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Proton Magnetic Resonance Spectroscopy Reveals Medial Temporal Metabolic Abnormalities in Adolescents With History of Preterm Birth

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ABSTRACT: Prematurity is associated with volumetric reductions in specific brain areas such as the hippocampus and with metabolic changes that can be detected by spectroscopy. Short echo time (35 ms) Proton magnetic resonance spectroscopy (^{1}H MRS) was performed to assess possible medial temporal lobe metabolic abnormalities in 21 adolescents with preterm birth (mean age: 14.8, SD: 1.3) compared with an age-matched control sample (mean age: 14.8, SD: 1.6). ^{1}H MRS spectra were analyzed with linear combination model fitting, obtaining the absolute metabolite concentrations for Creatine (Cr), and myo-inositol (Ins). In addition, the following metabolite sums were measured: total Cho (glycerophospho-choline + phosphocholine), total *N*-acetyl-aspartate + *N*-acetyl-aspartylglutamate (NA), and total Glx (glutamate + glutamine). A stereological analysis was performed to calculate hippocampal volume. Absolute Cr, and total NA values were decreased in the preterm group ($p = 0.016$; $p = 0.002$, respectively). The preterm also showed a hippocampal reduction ($p < 0.0001$). Significant relationships were found between gestational age and different metabolites and the hippocampal volume. Moreover, hippocampal volume correlated with brain metabolites in the whole sample. Results demonstrate that prematurity affects medial temporal lobe metabolites, and that the alteration is related to structural changes, suggesting that the cerebral changes persist until adolescence. **(*Pediatr Res* 64: 572–577, 2008)**

In vivo proton magnetic resonance spectroscopy (^{1}H MRS) is a neurochemical technique used to investigate specific brain metabolites, which can expand on the structural and functional information obtained by other neuroimaging techniques. Volumetric magnetic resonance imaging (MRI) analyses of subjects with history of preterm birth showed temporal gray matter (GM) reductions (1) and hippocampal changes that persist until the adolescence (2,3).

A previous study reported that preterms evaluated at 40 gestational weeks showed increased *N*-acetyl-aspartate (NAA) compared with the concentrations at birth, and that the levels at the second examination did not differ from those of the full-term control group (4). These data suggest that metabolic decreases in the immature brain may normalize. In addition, a study in adolescents with preterm birth (<30 wk of gestation) found a NAA/Cho + Creatine (Cr) reduction in the right temporal lobe in a subsample of preterms ($n = 9$) compared with full-term subjects, suggesting a persistent deficit (5).

No investigations to date have assessed abnormalities in the absolute metabolic concentrations by means of the user-independent frequency domain-fitting program LCModel in a healthy preterm sample at long-term or their relationship with hippocampal volumetric atrophy. The goal of our study was to determine whether single-voxel ^{1}H MRS is able to detect alterations in the medial temporal lobe region in adolescents with preterm birth and normal MRI. We hypothesized that volume reduction of the hippocampus could be related to metabolic abnormalities and that MRI volumetry and spectroscopy could be used as complementary techniques to assess long-term consequences of prematurity.

METHODS

Subjects. The sample comprised 21 healthy adolescents born prematurely (all ≤34 wk' gestation) and without perinatal complications. Exclusion criteria were: a) history of focal traumatic brain injury; b) cerebral palsy or neurologic diagnosis (including seizure and motor disorders); c) presence of global mental disabilities; and d) antecedents of intraventricular hemorrhages or hypoxic episodes. The preterm group was matched by age to 21 healthy normal gestation controls. All subjects attended normal school. Characteristics of the groups are summarized in Table 1. The study was approved by the ethics committee of the University of Barcelona and by a national research committee. All subjects or their family gave written informed consent before participation in the study. This investigation forms part of a larger project on the long-term consequences of prematurity underway at the University of Barcelona (3,6,7).

Magnetic resonance imaging and spectroscopic acquisition. Data were obtained on a 1.5 Tesla whole body MR scanner (General Electric Signa System; Milwaukee, WI). A set of high-resolution T1-weighted images was acquired with fast spoiled gradient recalled acquisitions with the following parameters: repetition time/echo time (TE) = 12/5.2 ms, inversion time 300 ms 1 nex, field of view = 24 × 24 cm, and 256 × 256 matrix. The

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Abbreviations: Cho, choline; **Cr,** creatine; **CSF,** cerebral spinal fluid; **Glx,** glutamate + glutamine; **GM,** gray matter; **^{1}H MRS,** proton magnetic resonance spectroscopy; **ICV,** intracranial volume, Ins, myo-inositol; **NA,** *N*-acetyl-aspartate + *N*-acetyl-aspartylglutamate; **NAA,** *N*-acetyl-aspartate; **TE,** echo time,**VOI,** volume of interest

Table 1. *Characteristics of the sample*

	Prematures Mean ± SD	Controls Mean ± SD
Age (y)	14.8 ± 1.3	14.8 ± 1.6
Gender (boys/girls)	8/13	11/10
Gestational age (wk)	30.0 ± 2.0	40.0 ± 1.8
Gestational weight (g)	1375.4 ± 348.1	3453.3 ± 473.8

whole-brain data were acquired in an axial plane yielding contiguous slices with slice thickness of 1.5 mm.

^{1}H MRS was obtained with a standard quadrature head coil.Proton spectra were obtained from a volume of interest (VOI) of a single 8 cm^3 voxel (2 cm × 2 cm × 2 cm) prescribed from a coronal plane. In all subjects, the VOI was placed on the T1-weighted image in the left medial temporal region, trying to include the hippocampus in all cases. The mid brain cistern was used as the landmark to locate the VOI in all subjects, although in some cases the VOI had to be moved to avoid bone and cerebral spinal fluid (CSF) contamination. This procedure was applied in the same manner in all subjects and care was taken to ensure standard placement. Spectra were acquired with the use of a double-spin echo point-resolved spectroscopy sequence with repetition time = 1500 ms and TE = 35 ms, data points 2048, number of scans 128, scan time 3 min 48 s, with automatic shimming and water suppression. Point-resolved spectroscopy sequence is a good method for a no-loss sequence if false signals can be minimized at short TE (8). With short TE, metabolites with both short and long T2 relaxation times are observed. Apart from NAA, Cho, and Cr, additional signals can be observed of compounds such as glutamate/glutamine and myo-inositol (Ins) (9,10).

Absolute metabolite quantification: the linear combination model-fitting (LCModel). For the quantification of the absolute concentrations, we used the user-independent frequency domain-fitting program LCModel (11,12) version 6.1–4A, applying an eddy current correction (13) and using internal water signal reference to calculate absolute metabolite concentrations. Finally, to correct the absolute metabolite values we applied the mass of water in the different compartments, assuming that the relative densities of MR-visible water in GM, white matter (WM), and CSF are 0.78, 0.65, and 0.97 (14) respectively. The correction factor used was: "[(GM% × 0.78) + (WM% × 0.65) + (CSF × 0.97)]/(GM% + WM%) plus use of tissue density of 1.05 kg/L." Only corrected metabolite concentration values in mM were used to perform the analyses.

Certain metabolites are quite difficult to resolve from others (12) and the sum of the concentrations of metabolites with similar spectra is much more accurate than the individual concentrations. So, apart from the individual analysis of the Cr and the Ins compounds, we studied the sum of three pairs: NAA + *N*-acetyl-aspartylglutamate, referred to as "total NA"; glycerophospho-choline + phosphocholine, referred to as "total Cho"; and glutamate + glutamine, referred to as "total Glx". We only considered the metabolite values when the coefficient of variation for the LCModel concentrations was below 20%, indicating that these metabolites could be reliably estimated (12). For the total Glx, three subjects (two preterm and one control) had a metabolic concentration with a SD >20%. So, in these cases the values for Glx were discarded. Figure 1 shows an example of the spectra analyzed with the LCModel.

Whole brain tissue segmentation. The values for whole brain GM, WM, and CSF were obtained through the segmentation function using SPM2 software, running in Matlab 6.5 (MathWorks, Natick, MA). We segmented the original brain files obtaining a partition into GM, WM, and CSF for each subject. We obtained a specific value for each tissue in mm^3. Intracranial volume (ICV) was calculated by the sum of the three values.

Calculation of the VOI composition. To remove any effect produced by tissue content or CSF contamination in the VOI, we calculated the VOI composition in percentages of GM, WM, and CSF for each subject. The percentages were obtained applying a specific mask including the VOI on each whole brain segmented tissue maps, described above. All the VOI contents are shown in Table 2.

Stereological volumetric analysis. To provide complementary volumetric analysis, we performed stereological measurements of the left hippocampus. Measures were carried out in a Linux workstation, using ANALYZE 6.0 software. First, images were interpolated from 1.5 mm slices to 0.5 mm slices to achieve better resolution; a voxel size of 0.5 mm^3 was generated. Afterward, images were aligned in accordance with the anterior commissure–posterior commissure orientation. The hippocampal volume was measured using a 7 × 7 mm^2 rigid grid with random starting position and angle of deviation from horizontal. The grid was superimposed on every third coronal slice. The coronal orientation was chosen to work with slices oriented perpendicular to the long axis of the hippocampus, a procedure reported to improve measurements (15). The interslice increment and grid size chosen yielded a coefficient of error in the 0.01–0.03 range. The *orthogonals* tool provided by ANALYZE 6.0 makes it possible to view every grid point in three orthogonal views simultaneously, which helps to decide whether a point is contained by the measured structure or not. With stereology, we can exclude adjacent parahippocampal cortices (see Fig. 2). We obtained direct values from the hippocampal volume in mm^3. All stereological measures were corrected by the ICV*100.

Statistical analysis. Metabolic and volumetric data were compared by the *t* test or by the nonparametric Mann–Whitney *U* test in the variables that did not fulfill the requirement for parametrical statistical tests.

We performed correlation analyses to relate the gestational age (GA) at birth and the metabolic and volumetric data for the whole sample (by Spearman, because the GA at birth for the whole sample did not fulfill the normality conditions), and separately for patients and controls (by Pearson).

Finally, we performed a correlation analysis (Pearson) in the whole sample relating the metabolic values found different between groups and the hippocampal volume to evaluate the relationship between the hippocampal volume and possible changes in the brain metabolites in this region. All statistical analyses were carried out with the SPSS 14.0 version.

RESULTS

Magnetic resonance imaging. T1 visual inspection carried out by two expert neuroradiologists (N.B., J.M.M.) revealed no brain MRI abnormalities in the whole sample. No visual differences were observed in cerebral development in either group.

Whole brain volumetric data. Segmentation analyses revealed that there were no significant differences between groups in GM, WM, CSF, or total ICV (see Table 2).

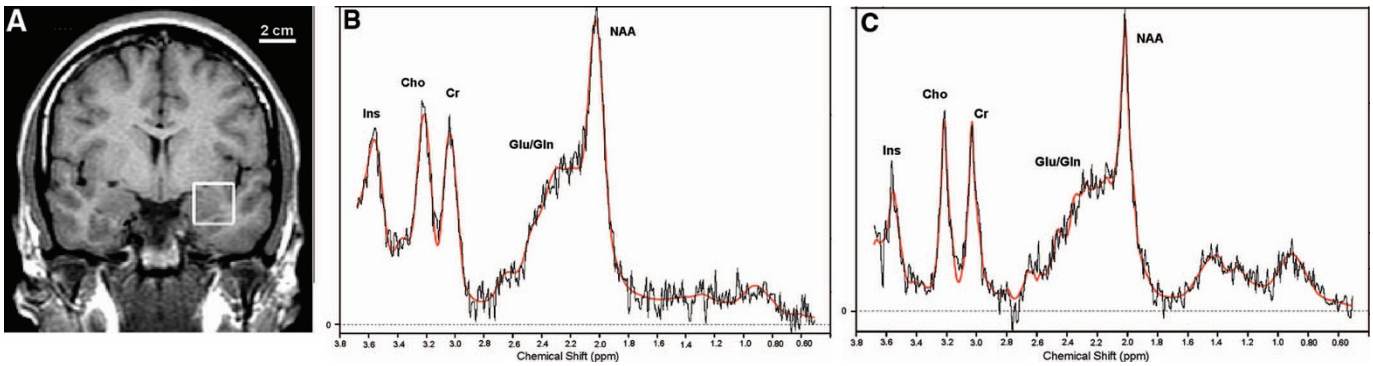

Figure 1. (*A*) Example of voxel placement. Magnification of the image ×0.4; (*B*) and (*C*) proton magnetic resonance spectra with LCModel obtained in the medial temporal lobe in a control (*B*) and in a preterm subject (*C*). Acquisition parameters: double-spin echo point-resolved spectroscopy sequence, with repetition time = 1500 ms and echo time = 35 ms. The values of the chemical shift are provided in parts per million (ppm).

Table 2. *Comparisons of whole brain volumetric data and composition of the VOI between groups*

	Preterms N = 21 Mean ± SD	Controls N = 21 Mean ± SD	Statistics (*p* value)
Whole brain volumetric data (dm^3)			
Gray matter	0.77 ± 0.07	0.80 ± 0.07	$t = -1.282$ (0.207)
White matter	0.38 ± 0.05	0.40 ± 0.04	$t = -1.562$ (0.126)
Cerebral spinal fluid	0.33 ± 0.04	0.34 ± 0.04	$t = -0.958$ (0.344)
Total intracranial volume	1.48 ± 0.13	1.54 ± 0.13	$t = -1.561$ (0.126)
Percentages of GM, WM and CSF into the VOI			
Gray matter	67.4 ± 5.9	68.7 ± 5.9	$t = -0.71$ (0.485)
White matter	22.6 ± 9.3	22.5 ± 7.4	$t = 0.01$ (0.989)
Cerebral spinal fluid	10.0 ± 5.6	8.8 ± 3.9	$t = -0.85$ (0.401)

Abbreviations: GM, gray matter; WM, white matter; CSF, cerebral spinal fluid; VOI, volume of interest.

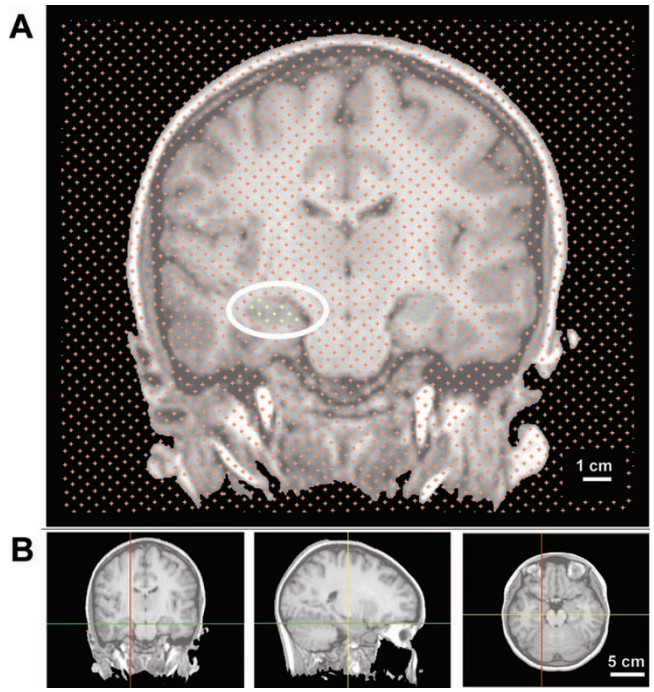

Figure 2. Illustrative stereological grid used for hippocampal measurements. Region of interest is based on a point counting estimation. Only the points inside the structure are considered in the measurements. (*A*) Circle showing left hippocampal region of interest. Magnification of the image ×0.6. (*B*) Orthogonal view option in stereology: coronal, sagittal and axial view of the same hippocampal point. Magnification of the image ×0.2.

VOI composition results. We did not find any difference in GM, WM, or CSF contained into the VOI between preterm and full-term adolescents (see Table 2).

Spectroscopy. ^{1}H MRS metabolite concentrations examined are shown in Table 3. The comparison between groups demonstrated that preterm subjects had significantly lower Cr and total NA levels than the control group. In contrast, no significant differences were found in the total Cho, the Ins or in the total Glx.

In addition, considering that NAA and Cr showed the most profound effects whereas Cho did not, as a complementary analysis, we calculated the following metabolites ratios: NAA/Cho and Cr/Cho. The results showed that the tendency and meaning of the results remain in the same way than those reported in the metabolite absolute value results (see Table 3).

Table 3. *Between groups comparison of the metabolite concentrations*

	Preterms N = 21 Mean ± SD	Controls N = 21 Mean ± SD	Statistics (*p* value)
Metabolite absolute values (mM)			
Creatine	3.5 ± 0.6 **6**	4.0 ± 0.5	$t = -2.52$ (0.016)
Total Cho	1.3 ± 0.3	1.4 ± 0.2	$t = -0.54$ (0.594)
Total NA	4.8 ± 0.8 **6**	5.6 ± 0.7	$t = -3.31$ (0.002)
Myo-inositol	3.9 ± 1.0	4.4 ± 0.9	$t = -1.90$ (0.065)
Total Glx*	9.1 ± 1.6	9.2 ± 1.2	$t = -0.23$ (0.816)
Metabolite ratio values			
Creatine/total Cho	2.7 ± 0.3 **6**	3.0 ± 0.4	$t = -2.60$ (0.013)
Total NA/total Cho	3.7 ± 0.5 **6**	4.2 ± 0.6	$t = -2.60$ (0.013)

* N = 19 preterm versus 20 controls.

Abbreviations: Total Cho, Glycerophosphocholine + Phosphocholine; Total NA, *N*-acetyl-aspartate + *N*-acetyl-aspartylglutamate; Total Glx, Glutamate + Glutamine; CSF, cerebral spinal fluid.

6: indicates lower levels of metabolite in comparison with the other group.

Hippocampal stereology. We found a significant left hippocampal volume loss in the premature group (volumes before standardization) compared with controls ($t = -5.81$; $p < 0.0001$; preterm (mean ± SD): 2335.5 mm^3 + 292.4; control (mean ± SD): 2848.1 mm^3 + 256.2). After standardization of hippocampal volume by ICV, the hippocampal volume reduction remained statistically significant ($t = -4.07$; $p < 0.0001$).

Gestational age relationships. Correlations analyses between GA at birth and the metabolic and volumetric data showed a significant positive correlation in the whole sample ($n = 42$) between GA at birth and Cr, total NA, Ins, and the volume of hippocampus (see Table 4 and Fig. 3). In the preterm group, we also observed significant positive correlations between GA at birth and total NA (see Table 4). No other correlations were observed either in the premature group and or in controls.

Hippocampal volume relationships. The study of the relationship between the volumetric data and the metabolite values revealed a significant positive correlation in the whole sample between total NA and hippocampal volume (a high significance with volume before standardization and a trend toward significance with the hippocampal volume corrected by the ICV): that is, the greater the volume of hippocampus, the higher the level of total NA (hippocampal direct values: $r = 0.51$, $p < 0.0001$; hippocampal values corrected by ICV: $r = 0.31$, $p = 0.053$). Moreover, we also found a relationship between Cr ($r = 0.43$, $p = 0.005$), Ins ($r = 0.38$, $p = 0.013$), and hippocampal volume in direct values.

DISCUSSION

In this ^{1}H MRS study, we found differences in absolute metabolite concentrations in the medial temporal lobe region between a group of adolescents with history of prematurity

Table 4. *Significant correlations between gestational age and metabolite values and volumetric data*

	Rho spearman (*p*)
Whole sample	
Metabolites	
Creatine	0.37 (0.015)
Total NA	0.47 (0.002)
Myo-inositol	0.31 (0.049)
Volumetric data	
Hippocampal volume (volume before standardization)	0.64 (<0.0001)
Hippocampal volume corrected by the Intracranial volume*100	0.37 (0.018)
Preterm sample	Pearson (*p*)
Metabolite	
Total NA	0.43 (0.052)

Abbreviations: Total NA, *N*-acetyl-aspartate + *N*-acetyl-aspartylglutamate.

and a control group. Total NA and Cr were lower in the preterm sample.

Metabolite concentrations of NA-containing compounds are thought to be localized mainly in mature neurons (16). NAA is a marker for either neuronal loss or cellular dysfunction (17). NAA values are decreased in several types of cerebral diseases (18,19), and depletion in total NA observed in this study can be interpreted as a reflection of neuronal dysfunction or significant neuronal damage (20). No previous studies in adolescent samples with preterm birth and without perinatal complications have been performed, but a study in a child sample with hypoxic-ischemic insults shows similar results (21). One study reported no differences in NAA values in a group of preterm infants compared with controls (4), but the present study did not find a normalization of total NA values at adolescence in the preterm group.

Cr is a marker of cell energy in neurons and glial cells (22). It has been suggested that low Cr concentrations in an immature brain may increase susceptibility to brain damage (*i.e.*, because of hypoxic episodes) (23). Cr depletion reported in this investigation agreed with other studies that demonstrated depletion of Cr in schizophrenic patients with hippocampal reductions (24) and in degenerative brain lesions (25). In addition, the loss of Cr may be secondary to a reduction in glial proliferation because glial cells have higher Cr levels than neurons. A previous study (26) showed that Cr levels in the developing brain reached adolescent values at 4 mo. In our case, the preterm sample did not reach control values at adolescence.

In contrast to our results, a previous investigation found no differences in brain metabolites between a group of preterm infants and a control sample (27). Most subjects in the present sample (19 out of 21) have a GA at birth <32 wk, whereas they studied infants with a GA at birth >32 wk. Moreover, they assessed the centrum semiovale for white matter, the thalamus, and the occipital GM.

Our preterm sample also showed a reduction in hippocampal volume compared with controls. This is in agreement with previous volumetric studies in adolescent samples (2,3). In the whole sample, the hippocampal volume reductions, like those of the three brain metabolites in the adolescents with preterm birth (total NA, Cr, and Ins), correlated significantly with GA at birth. In the preterm group, total NA also showed a significant correlation with GA. It may indicate that the degree of prematurity is relevant for long-term neurochemical status.

In addition, our finding about correlations between different metabolites and hippocampal volume suggests that ^{1}H MRS studies can be used to complement information about the

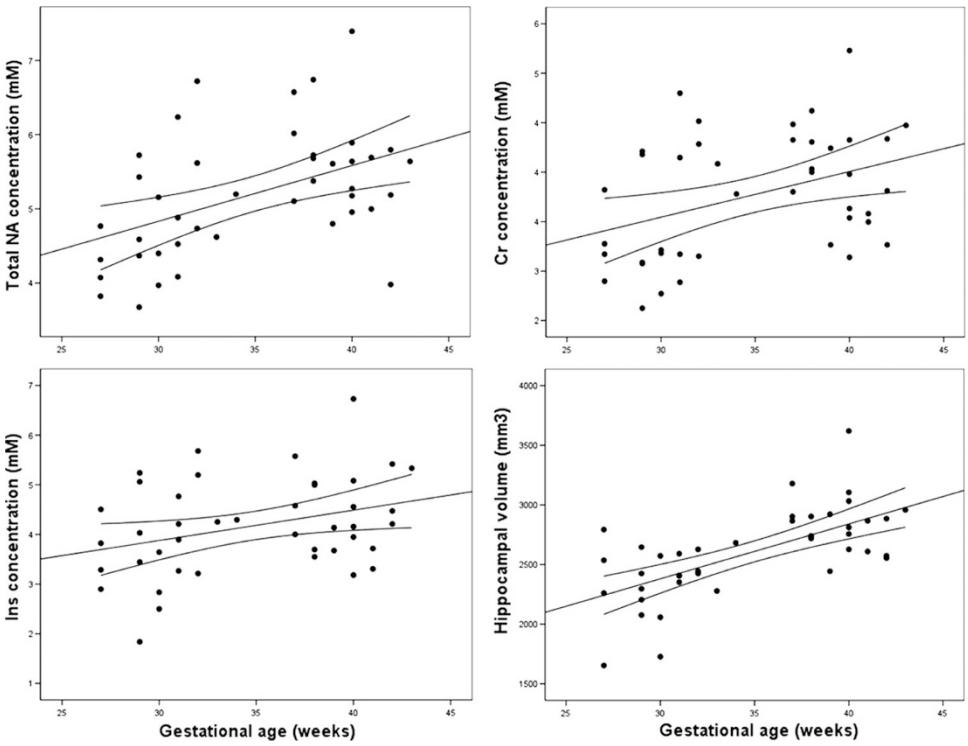

Figure 3. Plots showing corrected metabolic values and the volume of the hippocampus against gestational age. The lines indicate a linear fit to the data, with upper and lower confidence levels (95%).

hippocampal integrity. Other studies of temporal pathologies have demonstrated a relationship between metabolic and hippocampal volumetric data (28,29). It is important to take into account that neonatal intensive care exposes preterm neonates to a series of repeated, randomly occurring invasive procedures and handling, resulting in acute pain, chronic pain, and prolonged stress (30). In fact, studies using animal models indicate that perinatal stress has been shown to change NAA concentrations (31). Moreover, Cr has been reported to protect immature brain from perinatal injury (32) and the fact we showed a relationship between Cr concentration levels and hippocampal atrophy (the loss of Cr, the reduction in the hippocampal volume) favors this hypothesis.

These differences in neuronal integrity between preterms and controls may be due to several factors, including a possible differential regional vulnerability and disruptions of brain maturation. The medial temporal region has been previously reported to be especially vulnerable in preterm children compared with controls (33).

Our sample size of only 21 preterms should be considered in the current study but other technical aspects can also be mentioned. Only the left medial temporal brain region was evaluated in this study, but it is possible that other regions that have structural deficits in preterm subjects such as thalamus (3,7) or caudate nucleus (34) may present biochemical evidence of neuronal dysfunctions at adolescence. Moreover, we cannot ensure that the entire hippocampus was included in all cases, because we used the visual inspection to avoid the inclusion of CSF of the ventricular system. More recent procedures allow isolating the hippocampus by using the coordinates from three planes (35). However, since relative tissue content can affect both metabolite levels and water intensity used for quantification (14), we studied the VOI composition in the current sample and no differences in GM, WM, or CSF were found between groups. Other studies have primarily used single-voxel approaches to obtain magnetic resonance spectra from the temporal lobe and some investigations support the use of single-voxel spectroscopy for reproducibility in studies of the medial temporal lobe metabolic characteristics (36,37). Regarding the left–right question, an *in vivo* short ET ^{1}H MRS study demonstrated that there were no significant left–right differences in the study of the temporal lobe metabolites in normal subjects (38).

This study is the first to demonstrate neurochemical alterations in adolescents with history of prematurity without perinatal complications and normal standard MRI. Consistent with previous spectroscopy findings in preterm adolescents (5), we found decreased metabolite levels in the medial temporal lobe. These changes may provide support for either neuronal dysfunction or neuronal loss and may be associated with reduced neuronal integrity. In addition, these ^{1}H MRS findings were related to the hippocampal volume. This study suggests a possible abnormality in brain metabolism in the medial temporal lobe in preterm that persists until adolescence. Although we found neurochemical differences in preterm adolescents compared with controls, these differences were apparently not relevant for daily living, since our subjects received a normal schooling. Therefore, the functional implications of neurochemical changes require further investigation.

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