

# Proton MR spectroscopy with metabolite-nulling reveals elevated macromolecules in acute multiple sclerosis

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## Summary

Proton magnetic resonance spectroscopy has shown elevated signals in the spectral region of lipids in acute multiple sclerosis lesions. The metabolite-nulling technique allows the separation of macromolecules from other metabolites, such as lactate, *N*-acetyl-aspartate, creatine, choline and *myo*-inositol. Using this technique in studies on multiple sclerosis patients, we were able to differentiate macromolecules biochemically in acute and chronic multiple sclerosis lesions. Ten patients with acute, contrast-enhancing multiple sclerosis lesions, 10 patients with chronic lesions and 10 healthy control subjects were investigated with a 1.5 T whole body system, using a stimulated echo acquisition mode (STEAM) sequence with metabolite-nulling and outer volume saturation. Metabolites and macromolecules were quantitated absolutely. The 0.9 and 1.3 parts per million (p.p.m.) resonances of the macromolecules were significantly elevated in acute lesions compared with chronic lesions and healthy controls ( $P < 0.001$  for 0.9 p.p.m.,  $P < 0.05$

for 1.3 p.p.m.). The macromolecular resonances at 2.1 and 3.0 p.p.m. in acute and chronic lesions were normal. *N*-acetyl-aspartate was significantly reduced in acute and chronic lesions compared with controls ( $P < 0.05$  and  $P < 0.01$ , respectively). Choline was significantly elevated in acute lesions compared with controls ( $P < 0.05$ ). Up to now, elevated resonances at 0.9 and 1.3 p.p.m. in acute lesions have been interpreted as lipids. In metabolite-nulled spectra, the macromolecular resonances did not fit those of lipids and might have been due to proteins or polypeptides containing the amino acids alanine, threonine, valine, leucine and isoleucine. These account for ~40% of the amino acids of myelin proteolipid protein and for ~20% of myelin basic protein. The increased macromolecular resonances at 0.9 and 1.3 p.p.m. may be interpreted as biochemical markers of myelin fragments and may be used as reliable markers of acute multiple sclerosis lesions as they provide clear discrimination among acute and chronic lesions and controls.

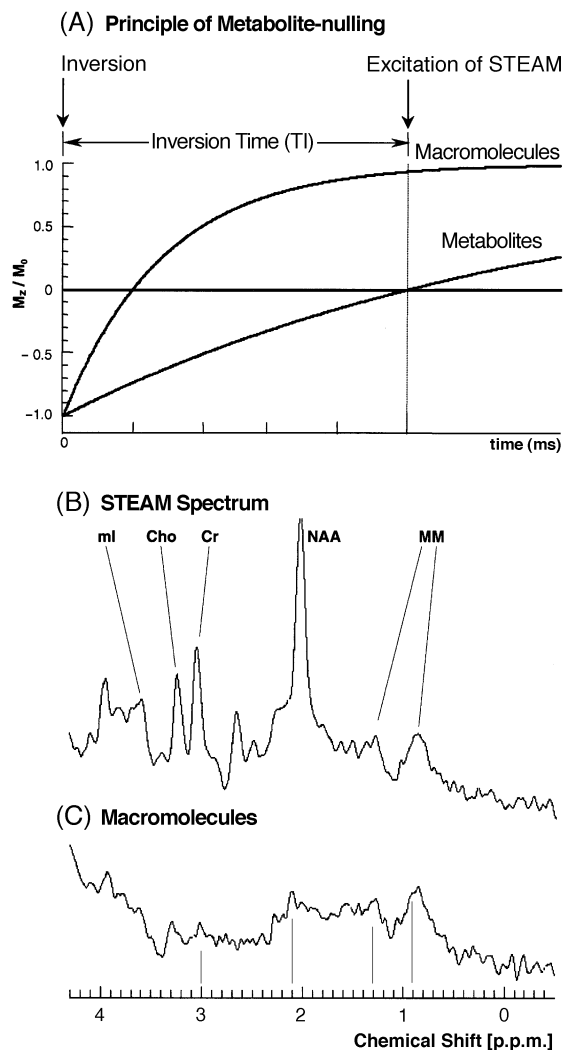
**Keywords:** NMR spectroscopy; multiple sclerosis; macromolecules; lipids; absolute quantitation

**Abbreviations:** Cho = choline; Cr = creatine; Glx = glutamine and glutamate; <sup>1</sup>H-MRS = proton magnetic resonance spectroscopy; Lac = lactate; MI = *myo*-inositol; NAA = *N*-acetyl-aspartate; p.p.m. = parts per million; STEAM = stimulated echo acquisition mode; TE = echo time; TE<sub>eff</sub> = effective echo time; TI = inversion time; TM = mixing time; TR = repetition time; VOI = volume of interest

## Introduction

In proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS) of the brain, separation of macromolecules (proteins and lipids) from metabolites with low molecular weight, such as lactate (Lac), *N*-acetyl-aspartate (NAA), creatine (Cr), choline (Cho), *myo*-inositol (MI) and glutamine and glutamate (Glx), is achieved by the metabolite-nulling technique according to different T<sub>1</sub> relaxation times, as shown in Fig. 1 (Behar

and Ogino, 1993). Prominent physiological macromolecular resonances detected on such localized metabolite-nulled <sup>1</sup>H magnetic resonance spectra have been described at 0.93, 1.24, 1.43, 2.05 and 3.00 parts per million (p.p.m.) (Behar *et al.*, 1994). Discrimination of metabolites from elevated macromolecular resonances in acute stroke (Hwang *et al.*, 1996; Saunders *et al.*, 1997) and human brain tumours has



**Fig. 1** (A) Principle of metabolite nulling. Abscissa, time; ordinate, magnetization. After an antecedent inversion pulse, the longitudinal magnetization is inverted. Because of the longer  $T_1$  relaxation time of the metabolites, the amount of longitudinal magnetization of the metabolites is zero at the beginning of STEAM excitation (arrow). At this time the longitudinal magnetization of the macromolecules has already recovered nearly fully, resulting in a spectrum in which only macromolecules are visible (C). (B) STEAM spectrum of a healthy subject without metabolite-nulling. The metabolites *N*-acetyl aspartate (NAA), choline (Cho), creatine (Cr) and *myo*-inositol (mI) are clearly visible. MM = macromolecules. (C) Metabolite-nulled spectrum of the same subject. The macromolecular resonances at 0.9, 1.3, 2.1 and 3.0 p.p.m. are visible without overlaying metabolites.

been reported (Howe *et al.*, 1995). Prior to the present study, there have been no published reports of the application of the metabolite-nulling technique to multiple sclerosis.

Differentiation between metabolites and macromolecules (lipids and proteins) has become important in acute and chronic multiple sclerosis because in the past the detection of elevated signals in the spectral region of lipids in acute multiple sclerosis lesions has been interpreted as an indicator of acute lesions (Wolinski *et al.*, 1990; Larsson *et al.*, 1991; Narayana *et al.*, 1992, 1998; Davie *et al.*, 1993, 1994;

Koopmans *et al.*, 1993; Roser *et al.*, 1996). In none of the studies was any outer volume saturation performed to exclude lipid contamination from the fat-containing structures of the skull and galea. Narayana and colleagues (Narayana *et al.*, 1992) and Davie and colleagues (Davie *et al.*, 1994) discussed this lipid contamination from outside the volume of interest (VOI) and thought it to be dependent on the distance between scalp and VOI. Seeger and colleagues, however, demonstrated that considerable lipid contamination was not dependent on that distance, and stressed the importance of outer volume saturation in single-voxel spectroscopy (Seeger *et al.*, 2000).

Furthermore, no quantitative determination of the macromolecules has yet been performed in any pathological condition.

In the present study, the metabolite-nulling technique was applied to acute and chronic multiple sclerosis lesions. By differentiating between macromolecules and overlying metabolites, we predicted the detection of macromolecules in acute lesions. Outer volume saturation was performed in order to achieve reliable detection and evaluation of the macromolecule resonances. Quantitation was achieved by a modified version of the LCModel software (Provencher *et al.*, 1993).

## Methods

### Subjects

We studied 10 patients with acute multiple sclerosis lesions (four men, six women, median age 30.5 years, range 22–39 years), 10 patients with chronic lesions (two men, eight women, median age 34.5 years, range 21–54 years) and 10 healthy control subjects (six men, four women, median age 27 years, range 19–62 years).

Of the ten patients with acute lesions, eight had clinically definite multiple sclerosis and two had laboratory-supported definite multiple sclerosis (Poser *et al.*, 1983). The mean duration of neurological symptoms before  $^1\text{H}$ -MRS ranged from 1 to 18 days (mean 6 days). All acute lesions showed contrast enhancement on  $T_1$ -weighted images.

Ten patients with chronic lesions had clinically definite multiple sclerosis, six had a relapsing–remitting course and four had a secondary progressive course. None of them showed a contrast-enhancing lesion on  $T_1$ -weighted images.

Eight patients with acute lesions had not received corticosteroid treatment and two had received corticosteroids for 2 and 3 days in advance of  $^1\text{H}$ -MRS. Effects of corticosteroids on the spectra were examined by measurements performed before and on day 5 of a 5-day course of corticosteroid treatment (500 mg methylprednisolone/day) and 7 days after the last dose.

The procedure was approved by the 'Ethik-Kommission der Medizinischen Fakultät des Universitätsklinikums Tübingen', and all subjects gave written informed consent for this investigation.

### **MRI and spectroscopy**

Both MRI and  $^1\text{H}$  MRS were performed on a 1.5 T whole body system. The study commenced with an axial native  $T_1$ -weighted spin-echo sequence [repetition time (TR) 600 ms, echo time (TE) 12 ms, 5 mm slice thickness with a 0.5 mm gap,  $192 \times 256$  matrix, one acquisition] and an axial  $T_2$ -weighted turbo spine-echo sequence [TR 5600 ms, effective echo time ( $\text{TE}_{\text{eff}}$ ) 119 ms, echo train length 15, 5 mm slice thickness with a 0.5 mm gap,  $210 \times 256$  matrix, three acquisitions].

All patients received intravenous contrast medium (gadolinium-DTPA) (Magnevist; Schering, Berlin, Germany) for contrast-enhanced  $T_1$ -weighted images. Contrast medium was applied at least 24 h before  $^1\text{H}$ -MRS in five patients with acute and eight patients with chronic multiple sclerosis, 4 h before  $^1\text{H}$ -MRS in three patients with acute lesions and 20 min before  $^1\text{H}$ -MRS in two patients with acute and two with chronic multiple sclerosis. The effects of contrast medium on the spectra were studied 1 day after contrast medium application in one patient with acute and one patient with chronic multiple sclerosis.

In the patients with acute multiple sclerosis, the known contrast-enhancing lesions were selected for spectroscopy; all were located in the supratentorial white matter. Lesions that appeared non-enhancing on  $T_1$ -weighted hypointense and on  $T_2$ -weighted hyperintense imaging were investigated in the supratentorial deep white matter of patients with chronic multiple sclerosis. In the controls, the cubic VOI was placed in the supratentorial deep white matter of the parieto-occipital region. The voxel size was always 8 ml.

$^1\text{H}$ -MRS was performed with a self-designed single-voxel stimulated echo acquisition mode (STEAM) sequence with the following parameters: TR 1500 ms; mixing time (TM) 10 ms; TE 15 ms; 128 acquisitions (Seeger *et al.*, 1999). A hyperbolic secant inversion pulse with an inversion time (TI) of 500 ms was used for metabolite-nulling. For outer volume saturation, three pairs of perpendicular saturation slices were applied for presaturation of fat-containing regions on each side of the VOI (Seeger *et al.*, 2000). Lac, NAA, Cr, Cho, MI and Glx were quantitated by using the user-independent frequency domain-fitting program LCModel (Provencher *et al.*, 1993). For additional quantitation of the macromolecular resonances, LCModel was modified. Averaged metabolite-nulled spectra of white matter in 10 healthy volunteers and 10 acute multiple sclerosis lesions acquired with outer volume saturation were parameterized in each case with resonance lines at 0.9, 1.3, 2.1 and 3.1 p.p.m. These independent curves were added to the initial basis set of 15 metabolite model spectra of the LCModel. The parameterized resonances were found to have a broader line width than the metabolites and could be used to give a sufficient fit of the macromolecules. The *in vivo* STEAM spectra without metabolite-nulling were fitted with the extended basis set, resulting in absolute concentrations of metabolites and macromolecules, the latter in arbitrary units.

In an acute multiple sclerosis lesion, an incidentally diagnosed lipoma of the corpus callosum of the same patient and in one control,  $T_1$  measurements of the resonance at 0.9 p.p.m. were performed by  $T_1$  variation. Inversion times of 100–4000 ms and a constant delay of 3000 ms between radio-frequency excitation and successive inversion pulse were used. The  $T_1$  relaxation times were calculated from the fits of the spectral signal intensity.

### **Statistics**

The normality of the distributions of metabolite and macromolecule concentrations in all three groups—acute lesions, chronic lesions and control subjects—was determined with the Kolmogorov–Smirnov test. Metabolite and macromolecule concentrations in all three groups were compared by analysis of variance (ANOVA), including Bonferroni correction for multiple comparisons.

### **Results**

#### ***Effects of contrast enhancement and steroid treatment***

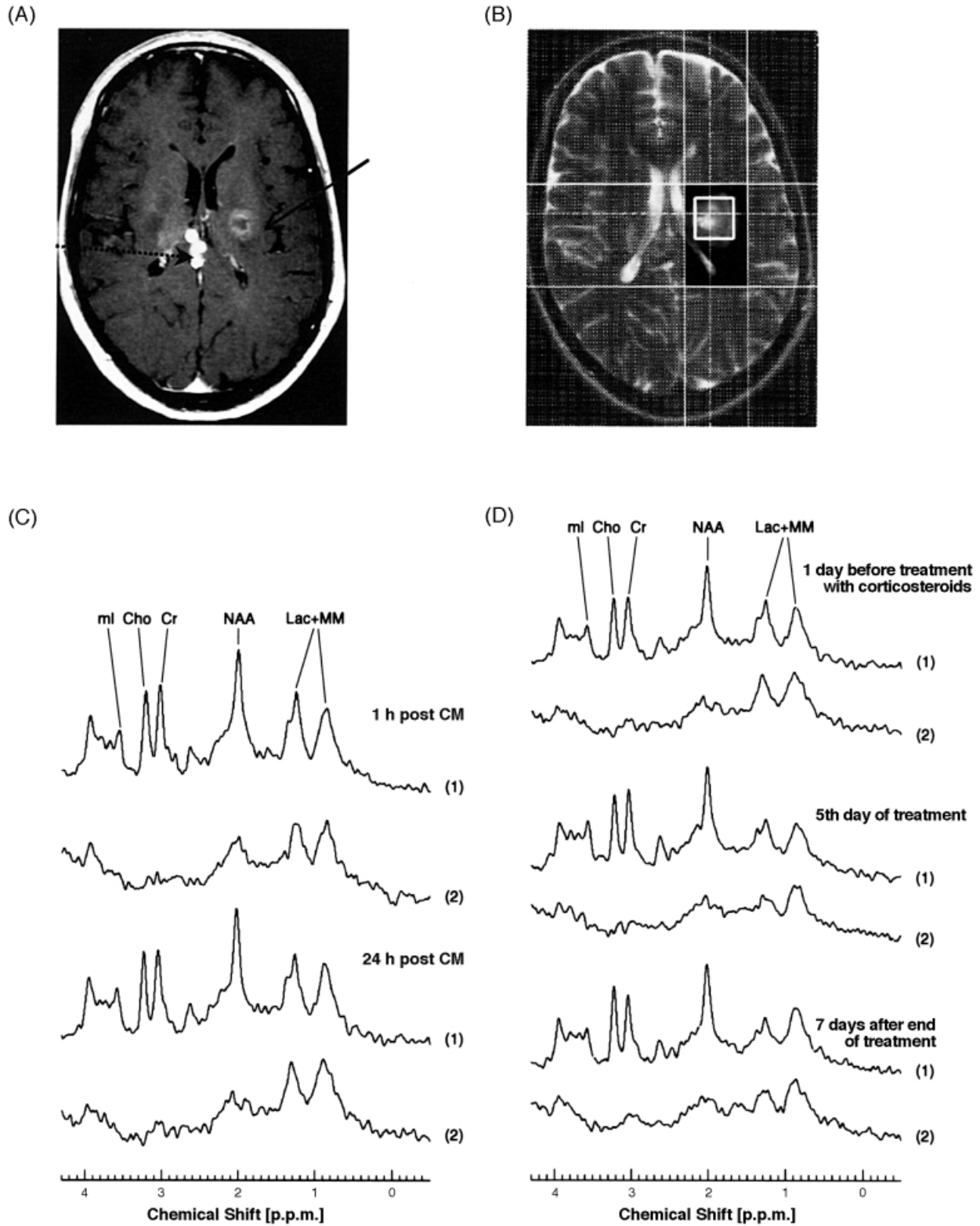
No substantial changes between the spectra 1 h and 1 day after contrast-enhancement were found (Fig. 2C), nor were differences found between spectra obtained before, during and after a 5-day course of corticosteroid treatment (Fig. 2D). All these spectra were obtained from a 19-year-old male with acute multiple sclerosis and an 8-day history of a hemiparesis of sensory and motor functions. A representative contrast-enhanced  $T_1$ -weighted image, showing a ring-shaped enhancing lesion in the left internal capsule (arrow) and a lipoma of the corpus callosum (dotted arrow) is shown in Fig. 2A. The VOI and the saturation slices are shown in Fig. 2B.

#### ***Metabolite and macromolecule concentrations***

NAA was significantly reduced in both the acute and the chronic multiple sclerosis lesion groups compared with the controls ( $P < 0.05$  for acute and  $P < 0.01$  for chronic lesions). Cho was significantly elevated in acute multiple sclerosis compared with the controls ( $P < 0.05$ ). MI showed a tendency to increase in the group with chronic multiple sclerosis but this did not reach significance. The other metabolite concentrations did not reveal any significant differences between the acute, chronic and control groups. The metabolite and macromolecule concentrations are given in Table 1.

The macromolecular resonance at 0.9 p.p.m. was significantly elevated in the acute group compared with the chronic group and the controls ( $P < 0.001$  in each case). The resonance at 1.3 p.p.m. was significantly elevated in acute lesions compared with chronic lesions and healthy controls ( $P < 0.05$  in each case). The macromolecular resonances at 2.1 and at 3.1 p.p.m. did not differ significantly between the groups.

Examples of spectra from an acute multiple sclerosis



**Fig. 2** (A) T<sub>1</sub>-weighted contrast-enhanced axial image of an acute ring-shaped enhancing lesion in the left internal capsule (arrow) and a lipoma of the corpus callosum (dotted arrow). (B) Corresponding T<sub>2</sub>-weighted turbo spin-echo image showing the lesion with high signal intensity. The volume of interest and the areas of saturation are indicated. (C) STEAM spectra (trace 1, TR 1.5 s, TM 10 ms, TE 15 ms) and metabolite-nulled spectra of the acute lesion (trace 2, TR 1.5 s, TM 10 ms, TE 15 ms, TI 500 ms) 1 and 24 h after contrast application, showing a similar spectral pattern. (D) STEAM spectra and metabolite-nulled spectra of the acute lesion (parameters as in C) 1 day before a 5-day course of treatment with glucocorticoids, on day 5 of treatment and 7 days after the end of treatment. No spectral changes were found in the follow-up. CM = contrast medium; MM = macromolecules.

**Table 1** Metabolite concentrations [mean (SD)]

	Acute lesions	Chronic lesions	Healthy controls
Lac	2.16 (1.94)	0.23 (0.08)	0.45 (0.09)
NAA	6.18 (1.24)*	5.65 (1.40) <sup>†</sup>	7.51 (0.85)
Cr	4.40 (0.95)	3.68 (0.89)	4.48 (0.62)
Cho	1.36 (0.26)*	1.15 (0.25)	1.09 (0.17)
MI	2.76 (1.04)	3.97 (1.09)	2.95 (1.16)
Glx	6.11 (2.45)	4.10 (1.79)	5.36 (1.23)
Macromolecules			
at 0.9 p.p.m.	17.11 (4.50) <sup>‡</sup>	8.56 (3.83)	9.57 (1.87)
at 1.3 p.p.m.	14.40 (6.35) <sup>§</sup>	8.40 (3.48)	8.96 (1.38)
at 2.1 p.p.m.	12.82 (1.72)	10.37 (4.29)	11.07 (2.91)
at 3.1 p.p.m.	1.51 (1.08)	2.01 (0.98)	1.39 (1.00)

The concentrations of the metabolites and the macromolecules are in arbitrary units, as the metabolites are affected by  $T_1$  saturation effect because of a TR of 1500 ms. \*Acute lesions versus healthy controls,  $P < 0.05$ ; <sup>†</sup>chronic lesions versus healthy controls,  $P < 0.01$ ; <sup>‡</sup>acute lesions versus chronic lesions and healthy controls,  $P < 0.001$ ; <sup>§</sup>acute lesions versus chronic lesions and healthy controls,  $P < 0.05$ .

lesion, a chronic lesion and a control are displayed in Fig. 3. In the STEAM spectrum of the acute lesion (Fig. 3A, top trace), two prominent resonances were visible, one at 0.9 and one at 1.3 p.p.m. After application of the inversion pulse of the metabolite-nulling technique (middle trace), the 0.9 p.p.m. resonance was more prominent than that at 1.3 p.p.m. because of intensity nulling of Lac. Subtraction of the two spectra (bottom trace) showed the presence of the Lac doublet at 1.33 p.p.m. The metabolite-nulled spectra of the chronic lesion did not differ from that of the healthy control. The NAA peak of the chronic lesion was reduced, while the Cho peak and the MI peak were increased compared with the control (Fig. 3B and C).

### Spectra of the lipoma

The lipoma was chosen to compare the spectrum of human lipids with the macromolecular resonances of the brain. Figure 4A shows the  $T_1$ -weighted images of the investigated curvilinear lipoma of the corpus callosum. The spectra of the lipoma (Fig. 4B) exhibited three resonances: the 0.9 p.p.m. resonance of the methyl  $\text{CH}_3$  groups, the 1.3 p.p.m. resonance of the methylene  $(\text{CH}_2)_n$  groups and the 2.1 p.p.m. resonance of the terminal methylene groups (Schick *et al.*, 1993). The peak of the methylene groups at 1.3 p.p.m. was seven-fold higher than that of the methyl groups at 0.9 p.p.m. In contrast to the spectrum of the lipoma, the macromolecular pattern of the acute multiple sclerosis lesion (Fig. 4B) was different; the peak intensities of the methylene and methyl groups were nearly equal, indicating a different chemical composition.

### $T_1$ measurements

The  $T_1$  relaxation curves of the macromolecules at 0.9 p.p.m. revealed a  $T_1$  time of 218 ms in the acute lesion and a  $T_1$  time of 185 ms in the healthy control. The  $T_1$  relaxation time of the corresponding resonance of the lipoma was

considerably longer (583 ms). The  $T_1$  relaxation curves of all three conditions (lipoma, acute multiple sclerosis, control) are shown in Fig. 4C.

### Discussion

The aim of this study was the characterization and quantitation of the increased macromolecular resonances at 0.9 and 1.3 p.p.m. by using the metabolite-nulling technique in conjunction with the modified LCMoDel program in acute and chronic multiple sclerosis lesions.

### Methodological considerations

By including the parameterized metabolite-nulled spectra in the basis set, LCMoDel provided a proper simultaneous fit of the macromolecules and metabolites in the STEAM spectra without omitting the macromolecular resonances as the so-called baseline.

The absolute values of the metabolites corresponded to millimolar concentrations, but they underlie a certain amount of  $T_1$  saturation due to the TR of 1500 ms. Thus, they are shown only as arbitrary units in Table 1. This should be taken into account in comparisons with published results.

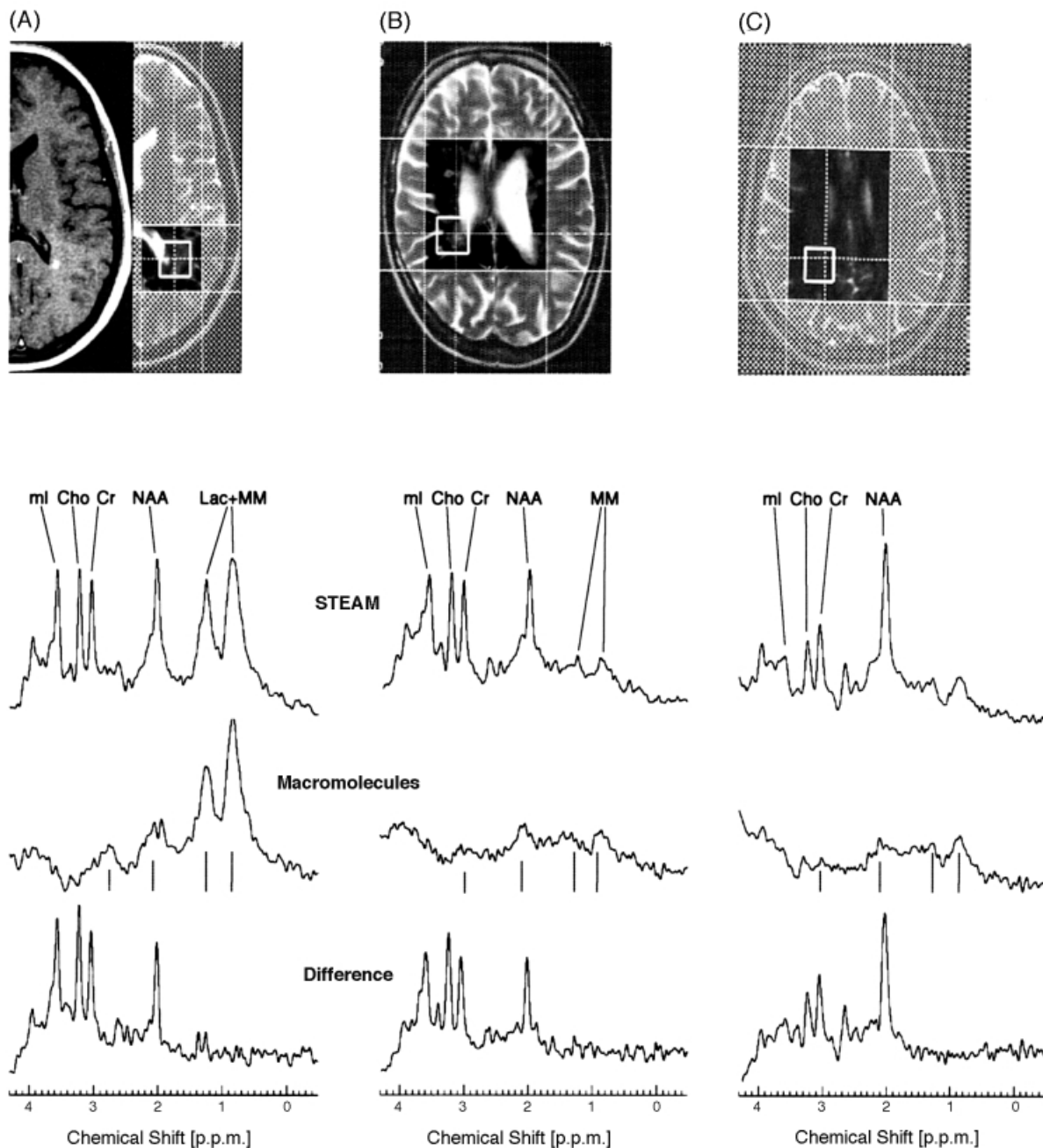
The definition of acute multiple sclerosis lesions is still a matter of controversy. An acute onset of clinical symptoms, and contrast-enhancement are criteria for the acute phase of lesion development. Enhancement usually accompanies inflammation (Katz *et al.*, 1993; Lucchinetti *et al.*, 1996; Brück *et al.*, 1997; Miller *et al.*, 1998). Thus, we chose acute clinical onset of symptoms accompanied by a contrast-enhancing lesion as criteria for an acute lesion.

Effects of contrast media on the spectra are critical issues. At long echo times, a reduction of Cho, unchanged NAA and Cr (Sijens *et al.*, 1998) and no changes in metabolites (Smith, *et al.*, 2000) have been reported. In short echo-time spectra, contrast media showed no effects (Narayana *et al.*, 1992; Taylor *et al.*, 1995; Sijens *et al.*, 1998). To investigate possible influences of contrast media on our short echo-time spectra, a follow-up was performed in one chronic and one acute patient 1 day after contrast application. No substantial changes were observed in these spectra. This supports the latter observation.

Effects of corticosteroid treatment on the spectra have been described as a reduction in the MI/Cr ratio (Auer *et al.*, 1997) and, in patients with Cushing's syndrome, a reduction in the Cho/Cr ratio (Khiat *et al.*, 1999). In both studies, metabolite ratios were considered and it is not clear whether these changes would also occur if an absolute quantitation were applied. In our study, the follow-up of one acute patient did not reveal changes in the spectra, so that two patients with corticosteroid treatment were allowed in the study.

### Metabolites and macromolecules

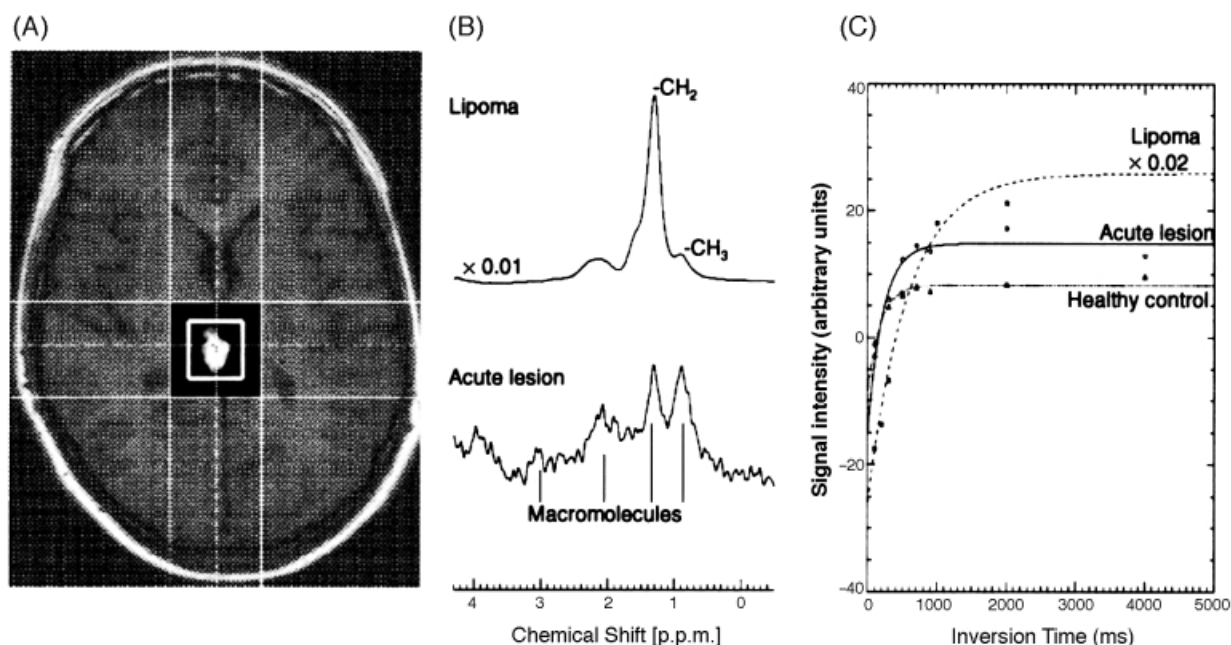
The reduction of NAA in acute and chronic lesions compared with controls was in agreement with other studies and was



**Fig 3** (A) T<sub>1</sub>-weighted contrast-enhanced and T<sub>1</sub>-weighted axial turbo spin-echo images of a 25-year-old female multiple sclerosis patient with a small acute contrast-enhancing and a large corresponding hyperintense lesion in the left occipital periventricular white matter. The saturation slices and the position of the 8 ml volume of interest (VOI) are indicated. Line 1, STEAM spectrum (TR 1.5 s, TM 10 ms, TE 15 ms); line 2, metabolite-nulled spectrum (TR 1.5 s, TM 10 ms, TE 15 ms, TI 500 ms); line 3, difference spectrum. The STEAM spectrum reveals reduced NAA and elevated MI. The macromolecules at 0.9 p.p.m. have nearly the same height in the STEAM and metabolite-nulled spectra. The resonance at 1.3 p.p.m. is lower on the metabolite-nulled spectrum because of intensity-nulling of Lac, which is clearly visible on the difference spectrum. (B) T<sub>2</sub>-weighted axial turbo spin-echo image of a 35-year-old female multiple sclerosis patient. The VOI contains a chronic lesion in the right periventricular white matter. In the STEAM spectrum, decreased NAA and elevated MI are visible (lines 1 and 3); the macromolecules in the metabolite-nulled spectrum are normal (line 2). (C) T<sub>2</sub>-weighted axial turbo spin-echo image of a 31-year-old male healthy control. The VOI contains normal white matter. STEAM spectrum, metabolite-nulled spectrum and difference spectrum.

interpreted as axonal loss or axonal dysfunction by these authors (Miller *et al.*, 1998; Narayana *et al.* 1998; Sarchielli *et al.*, 1998; Bitsch *et al.*, 1999; Brex *et al.*, 1999; Kornek

*et al.*, 2000). Increased Cho in acute lesions and elevated MI in chronic lesions have been described (Bruhn, *et al.*, 1992; Davie *et al.*, 1993, 1994; Koopmans *et al.*, 1993; Brex



**Fig. 4** (A)  $T_1$ -weighted native axial image of lipoma of the corpus callosum in the patient in Fig. 2A. The saturation slices and the position of the 8 ml volume of interest are indicated. (B) STEAM spectrum of the lipoma (upper trace, TR 1.5 s, TM 10 ms, TE 15 ms) and metabolite-nulled spectrum of the acute multiple sclerosis lesion (lower trace, TR 1.5 s, TM 10 ms, TE 15 ms, TI 500 ms). Note the difference in peak heights between the  $\text{CH}_2$  and  $\text{CH}_3$  resonances of the lipoma compared with resonances at 0.9 and 1.3 p.p.m. in the spectrum of the acute lesion, which have nearly the same peak height. (C) Fitted  $T_1$  relaxation curves of the lipoma (asterisks), an acute multiple sclerosis lesion (diamonds), and a healthy control (triangles). The  $T_1$  relaxation times for the acute lesion (218 ms) and the controls (185 ms) differ from that of the lipoma (583 ms).

*et al.*, 1999; Sarchielli *et al.*, 1999). The significantly elevated Cho in acute lesions compared with controls in the present study was in agreement with the literature, whereas the non-significantly elevated MI only slightly resembled the previously published results.

The elevation of the macromolecules at 0.9 and 1.3 p.p.m. in acute lesions was significant. Both resonances seemed to be reliable markers of acute multiple sclerosis lesions, providing clear distinction between acute and chronic lesions and between acute lesions and controls ( $P < 0.001$  for 0.9 p.p.m. and  $P < 0.05$  for 1.3 p.p.m.).

In the literature, elevated lipids in acute multiple sclerosis lesions have been found to vary in the spectral regions of their occurrence. They have been reported to lie between 0.8 and 1.5 p.p.m. (Wolinski *et al.*, 1990), between 0.9 and 1.4 p.p.m. (Larsson *et al.*, 1991), between 0.5 and 2.0 p.p.m. (Narayana *et al.*, 1992, 1998) and between 0.8 and 2.0 p.p.m. (Koopmans *et al.*, 1993). According to our own experience, broad resonances between 0.5 and 2.0 p.p.m. can be linked in whole or in part to lipid contamination from outside the VOI if appropriate outer volume saturation is not applied. Davie and colleagues (Davie *et al.*, 1993, 1994) found a lipid peak at 0.9 and at 1.3 p.p.m., whereas Roser and colleagues (Roser *et al.*, 1996) described only one significant peak, at 1.3 p.p.m. In agreement with Davie and colleagues (Davie *et al.*, 1993, 1994), we also found elevated peaks at 1.3 and 0.9 p.p.m. without metabolite-nulling. Interestingly, after the application of metabolite-nulling the 1.3 p.p.m. resonance

was reduced, because of intensity nulling of the Lac that was present. In the difference spectra, Lac was visible at 1.33 p.p.m. Thus, the metabolite-nulling technique is necessary in order to determine the true proportions of Lac and macromolecules at 1.3 p.p.m.

### *T<sub>1</sub> measurements and the spectrum of the lipoma*

The measured  $T_1$  relaxation times of the macromolecule resonances at 0.9 p.p.m. in controls and acute multiple sclerosis lesions (185 and 218 ms, respectively) were similar to the measured  $T_1$  relaxation time of 250 ms at 2.1 T published by Behar and colleagues (Behar *et al.*, 1994). The  $T_1$  relaxation time of the corresponding resonance of the lipoma (583 ms) was much longer and obviously did not arise from the same molecular source.

The spectrum of the lipoma of the corpus callosum showed a spectral pattern that was strikingly different from the metabolite-nulled spectra of the acute lesion (Fig. 4B). Human lipids possess a methylene resonance at 1.3 p.p.m. and a methyl resonance at 0.9 p.p.m. (Callies *et al.* 1993; Schick *et al.*, 1993). Because of the chemical composition of lipids, the methylene resonance is seven- to eight-fold higher than the methyl resonance. In the present study, the resonances of macromolecules at 0.9 and 1.3 p.p.m. in acute multiple sclerosis lesions had nearly the same height, indicating that the methyl and methylene groups are not only due to a lipid component.

The three-fold longer  $T_1$  relaxation time of lipids than that of macromolecules, and the difference in chemical composition between lipids and the elevated macromolecules lead to the conclusion that the elevated resonances seen in acute lesions were not due entirely to lipids but might also have been caused by other macromolecules, e.g. proteins or polypeptides, which consist of amino acids with the same chemical shift. In *in vitro* measurements of rat brain, Behar and Ogino assigned the amino acids alanine and threonine resonances to 1.42 and 1.22 p.p.m., respectively, and the valine, leucine and isoleucine resonances to 0.88 and 0.94 p.p.m., respectively (Behar and Ogino, 1993). They confirmed macromolecular resonances by metabolite-nulling at 0.93, 1.24 and 1.43 p.p.m. with similar spectroscopic characteristics (Behar *et al.*, 1994). Because of lower field strengths in our study, the resonances at 1.24 and 1.43 p.p.m. were observed as one broad resonance at 1.3 p.p.m. The amino acids mentioned account for ~40% of the myelin proteolipid protein and for ~20% of the myelin basic protein (Lees and Brostoff, 1984). Thus, the significant elevation of the 0.9 and 1.3 p.p.m. resonance in acute lesions might be due to the cleavage of these myelin proteins, which have molecular weights of 29 869 and 18 500 Da, respectively, into smaller, less rigid polypeptides which become visible spectroscopically. These assignments are supported by a study of Bhakoo and Styles, who reported that alanine, valine, leucine and isoleucine were present in cultures of mature oligodendrocytes (Bhakoo and Styles, 2000). Therefore, the visibility of these proteins in acute multiple sclerosis plaques might be a result of oligodendrocyte pathology (Lassmann, 1998; Lucchinetti *et al.*, 1999; Wolswijk *et al.*, 2000). The possibility that these proteins appear as a result of remyelination is unlikely, as remyelination ensues 12 weeks after clinical onset, following the repopulation of the plaque by oligodendrocytes (Prineas *et al.*, 1993). Nevertheless, the elevated concentrations of macromolecules might also arise from a more complex source containing mobile lipids and the polypeptides mentioned above.

In conclusion, the application of outer volume saturation in localized  $^1\text{H}$ -MRS permitted the reliable, contamination-free detection of elevated resonances at 0.9 and 1.3 p.p.m. in acute multiple sclerosis lesions. The metabolite-nulling technique allowed us to differentiate between elevated macromolecular resonances and Lac, and thus made it possible to distinguish between the 'true' chemical compounds in the aliphatic region. The elevated resonances are very likely to be due, at least in part, to macromolecular proteins containing amino acids that are also present in myelin proteolipid protein and myelin basic protein. The correlation between the detectability of these resonances and the acuteness of a lesion may be the result of oligodendrocyte pathology or of the degradation of myelin into smaller, spectroscopically visible polypeptides or proteins. Thus, regardless of their immediate source, the resonances at 0.9 and 1.3 p.p.m. are biochemical markers useful for

discrimination among acute and chronic multiple sclerosis lesions and healthy controls.

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