1.44 ± 0.16, p = 0.034) and Cho/Cr (1.08 ± 0.18 vs. 0.96 ± 0,14, p = 0.013) ratios were higher in the medial temporal lobe voxel in women.

Discussion

Our study was conducted with a commonly used clinical MRI, commercial data acquisition software and quantification of metabolic ratios. In this context, the ratios varied, in general, depending on the anatomic area. Only the ratios of NAA (NAA/Cr, NAA/mI and NAA/Cho) decreased with age in the left frontal lobe and the PMB. The gender differences were sporadic and were only found to be significant in the left medial temporal lobe.

Changes in metabolic measures depending on the location of the voxel have been the rule in previous studies.[20-22] Our data (significant differences in 28 of 36 comparisons) corroborate this evidence, which is due, probably, to both the heterogeneity of tissue in the voxel (the proportion of white matter and gray matter)[20,22,23] and functional differences among the various brain areas.[24] The influence of age, however, has been more controversial. The results amongst publications have often been conflicting, including those of recent studies using 3T and 4T magnetic fields.[5-8] Despite their variability, the overall impression taken is that the NAA and its ratios tend to decrease with age, while choline, creatine, myo-inositol and to a lesser extent, the Cho/Cr and mI/Cr ratios, tend to increase. A recent systematic review has examined the age-dependent changes in ratios (except for NAA/mI) and absolute concentrations.[25] Although purely descriptive, the results of the ratios in this review suggest a marked variability. Despite this, one can gather that regardless of where a measurement is conducted, the mI/Cr ratio tends to decrease with age, whereas the NAA/Cr, NAA/Cho and Cho/Cr ratios tend to remain stable, although the first two can also decrease.[25] Nevertheless, the fundamental result of the meta-analysis was the statistical demonstration of a trend for a decrease in the absolute concentration of NAA with age (mainly by changes in the frontal lobe) and, particularly, an increase in the concentrations of choline and creatine (in this case by changes in the parietal lobe).[25] Therefore, the directions of change of the three metabolites support the trends of the ratios in our series: a decrease in the main NAA ratios and stability of the Cho/Cr ratio. Regardless of its stability, the latter showed a weak negative correlation with age within the age range of group 1 in the frontal area. This result suggests that conventional MRS can also be sensitive to changes in metabolites over short periods of time and is consistent with previous studies indicating that changes in certain metabolites can be overtly apparent from one decade to another[8] and even over much briefer periods of time.[26] Creatine in the frontal lobe or in the parietal white matter of elderly subjects may increase significantly, even over the course of a decade, in the absence of a comparable increase in choline or myo-inositol or decrease in NAA.[8,21,26-28] This suggests that the correlation between age and the frontal Cho/Cr ratio in group 1 can be determined by the increase of creatine.

In the temporal lobe, a decreased in NAA and its ratio with creatine have been previously considered as markers of aging.[29-31] In the review by Haga et al.[25], the NAA/Cr ratio decreased with age in two of the three papers exploring the hippocampus, while the Cho/Cr ratio remained stable in all three. In contrast, in our sample, the differences were not significant for any of these parameters. However, it was also in the temporal lobe where our two groups of subjects showed virtually all significant correlations between the ratios and age within the range of each group. The homogeneity of the older participants in our study can make us think that the metabolic changes described in the age range of group 1 may be more related to functional changes than to age.[32] In addition, it could suggest that a difference between the samples of the different previous studies may be responsible for the variability between the results published in those articles. Moreover, it was in the temporal lobe that we found the only metabolic differences between genders where NAA/Cr and Cho/Cr ratios were found to be higher in women. Unlike our data, most of the previous results indicate no differences between genders,[5,20,21,23,31,33,34]



	Group 1	Group 2	P
NAA/Cr	1.52 ± 0.11	1.63 ± 0.10	0.001
NAA/ml	2.37 ± 0.29	2.66 ± 0.31	0.001
NAA/Cho	2.42 ± 0.27	2.70 ± 0.38	0.003

	Group 1	Group 2	P
NAA/Cr	1.58 ± 0.16	1.97 ± 0.20	< 0.001
NAA/ml	2.14 ± 0.63	3.00 ± 0.68	< 0.001
NAA/Cho	1.71 ± 0.27	2.19 ± 0.35	< 0.001

Figure 3 Graphics of the inter-group differences of the NAA ratios in: A) the posteromedial region of the brain, and B) the left frontal lobe. Group 1, patients over 55 years; Group 2, subjects under 30 years.

although interactions between gender and metabolic quantification have also been described.[26,35] In any case, caution should be exercised when evaluating our results in the medial temporal lobe. The existence of a difference in the robustness and reproducibility of the values of the ratios according to the location of the voxel is known. Therefore, in a conventional MRI system for clinical application and with commercial software that automates the entire process, the values of ratios are more reliable in the PMB.[15] In our case, loss of data by distortion of the spectra in the frontal lobe and the medial temporal lobe decreased the sample size in those locations. This was especially important in the medial temporal lobe, where the decline of the homogeneity of the magnetic field caused by the transition between the brain and the air of the petrous bone[15] may have altered the metabolic quantification in our sample.

Sample selection is one of the main strengths of our study. However, its size is also a limitation that must be included because this is a study of normality. Nevertheless, constructing a large, disease-free sample of elderly subjects is complex in a single-center study, and the significant differences in our results are sufficient to show the trends of the metabolic ratios.[36] Our objective was to confirm the existence of these trends in the routine clinical environment and not to build tables of normality. The higher ischemic burden found in the subjects in group 1 may have also influenced the differences found, and we cannot clearly separate the effect of age with that of white matter ischemia. However, the increase in ischemic burden, despite not being physiological, is closely related to aging[37] (as is atrophy), and thus the degree of incidence in the sample of elderly subjects was fairly irrelevant. The difference in the clinical and neuropsychological assessment between the two groups is another limitation that should be highlighted, although it is highly unlikely that the sample of young subjects presents factors that would cause the measurements to deviate from normality. Unfortunately, in this study, we did not take into consideration other possible sources of variability which could be technical, physiological, or dependent upon the habits of the subjects.[38,39] Although it is unlikely that these factors invalidate our results, they could introduce effects that we can assess in future studies. Finally, the conventional technique and software used in this study are additional limitations and did not allow us to obtain reliable results in the medial temporal lobe. Moreover, an absolute quantification would have allowed us to obtain better adjusted data for changes in the metabolites.[40] However, absolute quantification is more complex, may introduce additional errors, and it is not a standard method in the clinical setting;[40] the focus of our study.

In short, in a standard technical and clinical context, age and location of the voxel result in changes in metabolic ratios in MRS studies. Therefore, these are factors that should be considered in the study of brain areas included in the Default Mode Network when they are affected by neurodegenerative and psychiatric diseases. However, technical difficulties, especially in the medial temporal lobe, can cause errors in metabolic measurements that the radiologist should also keep in mind.

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Author contributions

The authors have participated in the preparation of this work as follows:

- José María García Santos: conception, design and integrity of the study, literature search, data collection, analysis and statistical processing and interpretation of results and final editing of the article.
- Luis Fuentes: conception, design and integrity of the study, discussion of the spectroscopic data and relevant contributions to the final manuscript.
- Juan Vidal: conception, design and integrity of the study, analysis of clinical data and provided relevant clinical input in the drafting of the article.
- Martirio Antequera: integrity of the study, final design of the sample, collection and analysis of cognitive tests and relevant contributions to the final manuscript.
- Silvia Torres: integrity of the study, literature search and relevant contributions to the final manuscript.
- Carmen Antúnez: conception, design and integrity of the study, discussion of neuropsychological data and relevant intellectual contributions to the final manuscript.
- Ginés Ortega: conception, design and integrity of the study, discussion of clinical data and provided relevant clinical input in the drafting of the article.

All authors have read the final version of the article and have given their approval.

Conflicts of interest

The authors declare no conflict of interest.

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