^{1}H-MR spectroscopy is sensitive to subtle effects of perinatal asphyxia

Article abstract—The authors performed neuropsychological and ^{1}H-MRS studies in 18 subclinical patients with antecedents of perinatal asphyxia (PA) and in 18 matched control subjects. Patients with PA showed reduced values of N-acetylaspartate (NAA) in both the basal ganglia and the midtemporal region (MTR) and reduced NAA/choline values in the MTR. Neuropsychological testing showed group differences in tasks related to attention and memory. These results indicate persistent dysfunctions in cerebral structures vulnerable to hypoxia and demonstrate the utility of MRS for the long-term evaluation of cerebral sequelae of neonatal asphyxia.

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Perinatal asphyxia (PA) produces a wide range of cerebral lesions and cognitive deficits.[1,2] However, some infants with PA will not present clear neurologic deficits or brain damage visible with structural neuroimaging,[3] although they may show subtle cognitive deficits at school age.[2]

Proton (^{1}H) MRS has proved to be a useful tool for the early evaluation of brain injury in asphyxiated neonates. High lactate levels and low *N*-acetylaspartate (NAA) levels are the most common findings.[3,4] A fall in the NAA/choline (Cho) ratio[3] also indicates adverse prognosis.

In this study, we sought to determine whether ^{1}H-MRS is sensitive to mild brain dysfunction in adolescents with antecedents of PA who exhibited no neurologic handicap during later development. A secondary aim was to relate neurochemical data with neuropsychological performance.

Methods. *Subjects.* The study sample was drawn from a total population of 647 children with documented PA, according to the criteria of two experienced child neurologists. All the children were born at term in two general hospitals in Barcelona (Catalonia, Spain) between 1978 and 1986. Clinical files were retrospectively reviewed to obtain information on the characteristics of the diagnosed asphyxia and the follow-up of each case. Only patients fulfilling at least two of the following criteria were included in the final sample: 1) umbilical artery pH of ≤7.15; 2) Apgar score at 5 minutes of ≤6; 3) meconium-stained amniotic fluid; 4) presence of intrapartum bradycardia; 5) presence of respiratory distress during the first 24 hours of life; 6) need for oxygen supply after delivery; 7) presence of neurologic anomalies during the first 48 hours of life (including tone abnormalities, neurologic depression, primitive reflex anomalies, convulsions, etc.).

From the total group with these characteristics, only patients without neurologic handicaps were considered. Patients with a diagnosis of cerebral palsy, mental retardation, or any neurosensorial deficit, as observed in the follow-up histories, were excluded. Of the total group of patients screened, clinical and outcome information could not be obtained for 165 patients (25.5%); 246 cases (38%) did not meet the inclusion criteria, because of the presence of neurologic handicaps or the lack of a clear diagnosis of PA; 34 children (5.3%) had died during the first months of life; updated address or telephone number was not available in 138 cases (21.3%); and parents or adult patients declined enrollment in 36 cases (5.6%). A group of 28 patients (4.3%) agreed to participate in the neuropsychological study. Eighteen of these patients (2.8%) also agreed to undergo the MRS study.

Patients' clinical data are shown in table 1. All the patients needed oxygen supply after delivery. Age of patients at the time of examination ranged from 13 to 21 years (mean 15.5 ± 2.83 years). Ten patients were boys and eight were girls. One patient was left-handed. The mean educational level of the group was 9.83 ± 2.5 years.

A group of 18 healthy adolescents matched for age, sex, educational level, and social background were recruited as a comparison group. All the subjects participated voluntarily. They all had histories of pregnancy with no major complications (as certified by the mother), normal birth, absence of respiratory, cardiac, or neurologic disease at present or during development, and no psychiatric antecedents or an IQ of ≤85. Subjects were aged 13 to 22 years (mean 16.33 ± 2.63 years). Eight subjects were boys and 10 were girls. Two subjects were left-handed. The mean educational level of the control subjects was slightly higher than that of the patients, but the difference was not significant (mean ± SD 10.89 ± 2.59 years; $t = -1.24$, $p = 0.22$).

Signed informed consent was obtained from all the participants or their parents.

^{1}H-MRS study. A 1.5 T MR scanner (Signa 5.4; General Electric, Milwaukee, WI) was used. Single-voxel ^{1}H-MRS was performed with two voxels ($2 \times 2 \times 2$ cm^3), one located in the left striatum from an axial section, including basal ganglia and parts of thalamus, and the other in the left midtemporal region (MTR; anterior hippocampus) from

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Table 1 *Perinatal clinical details of patients*

Case no.	Birth wt, g	Apgar 1 min (5 min)	Umbilical artery pH	Bradycardia	Meconium in AF	Respiratory distress	Neurologically depressed	Tone	Primitive reflexes	Seizures	EEG	Systemic/metabolic complications	HIE grade
1	3.100	4 (5)	7.26	No	No	No	No	N	Ab	No	N	Yes	2
2	3.620	9 (10)	7.11	No	Yes	Yes	No	Hyper	N	No	N	Yes	2
3	3.100	2 (5)	—	No	No	No	Yes	—	Ab	Yes	Ab	Yes	2
4	4.370	8 (9)	7.06	No	No	No	No	Hyper	N	No	Ab	Yes	2
5	2.650	3 (8)	7.27	No	Yes	Yes	Yes	N	Ab	No	N	Yes	2
6	2.920	8 (10)	7.09	Yes	No	No	No	Hypo	N	No	N	Yes	2
7	2.740	4 (8)	7.14	Yes	Yes	No	Yes	—	Ab	No	Ab	Yes	2
8	3.900	2 (6)	—	No	Yes	No	Yes	Hyper	Ab	No	N	Yes	2
9	3.340	9 (10)	7.16	No	Yes	No	No	N	N	No	N	No	0
10	3.660	9 (10)	7.12	No	No	No	Yes	N	N	No	Ab	Yes	2
11	3.500	8 (9)	7.17	No	No	No	No	N	N	No	N	No	0
12	3.350	1 (6)	6.98	Yes	Yes	No	Yes	Hypo	Ab	No	N	Yes	2
13	3.850	5 (7)	—	No	Yes	No	No	N	Ab	No	N	Yes	1
14	3.270	4 (10)	—	No	Yes	No	No	Hypo	N	No	N	No	1
15	3.020	5 (7)	7.28	No	Yes	Yes	No	Hypo	—	No	N	Yes	2
16	3.220	3 (8)	—	Yes	No	No	Yes	Hypo	Ab	Yes	Ab	No	2
17	2.980	3 (5)	—	No	No	No	—	Hypo	Ab	Yes	Ab	Yes	2
18	3.300	1 (3)	7.30	No	Yes	Yes	Yes	Hypo	Ab	Yes	—	Yes	2

AF = amniotic fluid; N = normal; Hypo = hypotonia; Hyper = hypertonia; Ab = abnormal; HIE = hypoxic–ischemic encephalopathy, graded by Sarnat and Sarnat[5]: 0 = no encephalopathy; 1 = mild; 2 = moderate.

a coronal section (figure). Water-suppressed spectra were acquired with the use of a double-spin echo point-resolved spectroscopy sequence (PRESS) with repetition time of 1500 milliseconds and echo times of 144 milliseconds for the basal ganglia voxel and of 35 milliseconds for the hippocampus voxel. For each location, NAA (at 2.0 ppm) and Cho (at 3.15 ppm) peaks were obtained, as well as the NAA/Cho ratio. Analysis of the spectra was performed using the manufacturer-supplied spectroscopy software package of the MR system.

Neuropsychological assessment. We included tests to assess cognitive functions associated with hippocampal and basal ganglia structures, as these structures are particularly susceptible to the effects of hypoxia detected by MRS[4]. The neuropsychological battery was as follows: Rey's Auditory Verbal Learning Test, Visual Reproduction Test from the Wechsler Memory Scale–Revised, Stroop Test, Controlled Oral Word Association Test, and Similarities and Vocabulary Subtests from the Wechsler Intelligence Scales.[6]

Statistical analysis. We used Student's *t*-test for independent samples to compare the means from the groups in both the neuropsychological and the MRS variables. Pearson's correlation with the neuropsychological variables was calculated. For all the analyses, we took the two-tailed significance.

Results. MR data from all the subjects evaluated were considered to be normal by experienced neuroradiologists. We found differences between the groups in the absolute

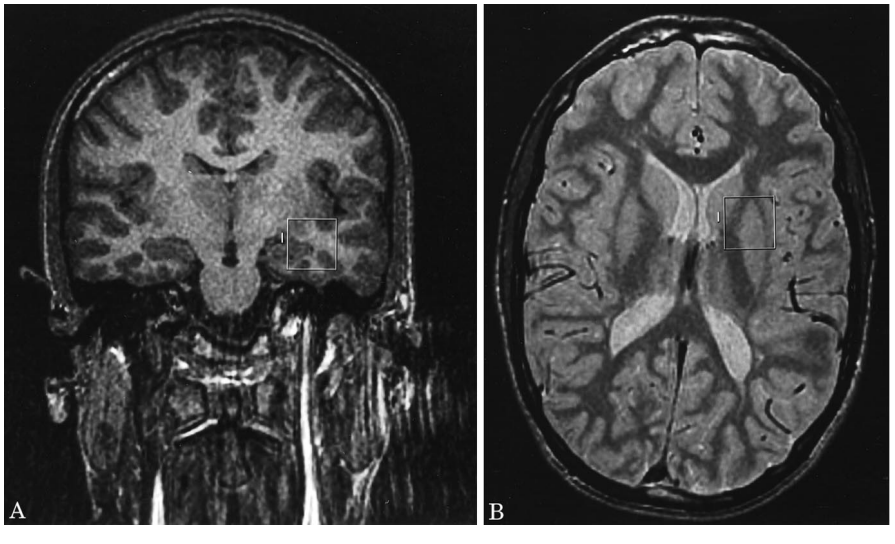

Figure. Coronal and axial ^{1}H-MR images show placement of the volume of interest in the left midtemporal region (A) and the left basal ganglia region (B).

Table 2 *Neurochemical and neuropsychological results*

Results	Voxel location	PA group, n = 18	Control group, n = 18	*t* Value	*p* Value
Metabolites					
NAA	Left basal ganglia	48.55 ± 6.31	53.11 ± 5.92	−2.23	0.032
	Left MTR	70.67 ± 12.92	80.94 ± 11.79	−2.49	0.018
Cho	Left basal ganglia	26.55 ± 4.99	28.05 ± 3.17	−1.08	0.29
	Left MTR	53.61 ± 10.85	56.11 ± 9.13	−0.75	0.46
NAA/Cho	Left basal ganglia	1.88 ± 0.37	1.89 ± 0.15	−0.17	0.86
	Left MTR	1.33 ± 0.18	1.45 ± 0.13	−2.17	0.037
Neuropsychological tests					
Similarities (direct score)		17.4 ± 3.7	18.0 ± 2.9	−0.49	0.62
Vocabulary (direct score)		47.1 ± 9.1	51.1 ± 5.5	−1.57	0.12
RAVLT					
Delayed recall		11.8 ± 2.1	13.1 ± 1.5	−2.11	0.042
Sum of words		52.1 ± 7.5	56.8 ± 5.7	−2.09	0.044
VR (WMS-R)					
Immediate		37.3 ± 2.4	38.4 ± 1.3	−1.8	0.08
Delayed		33.9 ± 5.4	37.3 ± 3.0	−2.32	0.026
COWAT		33.7 ± 11.9	40.3 ± 9.6	−1.85	0.07
Stroop Test, interference condition		34.3 ± 9.7	43.0 ± 9.4	−2.74	0.01

PA = perinatal asphyxia; MTR = midtemporal region; NAA = *N*-acetylaspartate; Cho = choline; RAVLT = Rey's Auditory Verbal Learning Test; VR (WMS-R) = Visual Reproduction Test, Wechsler Memory Scale–Revised; COWAT = Controlled Oral Word Association Test.

values of NAA from both the basal ganglia and the MTR and in the NAA/Cho ratio from the MTR location (table 2).

We also observed significant group differences in several neuropsychological tests (see table 2). Pearson correlations between cognitive scores and MRS did not reach statistical significance at the level of 0.01 in either group.

Discussion. This is the first study of the long-term neurochemical consequences of PA. We found levels of NAA in the basal ganglia and MTR to be lower in a sample of "subclinical" patients with antecedents of PA than in a well-matched control group.

Previous studies of PA using ^{1}H-MRS have shown that NAA/Cho ratios are significantly lower in patients with an adverse outcome than in survivors without handicaps[3] and that this ratio relates to neuromotor and cognitive performances at 12 months of age.[4] However, these studies compared subsamples of asphyxiated subjects but did not compare their results with those of a sample of subjects drawn from the normal population.

We found reduced values of NAA in the basal ganglia and hippocampus. This may be indicative of neuronal loss subsequent to the anoxic episode, as in the newborn, both regions are highly susceptible to hypoxia, probably because of the distribution of NMDA receptors and the high-energy requirements of these structures during development.[7] Decreased NAA in the hippocampus has been described in subjects with midtemporal neuronal loss, such as patients with AD[8] or epilepsy.[9]

Neuropsychologically, all the subjects were within the normal range for vocabulary and abstract reasoning, but their performance in tasks of verbal/visual memory and on the Stroop Test differed significantly from that of their peers. These results agree with others obtained in the long-term follow-up of neonates with PA assessed at 8 to 9 years.[2]

Previous studies have found that NAA is associated with overall neuropsychological performance in normal subjects.[10] However, we did not find this association, probably owing to the small size of the sample.

Some limitations of this study should be mentioned. First, the design is prospective with regard to the neuropsychological and MRS data but retrospective regarding the initial clinical information. This procedure of data recruitment may have introduced a bias. Second, the study was performed with 2.8% of the total screened sample. Thus, subjects who participated and those who did not may possibly differ in some aspects. To generalize the results, new data should be obtained from a larger, prospective sample. Finally, a multivoxel ^{1}H-MRS approach would be of great interest to assess other cerebral regions probably involved in PA.

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Diagnostic accuracy and certainty from sequential evaluations in peripheral neuropathy

Article abstract—Three masked neuromuscular experts analyzed the contribution of the data from sequential evaluations in predicting specific varieties of peripheral neuropathy in 72 patients. The largest improvement (16%) in diagnostic accuracy resulted from presentation of neurologic history. By contrast, diagnostic confidence increased gradually with presentation of additional medical information. Therefore, the authors conclude that for diagnostic accuracy and certainty, expert neuromuscular judgment and extensive characterizing or discriminative testing are needed.

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The methodology that physicians use to diagnose a specific health problem such as peripheral neuropathy usually involves sequential evaluations (medical history, neurologic examination, and characterizing or diagnostic tests), formulation of a short list of diagnostic categories, and then exclusion of diagnostic categories, arriving at a final diagnosis based on previous experience, published data, and judgment.[1] The contribution of the evaluative steps in making the final diagnosis with a high degree of confidence has not received the attention it deserves. Knowing which evaluative procedures best predict the final diagnosis and which provide confidence that the inference is correct is important information, useful in neurologic practice, physician training, and healthcare management.

Patients and methods. The patient cohort studied was 72 consecutive patients, mostly with neuromuscular problems, previously referred to one of us and evaluated between the dates of January 1, 1992, and June 30, 1992. The information from each evaluative step (referral medical history, referral neurologic examination, referral electrophysiologic test results, Mayo Clinic Rochester [MCR] medical history, MCR neurologic examination, and the other evaluative procedures listed in figure 1) was sequentially presented to a panel of neuromuscular physicians (the expert panel) in advanced training in peripheral nerve disease by one of us. After the presentation of the data for each evaluative step, each of the expert panel chose the likely diagnosis from a list of diagnostic possibilities (see the Appendix) and estimated their confidence that this would be the correct final diagnosis. For each evaluative step, we estimated the percentage of cases correctly identifying the final diagnosis (diagnostic accuracy).

The final diagnosis was categorized as shown in the appendix based on comprehensive medical and neurologic evaluation, electromyography, quantitative sensory testing, quantitative autonomic tests, and additional laboratory investigations. The laboratory investigations included, in most cases, complete blood counts, chemistry profiles including thyroid-stimulating hormone, serum and urine immunoelectrophoresis, immunologic markers (sedimentation rate, rheumatoid factor, antinuclear antibodies, extractable nuclear antigens, anticytoplasmatic nuclear antibodies [ANCA], and cryoglobulins), paraneoplastic antibodies (ANNA-1 and -2), chest radiograph, metastatic bone survey, serologic studies for hepatitis B and C, HIV,

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