

## **Proton magnetic resonance spectroscopy and electroencephalographic activity in attention deficit disorder**

**Arturo Alvarado, Germán Zapata,  
Larry Díaz, Zhilma Sucre, Gladys Veracochea,  
Raiza Pérez, Maritza Hernández, Francisco Itriago.**

### **Abstract**

Attention Deficit Disorder (ADD) could be considered a clinically heterogeneous condition that involves three sets of symptoms: inattention, impulsive behaviors, hyperactivity or a combination of them. Magnetic Resonance Imaging (MRI) has demonstrated size changes at cerebellum and basal ganglia of ADD in comparison with healthy child. The purpose of our work was to evaluate the activity of the brain in ADD child using Proton Magnetic Resonance Spectroscopy (1HMRS) and Electroencephalographic (EEG) activity. EEG studies were done in a Digital PL-EEG. MRI/ 1HMRS data was acquired on a 1.5 Tesla system (Magnetom Vision, Siemens Medical Systems). More than 50% of ADD child (classified as inattent) showed slow type alterations in EEG. 1HMRS study demonstrated that the main difference between the two study groups was the variation in the Cho/Cr ratio. The observed average for this ratio is significantly lower in ADD when compared with Healthy References Subjects (HR). These results suggest that a two techniques combination could be useful for the evaluation and future biochemical therapeutic trials in ADD.

**Key words:** Attention Deficit Disorder, Magnetic Resonance, Spectroscopy, and Electroencephalogram

### **Introduction**

Attention deficit disorder (ADD) could be considered as an early onset, clinically heterogeneous condition that involves three sets of symptoms: inattention, impulsive behaviors, hyperactivity or a combination of them (1,2). ADD is the most commonly diagnostic behavior disorder of childhood, estimated to affect 3 to 5 percent of school-age children. Some authors reported male-female ratios between 4:1 to 6:1 (3). The diagnosis of this condition seems easy, but on contrary is quite difficult due to the low particular organic signs or neurologic indicators that definitely indicate the presence of ADD. The most common features associated with ADD include emotional immaturity, distractibility, impulsivity, social alienation, low self-esteem, mood lability, temper outbursts, irregular academic performance and low frustration tolerance among others.

From the biochemistry point of view, there are little evidences that suggest metabolic implications associated with ADD. Some studies suggest a correlation between cerebral hypoxia or anoxia and greater frequency of attention deficits and hyperactivity (4,5). ADD characteristics have been observed in children with epilepsy and seizure disorders (6).

Previous imaging studies have tried to indicate which brain regions might miss or overfunction in ADD. Magnetic Resonance Imaging (MRI) has demonstrated that the size of the posterior vermis of the cerebellum was significant decreased in males with ADD (7) as well as significant loss of normal right/left symmetry in the caudate, smaller right globus pallidus, smaller right anterior frontal region and reversal of normal lateral ventricular asymmetry (8). Swanson et al. (9) report a 10% decrease in the basal ganglia size (caudate nucleus and globus pallidus) in ADD. Volumetric studies report localized hemispheric structural anomalies, which are concordant with models of abnormal frontal-striatum and parietal function (10). However the

most relevant information comes from the frontal regions. Previous studies from Functional Magnetic Resonance Imaging (fMRI) suggest an inability of the basal ganglia in the right frontal-striatum circuitry in suppressing outgoing stimulus (11) or subnormal activation of the prefrontal systems responsible for higher-order motor control (12). The executive functions and working memory observed in ADD children show similar patterns to those observed in patients with some kind of mistake in the function of the frontal lobe or in the neuroanatomic regions there projected. Positron Emission Tomography (PET) realized at the premotor and superior prefrontal cortex show decreased glucose metabolism in adults with ADD (13). On the other hand, many studies report differences in the Electroencephalogram (EEG) patterns in ADD. Thus, adolescents were found to have increased anterior EEG absolute theta activity and reduced posterior relative beta activity compared with controls. These support the continuation of a maturational lag and reduced cortical arousal in adolescent ADD (14). Concepts from Niedermeyer and Naidu have relationated EEG patterns in ADHD with, not a damaged but, a lazy frontal lobe, which results in uninhibited motor activity and disturbed attention (15).

The purpose of our work was to determine 1H Magnetic Resonance Spectroscopy (1H-MRS) profile in children with ADD and compare them with Healthy Children (HC) and to correlate the findings with EEG patterns on the frontal lobe of ADD children.

## **Materials and methods**

### Children with ADD and Healthy Subjects

Forty children with ADD (31 males and 9 females, mean age  $11 \pm 4$  years) and twenty children considered as healthy (14 male and 6 female, mean age  $12 \pm 2$  years) were studied. ADD diagnosis was made based on the DSM IV definition.

Four clinical specialists evaluated the children suspected as ADD:

The first evaluation consisted on a psychological interview in which the following tests were applied: Wechsler Intelligence Scale for Children-Revised (WISC-R), Bender test (motor visual), Harris test (laterality), Wepman evaluation (auditory discrimination), Write and Lecture examination, Auditive Perception evaluation and Family Paint test. A neurologist performed the second evaluation, consisting of a physical evaluation and Electroencephalogram (EEG). These criteria allowed us to exclude any child with neurological compromise.

The next step was the psychiatric examination in order to evaluate the child's mental status.

The biochemical, pharmacology and neurospectroscopy team, who validated the three previous criteria, did the fourth interview.

### EEG

All studies were done in a Digital PL-EEG, which allows us to watch 32 views (Bipolar, referential and average referential according with the International System 10-20). EEG recordings were obtained on both HR and ADD, awoken and under spontaneous sleep. The activation was done by means of Berger Technique, Lung Hyperventilation and Intermittent Light Stimulation. The frequency or spectral content of the EEG was evaluated in order to identify the four broad spectral band of clinical interest: delta (0-3 Hz), theta (4-7 Hz), alpha (8-13 Hz) and beta (above 14 Hz).

### Magnetic Resonance Imaging

All studies were performed with a 1.5 Tesla system (Magnetom Vision, Siemens Medical Systems, Erlangen, Germany) equipped with a standard CP Head coil. Sagittal T1 weighted, Coronal T2 weighted, Axial T2 weighted and T2 Fluid Attenuated Inversion Recovery (FLAIR) images were performed in ADD and HR subjects.

### Magnetic Resonance Spectroscopy

As in the MRI studies, all spectroscopy data was acquired on a 1.5 Tesla system (Magnetom Vision, Siemens Medical Systems, Erlangen, Germany). Single voxel proton MR spectra were obtained from 1.80 x 1.80 x 1.80 cm<sup>3</sup> in each of the frontal lobes. 1H-MRS data sets were acquired using a 90-180-180 spin-echo sequence (TR=1500 ms, TE=135 ms, 128 acquisitions) with three orthogonal selective radio-frequency pulses (RF). Water suppression was achieved by a 90° selective Gaussian RF pulse (CHESS). The regions of interest were selected from coronal, sagittal and axial MR images.

After voxel positioning, the following procedures were performed:

1. - Manual adjustment of the receiver for maximum water signal (CHESS pulse amplitude = 0 Volts).
2. - Automatic shimming for global field homogeneity improvement.
3. - Frequency adjustment.
4. - Manual shimming for local voxel homogeneity improvement until achieving a water bandwidth < 11 Hz.
5. - Second frequency adjustment.
6. - Transmitter adjustment.
7. - Manual adjustments of the CHESS pulse amplitude until the voltage of the water signal reached a value < 100 mV on the Analog to Digital Converter.

The combined MR imaging and MR spectroscopic data were acquired in approximately 30 minutes.

### Magnetic Resonance Spectroscopy Data Analysis

After acquisition, the MR spectroscopic data was post-processed by the following custom protocol: Apodization, Water Signal Averaging and Subtraction, Fourier Transformation, Polynomial Baseline Correction and Phase Correction.

Curve Fitting was done for N-Acetylaspartate (NAA) at 2.00 ppm, Choline (Cho) at 3.20 ppm and Creatine (Cre) at 3.00 ppm. The signal intensity was expressed as arbitrary units and the results were shown as metabolite ratios.

### Statistical Analysis

NAA/Cho+Cre, Cho/Cre, NAA/Cho and NAA/Cre ratios were calculated. Metabolite ratios were tested between ADD and HR at the right and left frontal lobe separately with repeated-measures analysis of variance with the Bonferroni correction for multiple comparisons. Also U Mann Whitney and Duncan test were applied.

## **Results**

### Electroencephalography

[Figure 1](#) shows an EEG from a HR. Note the good beta activity at the frontal regions in presence of a very well modulated alpha rhythm. In comparison, [Figure 2](#) show an EEG from an ADD. Note the increase in slow activity (theta) in presence of well-modulated alpha rhythm.

Fifty percent (50%) of ADD (classified as inattent) showed slow type alterations in EEG either paroxistic or not, located at the frontal temporal or frontal central regions.

Thirty five percent (35%) of ADD (classified as combined type) showed normal rhythms (20%), spike type or slow wave spike abnormalities (10%) and slow paroxistic at frontal temporal region (5 %).

Fifteen percent (15 %) of ADD (classified as unspecific type) showed slow wave spike frontal polar or parietal temporal (10%) and slow paroxistic bifrontal temporal (5%).

### Magnetic resonance spectroscopy

Figure 3 shows an example of 1H-MRS curves obtained in HR and Figure 4 shows 1H-MRS in ADD. Note the Cho signal intensity decrease in relation to Cre signal intensity in the ADD profile selected when compare with the HR profile. Metabolite ratio averages for the two groups of children are listed in Table 1. The results were analyzed with analysis of variance (ANOVA) and, the Bonferroni correction was applied for multiple comparisons among means. The Bonferroni p value adjusts for the fact that six comparisons were made. Thus the probability is only 5% that one or more of the comparisons would be significant with  $p < 0.05$  by chance alone.

Thus, no significant differences were found among the means of NAA/Cho+Cre and NAA/Cho ratios within all groups ( $p > 0.05$ ). Therefore, when the test for the NAA/Cre average was performed, a significant difference was found between results from ADD and HR at the left frontal lobe ( $p < 0.05$ ). The most significant ratio difference was the Cho/Cre ratio. The results showed strong differences between ADD and HR in both lobes. At the right and left frontal lobes, the p value was less than 0.01. No significant differences were found between right and left frontal, neither in ADD nor HR groups.

	NAA/Cho+Cre	NAA/Cho	NAA/Cre	Cho/Cre
Right Frontal Lobe				
ADD (n=40)	0.84±0.20	2.08±1.00	1.48±0.33	0.79±0.27
HR (n=20)	0.82±0.16	1.68±0.39	1.65±0.34	1.00±0.18
Left Frontal Lobe				
ADD (n=40)	0.79±0.20	1.90±0.61	1.41±0.37	0.80±0.26
HR (n=20)	0.80±0.25	1.70±0.41	1.74±0.42	1.02±0.14
<b>Table 1</b>				
Metabolite ratios values expressed as mean plus minus standard deviation.				

### Discussion

The most interesting finding of the current study is that single voxel 1H-MRS of the frontal lobes demonstrates some differences between ADD and HR. From the biochemistry point of view, the NAA/Cho+Cre ratio shows no significant difference between ADD and HR. High signal intensities from NAA suggest that neuronal injury is not present at ADD. As it is known the disappearance of NAA is associated either with neuronal death or axonal loss (16).

The main difference between the two study groups is the variation in the Cho/Cre ratio. The observed average for this ratio is significantly lower in ADD when compared with HR. Some interpretations can be considered for this subject. It suggests that Cho signal intensity

decreases in ADD. This may reflect either great choline containing phospholipids utilization or increase in their turnover. As well, it can also be supposed that most of the choline detected by 1H-MRS comes from the phospholipids containing choline, mainly the sum of phosphorylcholine and glycerophosphorylcholine. The fact that the ultimate source of choline for brain metabolism comes from the diet, moves us to think that future clinical trials in which cholines supplements are added could be intended in ADD children.

The second aspect is the decrease in the NAA/Cre ratio in the left front lobe of the ADD when compared with HR. Castillo et al. (17) in an early experience reported higher ratios of NAA/Cre, Glu/Cre and Cho/Cre in the left and right frontal lobes of ADD when compared with the age-matched controls. These results disagree with those reported in the present work.

The increase level of the Cre signal intensity observed in the profile of the ADD children may be related to the high levels of phosphocreatine and creatine related with brain energy or oxidative metabolism (18). This opens the possibility to correlate some changes in the brain energy with the hyperactivity in some patients with ADD.

Lactate (Lac) detection (1.30 ppm) was observed in two cases. Under physiological conditions, about 15% of the glucose metabolism in the brain leads to Lac formation. Its low resting concentration in human brain is about 1mM (19) and little variations can be documented in vivo (20). It is possibly that some alterations in the normal aerobic metabolism of the glucose are occurring in ADD. The three most common causes for the appearance of Lac in the brain spectrum are interruption of the Krebs cycle, inhibition of pyruvate dehydrogenase and an increase in the speed of glycolysis itself (21). Another interpretation for the Lac detection in ADD could be its elimination from the brain at a lower rate than in HR. Lac elimination in the brain is in proportion to the transport system saturation (18).

The last observation that will be discussed is the correlation between EEG and 1H-MRS. In this work the more frequent pattern obtained was the increase in slow activity at the frontal or frontal temporal regions. It was decided to study frontal lobe according to previous 1H-MRS (17) and fMRI reports (22), but the final decision was based on the 50% ADHD children that presented slow frontal EEG. In this study it was found 50% correlation between the EEG frontal pattern and 1H-MRS with decreased Cho/Cre, suggesting an important linkage between electric and biochemical activity of the frontal lobe. Some degree of correspondence between EEG and 1H MRS has been reported in epilepsy (23).

In conclusion single voxel 1H-MRS may be a useful tool in evaluation of ADD. Further studies are directed to correlate 1H-MRS, fMRI and EEG in order to find new directions for understanding the ADD epiphenomena.

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Figure 1

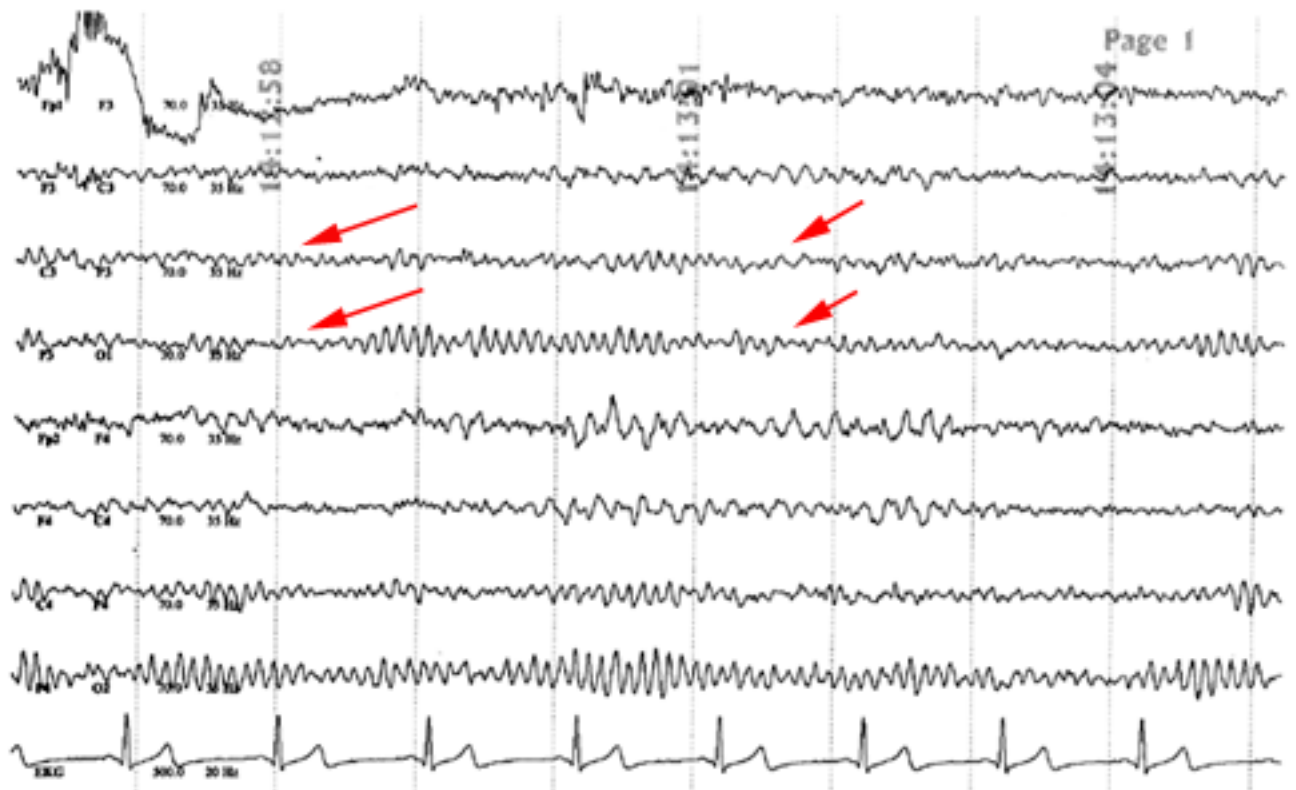


Figure 2



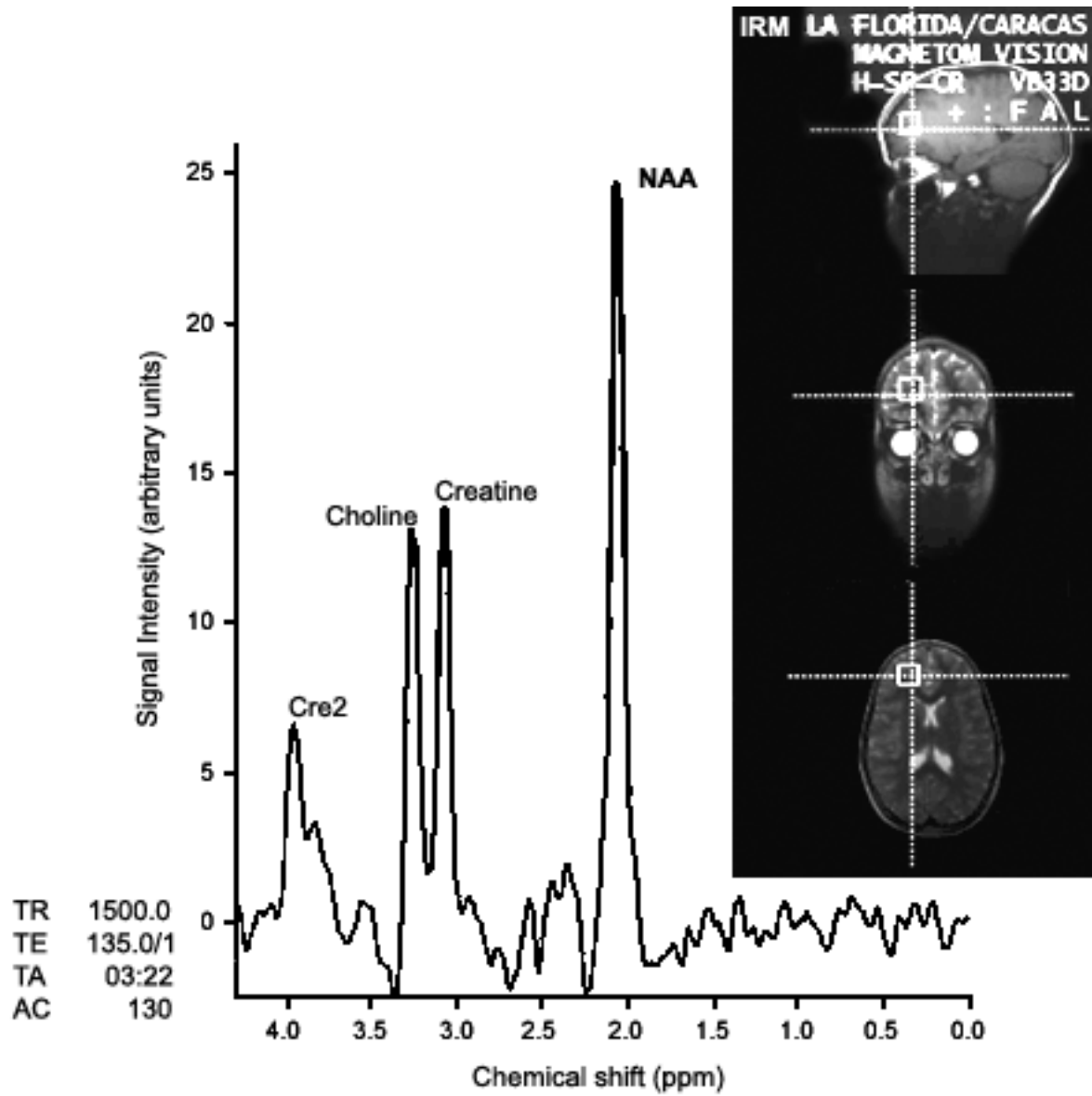


Figure 3

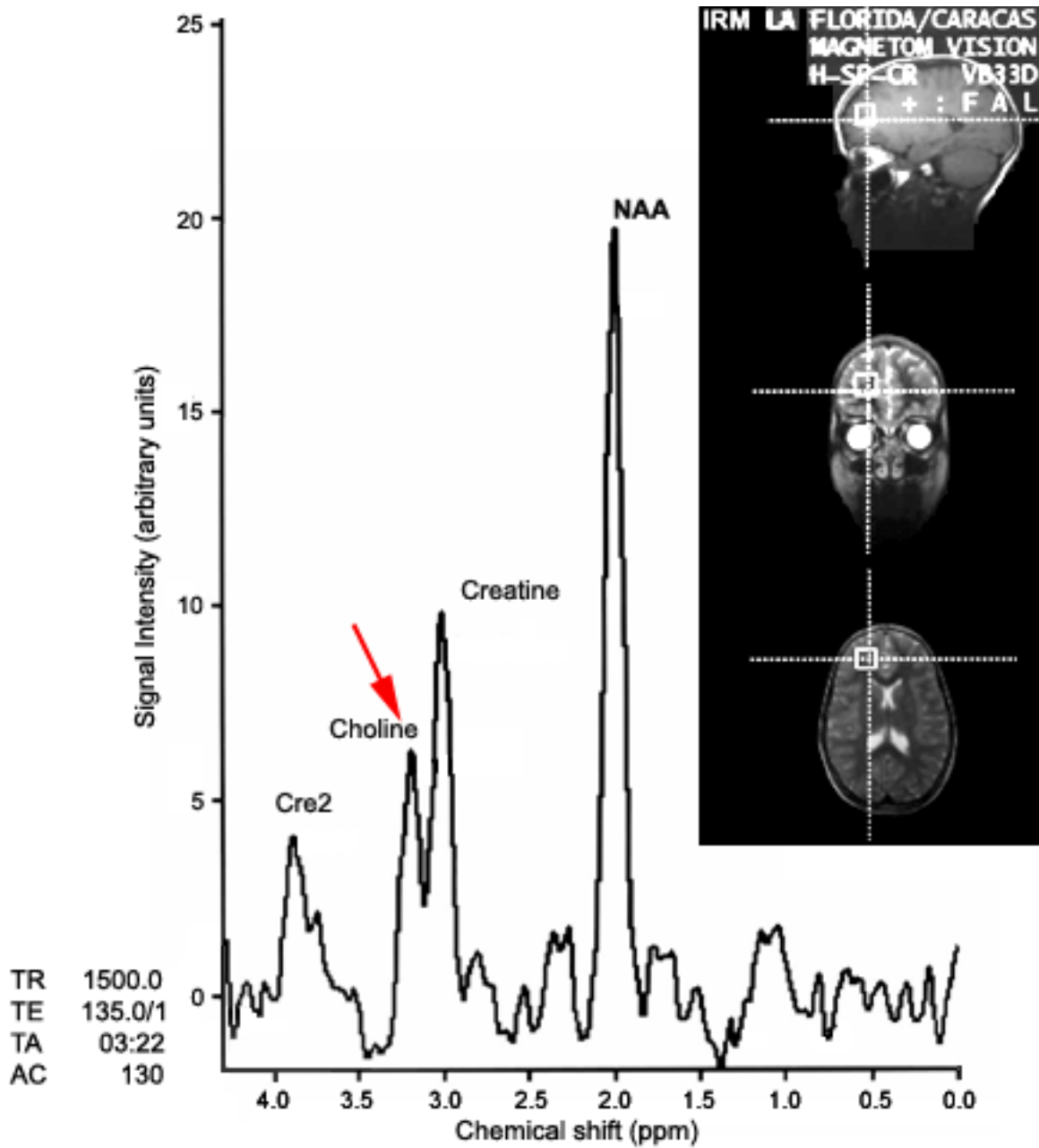


Figure 4