Multiple Sclerosis in Children: Cerebral Metabolic Alterations Monitored by Localized Proton Magnetic Resonance Spectroscopy In Vivo

H. Bruhn, MD, PhD,* J. Frahm, PhD,* K. D. Merboldt, PhD,* W. Hänicke, DiplMath,* F. Hanefeld, MD,† H. J. Christen, MD,† B. Kruse, MD,† and H. J. Bauer, MD†

In vivo proton magnetic resonance spectroscopy of 8 children (7–16 years) with established multiple sclerosis revealed distinct alterations in regional cerebral metabolism associated with different aspects of the disease: (1) Localized proton spectra (2 to 4-ml volumes of interest) from multiple sclerosis plaques were generally characterized by a decrease in *N*-acetylaspartate and creatines, and an increase in cholines and *myo*-inositol relative to age-matched control subjects, (2) neither chronic nor enhancing plaques (by gadolinium–diethylenetriamine pentaacetic acid) during an acute exacerbation showed elevated levels of lactate or lipids, (3) spectra from adjacent white matter that did not appear suspicious in magnetic resonance images were similar to those of normal control subjects, and (4) cortical gray matter related to neighboring multiple sclerosis lesions showed a notable reduction of *N*-acetylaspartate. The present results show that functional impairment in multiple sclerosis is linked to gross metabolic disturbances of neuronal cell chemistry. We suggest that focal demyelination is accompanied by increased membrane precursors of proliferative turnover and is associated with secondary neuronal shrinkage or loss, perhaps extending into related cortical gray matter.

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Recent progress in the development of localized proton magnetic resonance (MR) spectroscopy (MRS) [1–3] allows the acquisition of in vivo spectra with an increasing number of metabolites and from volumes of interest (VOIs) that are sufficiently small to assess focal alterations in cerebral metabolism associated with multiple sclerosis (MS). This inflammatory disease causes multiple plaques of demyelination, predominantly in white matter with a typical size of up to a few millimeters. Previous proton MRS studies of MS plaques in adults [4–10] mainly used large VOIs that compromise the spectroscopic results by significant partial volume effects with normal brain tissue. In addition, localization sequences with long echo times (TEs) (e.g., 135–270 msec) have sacrificed all resonance signals from metabolites with short T2 relaxation times and/or with a complex modulation pattern due to strong spin-spin coupling between nuclei from the same molecule, for example, mobile lipids, inositols, γ-aminobutyrate (GABA), glutamate, glutamine, and glucose.

As part of an ongoing longitudinal study of MS in childhood [11], the noninvasive technique of image-guided, short TE, proton MRS was applied to gain new insights into alterations of cerebral metabolism associated with the progression of the disease in vivo. Although MS is a rare disorder in children and adolescents, accounting for less than 1% of all cases [12–14], information gathered at an early stage is considered to be most useful as it may provide clues to its pathogenesis. A preliminary account of part of the work has been given [15].

Materials and Methods

Subjects

Eight patients (5 girls, 3 boys; age range, 7–16 years; median age, 14 years) with established MS [16] were investigated by

From *Max-Planck-Institut für biophysikalische Chemie, Postfach, and †Abt. Kinderheilkunde, Schwerpunkt Neuropädiatrie, Georg-August-Universität, Göttingen, Federal Republic of Germany.

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Address correspondence to Dr Bruhn, Max-Planck-Institut für biophysikalische Chemie, Postfach 2841, D-3400 Göttingen, Federal Republic of Germany.

Table 1. Clinical Data of Multiple Sclerosis Patients

Patient No.	Sex	Age at Onset (yr, mo)	Age at MRS Exam (yr, mo)	Disease at MRS	Clinical Course	Score on EDSS
1	F	9	14, 9	S	I	7.0
			15, 10	PA		
2	F	11, 6	14, 6	PA	I	2.5
			15, 6	S		
			15, 10	A		
3	F	15	16, 10	S	CP	2.5
4	M	13, 1	13, 8	A	I	2.0
			14, 9	S		
5	M	3, 7	7, 5	PA	I	2.0
6	F	13, 3	16, 9	S	IP	7.5
			16, 10	A		
7	M	9, 6	14, 1	S	I	3.5
8	F	8, 6	15, 5	PA	IP	3.5

MRS = magnetic resonance spectroscopy; EDSS = expanded disability status score; A = acute exacerbation; PA = postacute; S = stable; I = intermittent; IP = intermittent progressive; CP = chronic persistent.

fast-scan magnetic resonance imaging (MRI) and short TE, localized proton MRS, 3 of them over a period of 1 year. Pertinent clinical information is summarized in Table 1. During the course of their disease all children had additional MRI examinations (6 children with enhancement by gadolinium–diethylenetriamine pentaacetic acid [Gd-DTPA]) mainly during clinically acute episodes. All children had vaccinations in infancy against diphtheria, pertussis, tetanus, and poliomyelitis; some also had vaccinations against measles, mumps, and tuberculosis. The children had mild to severe disease judged by clinical criteria according to the expanded disability status scale (EDSS) [17]. The known duration of the disease varied between 7 years to a few weeks prior to the MRS examination. Data from cerebrospinal fluid (CSF) analyses at acute episodes showed increased local immunoglobulin in all patients, and oligoclonal bands in all but 1 (Patient 6). At the time of the MRS investigations, all but 2 patients (Patients 4 and 5) had cortisone therapy. Three children were investigated during an acute episode with use of an extended MRI protocol (Gd-DTPA) to identify enhancing plaques. A number of children of adolescent age (10–17 years, n = 10) served as control subjects for MRS. The spectra matched those of 300 young adult volunteers (20–30 years) investigated in this laboratory and showed a reproducibility of ±5% for major metabolite signals in white and gray matter. MRI and MRS examinations were performed after informed consent was obtained from the parents. No sedation was necessary.

Magnetic Resonance Imaging and Spectroscopy

The examination started with a fast-scan MRI protocol comprising T1-weighted fast low-angle shot (FLASH) sequences and T2-weighted contrast enhanced-Fourier acquired steady state (CE-FAST) sequences [18]. In cross-sectional two-dimensional (2D) images (256 × 256 matrix), the slice thickness was 4 mm and the field of view, 250 mm. In some cases three-dimensional (3D) FLASH (128 × 256 × 256 matrix) was used, yielding an isotropic resolution of 1 mm within a measuring time of 8 minutes. MS lesions with impaired blood-brain barrier were identified by the uptake of Gd-DTPA (Magnevist, Schering, Berlin, Federal Republic of Germany) following intravenous application [19–21]. In these patients, proton MRS was performed in a second session a few days later, to avoid spectral distortions due to Gd-DTPA–induced magnetic field inhomogeneities.

Water-suppressed localized proton MRS was accomplished using a stimulated echo acquisition mode (STEAM) sequence with a TE of 20 msec and a mixing time (TM) of 30 msec [2]. In general, spectra from 2- to 4-ml VOIs were obtained within 6.5 minutes using a repetition time (TR) of 3,000 msec and 128 scans. Data processing included zero filling, 0.5-Hz line broadening for filtering (gaussian multiplication of the time-domain data), Fourier transformation, and zero and first-order phase correction. No further smoothing, baseline manipulation, or resolution enhancement was applied. Typically, two to six locations could be studied during one examination taking 30 to 90 minutes. All studies were performed at 2.0 tesla (Siemens Magnetom, Erlangen, Federal Republic of Germany) using the standard head coil for both MRI and MRS.

Results

Normal Brain

Proton MR spectra of white and gray matter of a healthy 12½-year-old girl are shown in Figure 1. These spectra are representative controls for the age group studied and facilitate the recognition of pathological changes. Pure white matter spectra were obtained from a supraventricular VOI of 20 × 30 × 20 mm^3 (12 ml). The 16 × 30 × 30-mm^3 (14.4-ml) VOI selected for gray matter was located symmetrically to the longitudinal fissure of the parietal lobe to ensure minimal contributions from white matter.

Resonances in proton MR spectra of brain tissue are assigned [3] to *N*-acetylaspartate (NAA; methyl singlet

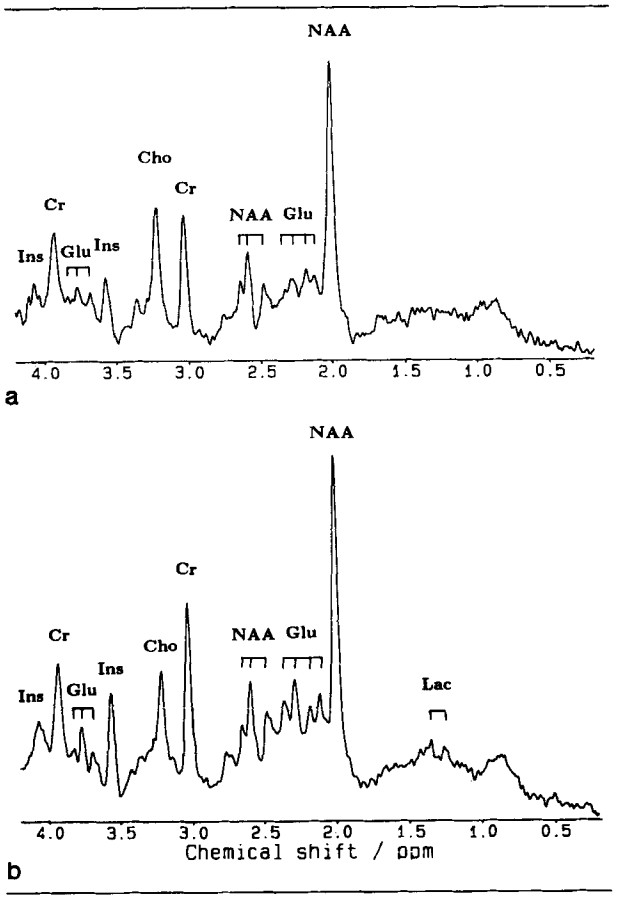

Fig 1. Proton magnetic resonance spectra (TR 3,000/TE 20 msec, 128 scans) of a 12½-year-old healthy control subject, originating (a) from a 20 × 30 × 20-mm³ (12-ml) volume of interest (VOI) in the left parietal supraventricular white matter and (b) from a 16 × 30 × 30-mm³ (14.4-ml) VOI in the parietal midline gray matter. Lac = lactic acid; NAA = N-acetylaspartate; Glu = glutamate; Cr = creatine and phosphocreatine; Cho = choline-containing compounds; Ins = myo-inositol. Chemical shifts (in ppm) are referenced to 2.01 ppm for the CH_3 group of NAA. The spectra are scaled to allow for direct comparison of peak intensities.

at 2.01 ppm, strongly coupled multiplet from the aspartyl group at 2.60 ppm), glutamate (Glu; strongly coupled methylene multiplets at 2.28 ppm, methine triplet at 3.77 ppm), creatines (Cr; creatine and phosphocreatine methyl singlets at 3.04 ppm, methylene singlets at 3.94 ppm), choline-containing compounds (Cho; trimethylammonium singlets at 3.22 ppm), and *myo*-inositol (Ins; strongly coupled methine pattern at 3.65 ppm and 4.06 ppm).

The physiological level of lactate (methyl doublet at 1.32 ppm, 7-Hz spin-spin splitting) fluctuated between a concentration below detectability and about 1 mM, as demonstrated in Figure 1b (relative to about 11 mM NAA in gray matter [22]). With the exception of lactate, the metabolite pattern in proton MR spectra of age-matched normal subjects was remarkably reproducible. Characteristic regional differences were seen, for example, in the spectra from white and gray matter in Figure 1. In particular, Cho is more concentrated in myelin-rich white matter than in gray matter, while the levels of NAA, Cr, Glu, and Ins are higher in gray matter.

The derivation of absolute metabolite concentrations from the resonance areas of fully relaxed (TR of 6,000 msec), short TE (≤ 20 msec) spectra [22] is hampered in the presence of T1 saturation (short TRs) as well as for a heterogeneous group of patients with different head sizes and variations of coil loading factors. However, experiments using different TRs revealed no relevant T1 changes of metabolite resonances in MS plaques. Thus, the magnitude of signal variations observed in spectra of MS lesions with a TR of 3,000 msec (see below) may be attributed mainly to changes in metabolite concentrations. Quantitative evaluations are presented in Table 2 as metabolite intensity ratios obtained from spectra with a TR of 3,000 msec and a TE of 20 msec. While this reduction to numbers may be useful in many circumstances, important aspects present in the original MR spectra may be obscured. This particularly applies to the overall quality and reliability of the data. To facilitate comparisons, all spectra in Figures 1 through 7 were processed and displayed in exactly the same way.

Table 2. Metabolite Ratios from Localized Proton Magnetic Resonance Spectra (TR 3,000/TE 20 msec) of Healthy Control Subjects (10–15 years) and Children with Multiple Sclerosis (MS)[a]

	NAA/Cho	NAA/Cr	Cr/Cho	Cr/Ins
Control gray matter	2.9	1.6	1.8	1.8
Control white matter	2.1	2.1	1.0	2.2
Chronic MS plaques	0.2–1.2	0.3–1.3	0.5–1.0	0.8–1.7
Mean values	0.8	1.1	0.8	1.3

[a]Reproducibility of gray and white matter results is ±5%. The range of values given for chronic MS plaques reflects the observed biological variability rather than statistical error.

NAA = *N*-acetylaspartate; Cho = choline-containing compounds; Cr = creatines; Ins = *myo*-inositol.

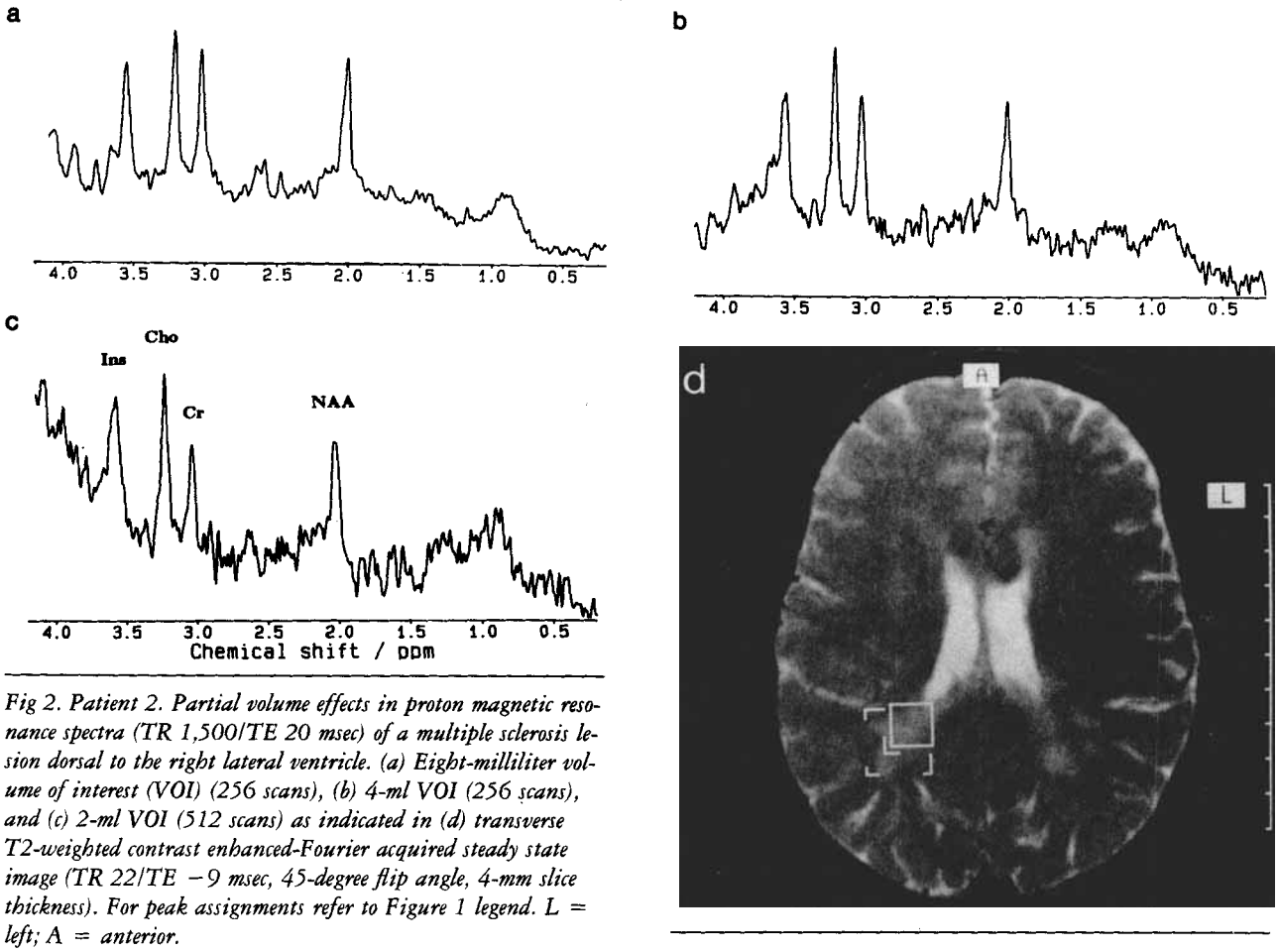

Fig 2. Patient 2. Partial volume effects in proton magnetic resonance spectra (TR 1,500/TE 20 msec) of a multiple sclerosis lesion dorsal to the right lateral ventricle. (a) Eight-milliliter volume of interest (VOI) (256 scans), (b) 4-ml VOI (256 scans), and (c) 2-ml VOI (512 scans) as indicated in (d) transverse T2-weighted contrast enhanced-Fourier acquired steady state image (TR 22/TE −9 msec, 45-degree flip angle, 4-mm slice thickness). For peak assignments refer to Figure 1 legend. L = left; A = anterior.

Chronic Multiple Sclerosis Plaques

A representative selection of resonance patterns found in localized proton spectra of MS plaques in paraventricular white matter is given in Figures 2 through 4. Figure 2 shows the influence of the size of the VOI in a dorsoventricular lesion (Patient 2) that appeared as a patchy hyperintense region on the T2-weighted image in Figure 2d. The spectrum of the lesion features a dramatically diminished resonance of NAA, notably decreased Cr, and elevated resonances of Cho and Ins when compared to normal white matter. No resonances from lactate or mobile lipids were seen in this lesion. A reduction of the VOI from 8 ml to 2 ml leads to a proportional decrease of the signal-to-noise ratio but also to a further decrease of NAA and Cr and a slight increase of Cho. A quantitative evaluation yields a decrease of the NAA/Cho ratio from 0.8 (8 ml) to 0.5 (2 ml) with a constant ratio of NAA/Cr of 0.9. These changes demonstrate a partial volume effect with surrounding white matter even in this relatively large lesion.

Intraindividual variability is illustrated in Figure 3a through c, depicting three spectra of different plaques in the same subject (Patient 1). The corresponding volumes are indicated in Figure 3d through f. The first spectrum (Fig 3a) originates from a lesion located behind the right lateral ventricle, as shown in Figure 2d (Patient 2). Using a 4-ml VOI matched to the extent of the lesion (Fig 3d), the spectrum exhibits a striking decrease of NAA and Cr and an increase of Cho. The second spectrum (Fig 3b) is from a 4-ml VOI of a prominent paraventricular plaque of the left hemisphere (Fig 3e). It shows somewhat more NAA and Cr compared to the first lesion as well as clearly enhanced Cho. This plaque had been investigated twice within a year without significant differences in the spectra. The third spectrum (Fig 3c) from a 2-ml VOI of a tiny right-sided parathalamic plaque (Fig 3f) again shows decreased NAA and Cr and enhanced Cho. Neither lactate nor lipid resonances were seen in the spectra.

Figure 4 demonstrates proton MR spectra from three plaques in two different patients. The spectra in Figure 4a and b originate from two 4-ml VOIs (Patient 2) placed on plaques in a dorsofrontal location

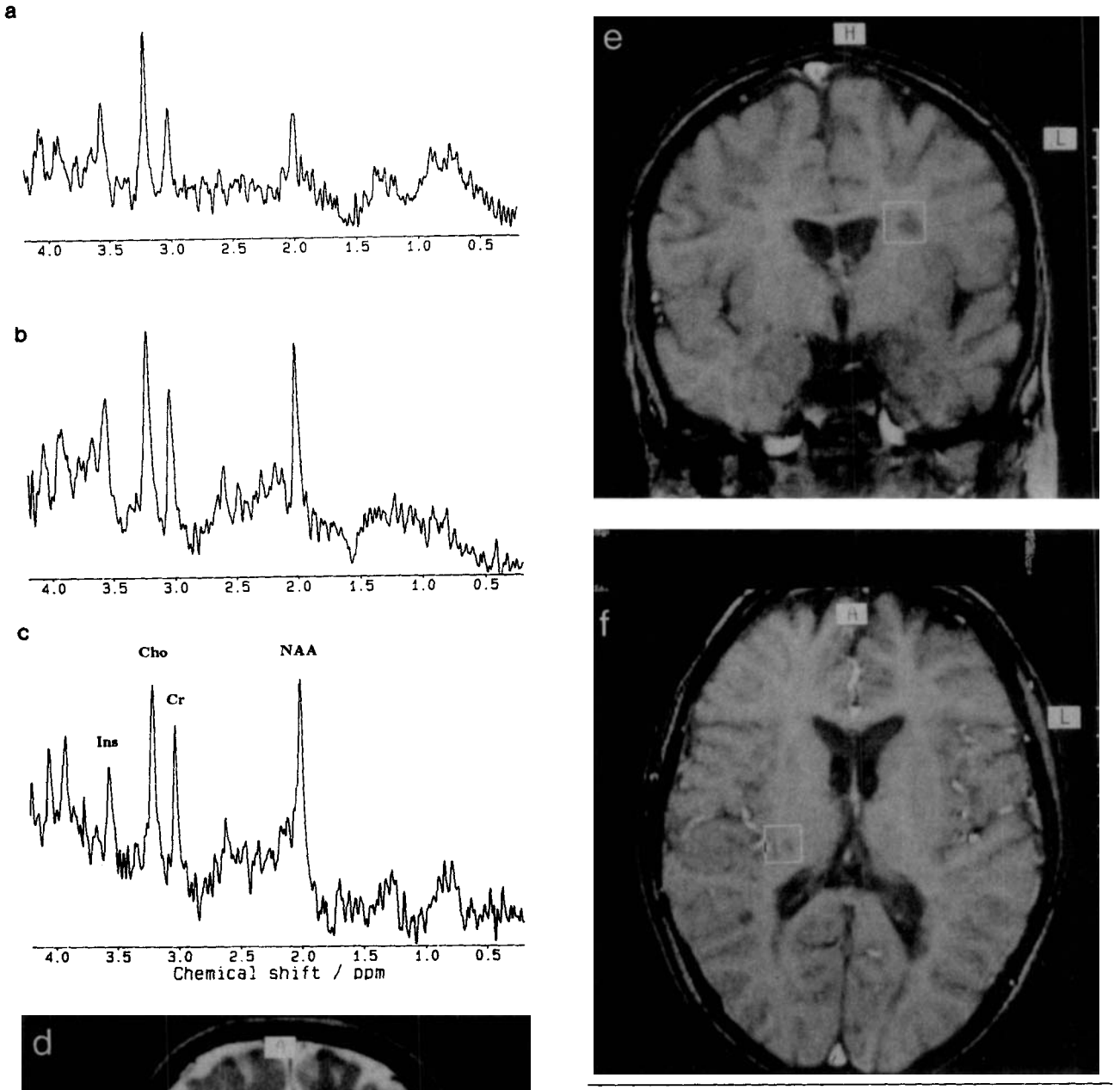

Fig 3. Patient 1. Variability of proton magnetic resonance spectra (TR 3,000/TE 20 msec) from different multiple sclerosis lesions. (a) Right dorsoparietal lesion (4-ml volume of interest {VOI}, 128 scans) as indicated in (d) transverse contrast enhanced-Fourier acquired steady state image (TR 13.6/TE −6 msec, 40-degree flip angle), (b) left paraventricular plaque (4-ml VOI, 128 scans) as indicated in (e) coronal fast low-angle shot (FLASH) image (TR 100/TE 6 msec, 70-degree flip angle), and (c) parathalamic plaque (2-ml VOI, 256 scans) as indicated in (f) transverse FLASH image (TR 100/TE 6 msec, 70-degree flip angle). For peak assignments refer to Figure 1 legend. L = left; A = anterior; H = head.

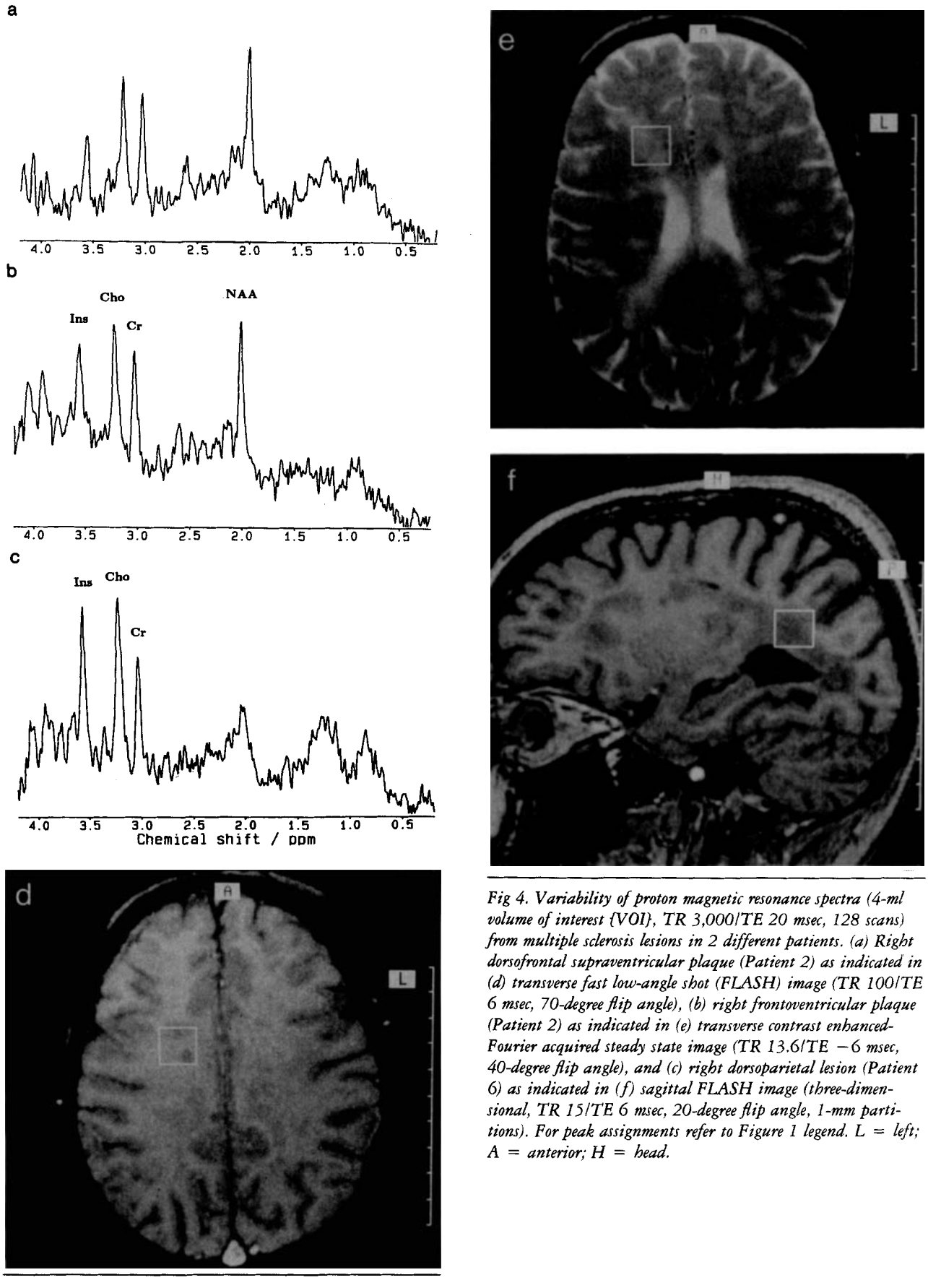

Fig 4. Variability of proton magnetic resonance spectra (4-ml volume of interest [VOI], TR 3,000/TE 20 msec, 128 scans) from multiple sclerosis lesions in 2 different patients. (a) Right dorsofrontal supraventricular plaque (Patient 2) as indicated in (d) transverse fast low-angle shot (FLASH) image (TR 100/TE 6 msec, 70-degree flip angle), (b) right frontoventricular plaque (Patient 2) as indicated in (e) transverse contrast enhanced-Fourier acquired steady state image (TR 13.6/TE −6 msec, 40-degree flip angle), and (c) right dorsoparietal lesion (Patient 6) as indicated in (f) sagittal FLASH image (three-dimensional, TR 15/TE 6 msec, 20-degree flip angle, 1-mm partitions). For peak assignments refer to Figure 1 legend. L = left; A = anterior; H = head.

(Fig 4d) and in front of the lateral ventricle of the right hemisphere (Fig 4e), respectively. The metabolite pattern is very similar to those seen in Figure 3 with a notable decrease of NAA. The plaque represented by Figure 4a and d had already been investigated 1 year earlier when Gd-DTPA enhancement was noted. Both investigations yielded identical spectra without any increase of either lipids or lactate in the enhancing lesion. The spectrum in Figure 4c originates from a 4-ml VOI (Patient 6) of an extensive lesion dorsal to the occipital horn of the right lateral ventricle (Fig 4f). The lesion is delineated in T1-weighted images as a hypointense region, with the spectrum showing depletion of NAA, decreased Cr, elevated Cho, and strongly elevated Ins. Resonances from cytosolic fractions of proteins and/or mobile lipids at about 0.8 ppm (methyl groups), 1.2 ppm (methylene groups), and around 2 ppm (methylene groups bound to carboxyl moieties) were slightly increased in this patient as well as in 1 other patient (Patient 8, not shown).

The metabolic disturbances observed in chronic MS lesions are summarized in Table 2. When compared to the metabolite ratios of white matter in healthy control subjects, all lesions exhibit significant reductions in the NAA/Cho (2.1 to 0.8), NAA/Cr (2.1 to 1.1), and Cr/Ins (2.2 to 1.3) ratios. The variability in the expression of the disease is indicated by the range of values observed in this study.

Enhancing Multiple Sclerosis Plaques

The lesion demonstrated in Figure 4c and f did not show Gd-DTPA enhancement, although the investigation of the patient (Patient 6) took place during an acute episode. However, damage of the blood-brain barrier was verified by the uptake of contrast agent in three other locations, one of which appeared at the frontal edge of the right posteroventricular lesion (see Fig 4f).

Proton MR spectra of two of the enhancing plaques are shown in Figure 5a and b, with the corresponding T1-weighted 3D FLASH images given in Figure 5c and d, respectively. Despite the fact that even a 2-ml VOI appears much larger than the actual damage to the blood-brain barrier, proton MRS obviously indicates a decrease of NAA over more extended regions than given by the bright spots of contrast agent uptake. This finding applies to both paraventricular lesions in the right and left hemispheres, although the reduction in NAA is less pronounced than in the large lesion characterized in Figure 4c. Again there is no indication for an increase of lipid resonances. The metabolite ratios for NAA/Cho (1.2) and NAA/Cr (0.9) fall within the range given in Table 2 for chronic MS lesions. However, in contrast to most older plaques, the ratios of Cr/Cho (1.3) and Cr/Ins (2.1) are close to those of control white matter.

White and Gray Matter Adjacent to Multiple Sclerosis Lesions

We also investigated the metabolic state of brain parenchyma adjacent to plaques that appeared unaffected on T1- and T2-weighted MRIs. In Figure 6 the spectrum from white matter of Patient 5 (Fig 6b), frontal to the lesion (spectrum in Fig 6a), was very similar to those found in control subjects (e.g., see Fig 1a). Respective metabolite ratios were within the ranges given in Table 2. Minor variations in Cr and Cho may be due to partial volume effects with gray matter or differences in age (7½ versus 12½ years) or both.

Figure 7 shows proton MR spectra from midline cortical gray matter of 2 different subjects with extensive MS lesions in neighboring white matter. In Patient 4 (Fig 7a, c) the spectrum from dorsofrontal gray matter exhibits notably decreased levels of NAA and Glu as well as an increase of GABA. The metabolite ratio of NAA/Cr (1.1) is significantly lower than that of control gray matter (1.6, see Table 2).

The spectrum in Figure 7b (Patient 2) was acquired from parietal gray matter in the vicinity of extensive dorsoventricular lesions, as indicated in the image shown in Figure 2d. The reduction in NAA is less pronounced than in the former example (Fig 7a), yielding a metabolite NAA/Cr ratio of 1.4. No cortical atrophy was evident in the MRIs of either subjects.

Discussion

Using state-of-the-art localization techniques suitable for the selection of adequately sized VOIs, proton MRS allows reliable insights into the chemical composition of MS plaques and their metabolic sequelae in vivo. The present findings are discussed in the following five theses.

(1) Proton MR spectra of MS plaques are indicative of significant neuroaxonal loss and membranoproliferative processes. Common observations in proton MR spectra of MS plaques are a striking decrease or even a depletion of NAA, a substantial decrease of the Cr pool, and an increase of Cho compounds and Ins. These findings become most pronounced in situations where partial volume effects with normal brain tissue can largely be excluded. Since NAA resides almost entirely in the cytosol of neurons and their processes [23], its reduction serves as a marker for neuronal impairment as evidenced in a number of other pathological states (e.g., see [24–27]). Since reversibility of the NAA decrease was not observed in MS lesions or in other pathological states, low NAA levels are unlikely to represent a reversible form of neuronal dysfunction. Residual NAA concentrations in MS lesions may thus reflect general shrinkage and partial loss of neuronal axons [28]. The increase in Cho and possibly Ins in MS plaques may find its pathological correlate in the inflammatory myelin breakdown and/or increased

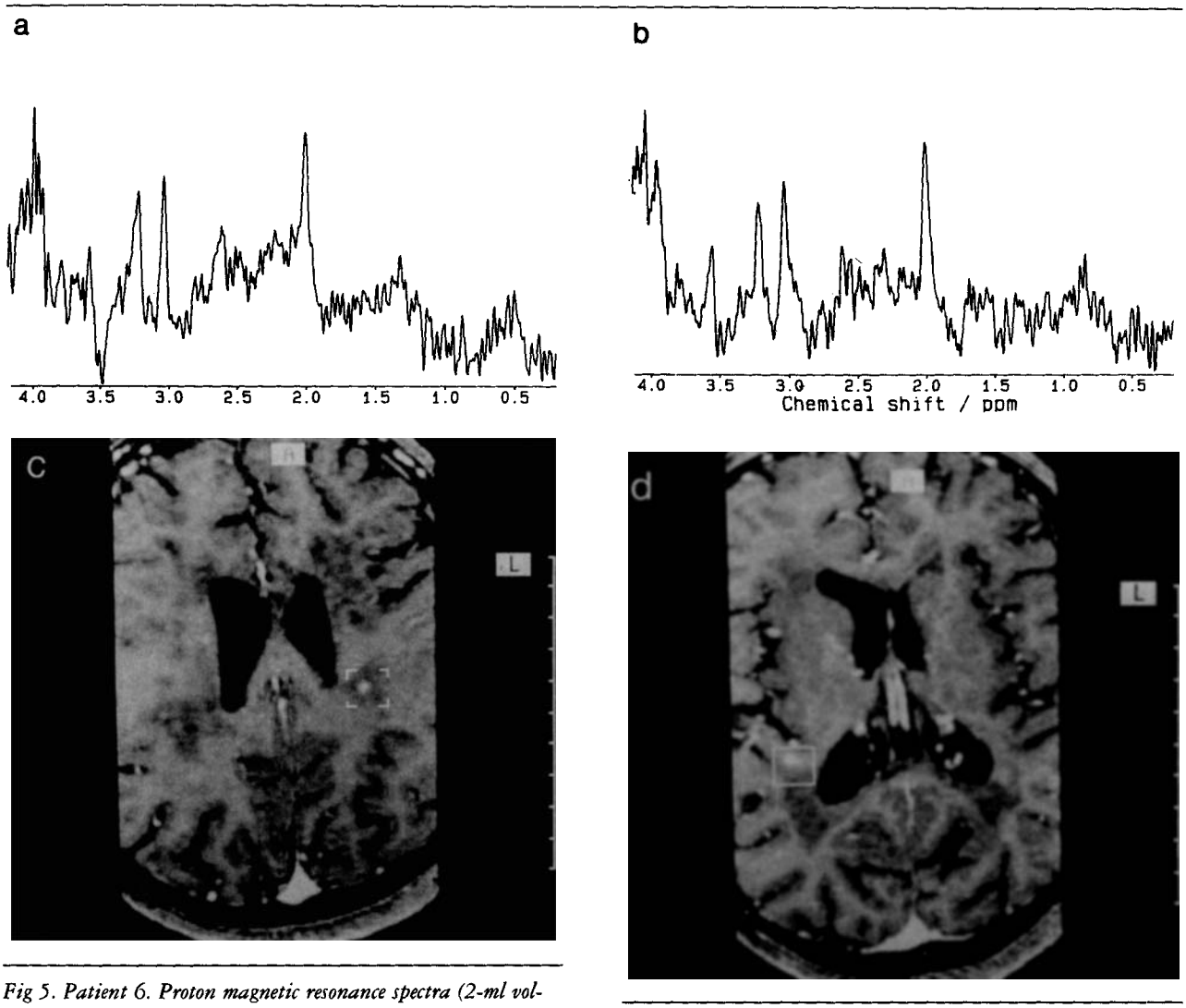

Fig 5. Patient 6. Proton magnetic resonance spectra (2-ml volume of interest [VOI], TR 3,000/TE 20 msec, 128 scans) of two different multiple sclerosis lesions showing gadolinium–diethylenetriamine pentaacetic acid enhancement during an acute exacerbation. (a) Left parietal plaque and (b) right parietal plaque as indicated in corresponding transverse fast low-angle shot images (c and d, respectively) (three-dimensional, TR 15/TE 6 msec, 20-degree flip angle, 1-mm partitions). For peak assignments refer to Figure 1 legend. L = left; A = anterior.

turnover of membrane constituents in proliferative oligodendrocytes and astrocytes, resulting in fibrillary gliosis. In favor of the latter hypothesis is the finding of increased Cho levels in membranoproliferative processes present in brain tumors but not during focal ischemic breakdown of myelin immediately following cerebral infarction [24–26].

When compared to previous proton MRS studies of MS in adulthood, the present results are in agreement with the observation of reduced NAA. However, in contrast to some reports, our study did not show elevated concentrations of lactate. Minor signals tentatively assigned to mobile lipids were seen in only 2 patients. Although neuronal loss in a variety of diseases is always indicated by a reduction of NAA, the entire metabolite pattern of MS plaques differs from those of other pathological states such as tumors, stroke, or leukodystrophies. Thus, proton MRS is expected to yield further clues for the differential diagnosis of, for example, brain tumor versus demyelination.

(2) Metabolite patterns in MS plaques show only minor intra- and interindividual differences and longitudinal changes. Qualitatively, all proton MR spectra of MS plaques exhibited a similar metabolite pattern. The range of quantitative variations in NAA, Cr, Cho, and Ins concentrations is demonstrated in Figures 2 through 6 and summarized in Table 2. Assuming that severe partial volume effects with unaffected brain parenchyma were avoided, spectral variations reflect changes in the metabolite pools due to alterations of cellular metabolism and/or cellular composition of the plaques (neurons, astrocytes, oligodendrocytes). The age of a chronic lesion had only minor or no impact

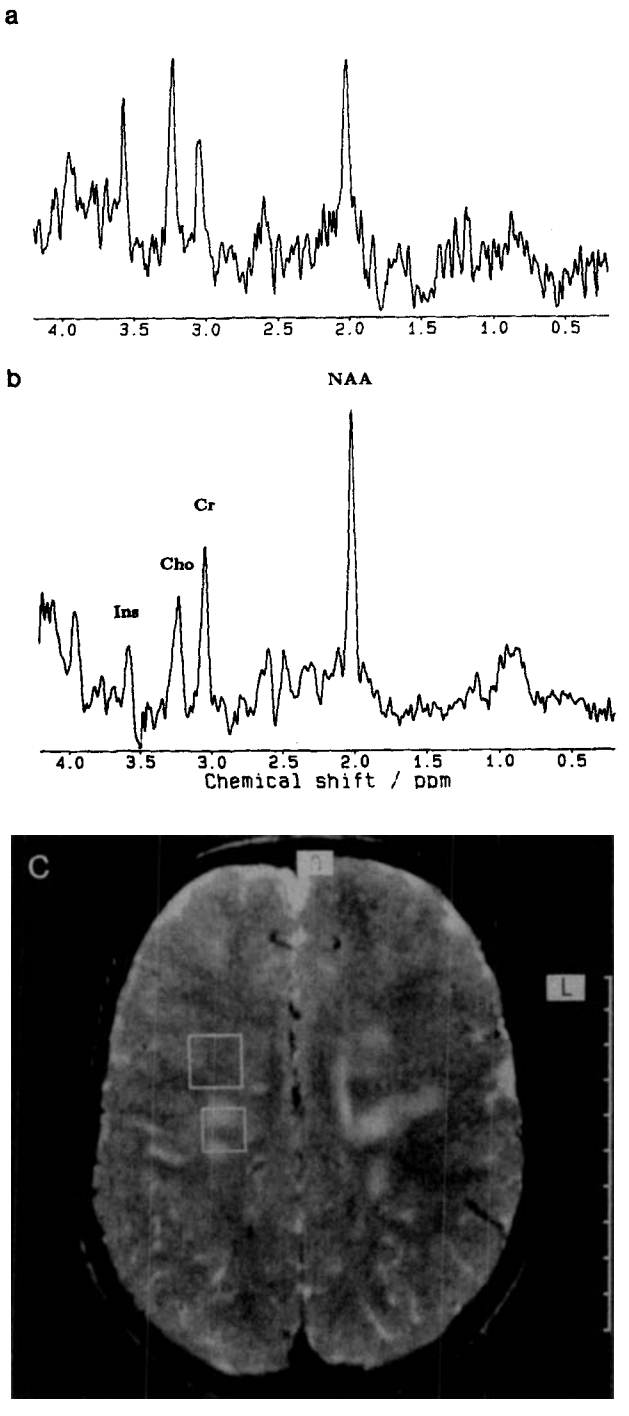

Fig 6. Patient 5. Proton magnetic resonance spectra (TR 3,000/TE 20 msec, 128 scans) of (a) a right supraventricular plaque (2-ml volume of interest [VOI]) and (b) adjacent white matter (4-ml VOI) that appears normal on MRI as indicated in (c) transverse contrast enhanced-Fourier acquired steady state image (TR 13.6/TE −6 msec, 40-degree flip angle). The resonance pattern from the lesion in (a) is similar to those in Figures 2 through 5, while that of the more anterior VOI resembles the characteristics of normal white matter as in Figure 1a. For peak assignments refer to Figure 1 legend. L = left; A = anterior.

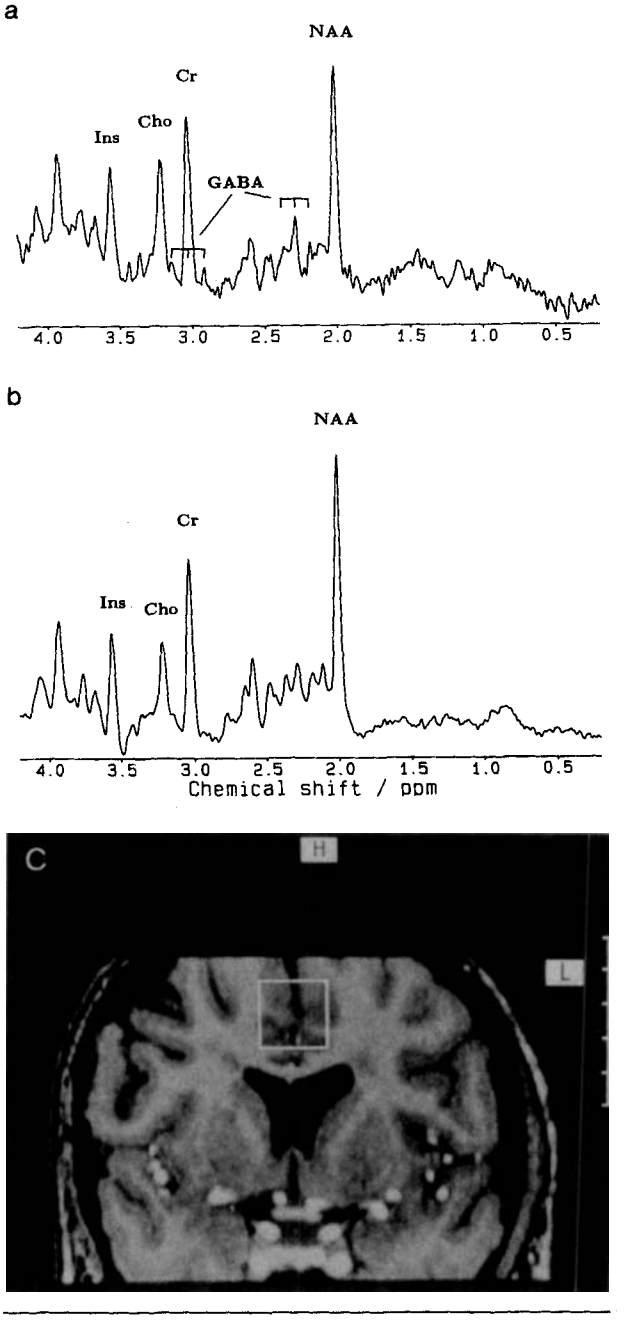

Fig 7. Proton magnetic resonance spectra (TR 3,000/TE 20 msec, 128 scans) of gray matter adjacent to multiple sclerosis lesions in 2 different subjects. (a) Dorsofrontal midline gray matter (8-ml volume of interest [VOI]) adjacent to frontoventricular lesions (Patient 4) as indicated in (c) coronal fast low-angle shot image (three-dimensional, TR 15/TE 6 msec, 20-degree flip angle, 1-mm partitions), and (b) parietal midline gray matter (18-ml VOI) adjacent to bilaterally dorsoventricular lesions (Patient 2) as shown in Figure 2d. For peak assignments refer to Figure 1 legend. GABA = γ-aminobutyric acid.

on the spectrum since no distinct differences occurred in a longitudinal study of some plaques over a year.

(3) Acute MS plaques show early loss of vital neuronal tissue. In acute episodes of the disease, Gd-DTPA enhancement on MRI identified foci of impaired blood-brain barrier and supposedly active inflammation (see Fig 5). In these cases, no increase of Cho and Ins and no decrease of Cr were (yet) apparent. However, a reduction of NAA, as found in chronic plaques, seems to be an early MRS-sensitive event. The absence of increased resonances from mobile short-chain fatty acids in enhancing plaques casts some doubt on previous suggestions that a specific time window of high lipid mobility in acute MS lesions (possibly by lipid-laden macrophages) should result in increased lipid visibility in proton MR spectra [6, 7]. In fact, the paracrystalline state of membrane lipids and cholesterol histochemically found in early plaques [29, 30] may not be broken down enough to allow for MR visibility. To clarify this point further, systematic longitudinal studies are required.

Other investigators reported an increase of lactate in acute versus chronic MS lesions [8]. Although slightly increased lactate has been an incidental finding in some extensive lesions of adults with progressive disease [9, 10], acute enhancing lesions as examined here in children do not necessarily accumulate lactate due to excessive continued anaerobic glycolysis caused by infiltrated macrophages and lymphocytes. On the other hand, even in healthy brain tissue slight elevations of lactate may eventually be observed (e.g., see Fig 1b).

(4) White matter adjacent to MS plaques and unsuspicious on MRIs exhibits a normal metabolite composition. In general, white matter adjacent to MS lesions was found to be spared from distinct MRS-sensitive metabolic disturbances. In the older literature, lipid abnormalities in the normal-appearing white matter were reported [31]. This concept was challenged as untenable in view of the fact that the crude techniques of preparation used 20 years ago could not exclude microscopic foci of demyelination in the normal-appearing white matter [32]. In more recent studies, histological abnormalities were demonstrated in 72% of such samples of white matter [33].

(5) Gray matter adjacent to MS plaques and associated with demyelinated axons shows neuronal loss. The reduction of NAA and Glu observed in a gray matter region of the motor cortex (Patient 4; Fig 7a, c) may be understood as a loss of functional neurons. Thus, the injury inflicted on neuronal axons by MS lesions is expected to affect the projections onto cortical gray matter. In cerebral forms of MS, plaques peripherally located in the white substance were found to be in contact with, or wholly within, cortical gray matter in 93.5% of a series of consecutively obtained specimens [31].

Similar distortions of the metabolic but not morphological integrity of gray matter have been observed in other degenerative brain diseases such as ceroid-lipofuscinosis (Bruhn H et al, unpublished observations, 1991) and Creutzfeldt-Jakob disease [27]. The incidental finding of an increase in the inhibitory neurotransmitter GABA (Patient 4) is not yet well understood. Clinically this patient had recovered completely from impaired neurological functions, particularly hemiparesis on the right side, 1 year prior to the MRS examination.

In conclusion, proton MRS reveals metabolic alterations associated with MS in both focal lesions and adjacent cortex. As a noninvasive tool, it could thus aid in the differentiation of MS plaques from other disorders, in understanding the progress of the disease, and in monitoring regional metabolic response to therapeutic interventions.

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