Absolute quantification of brain metabolites by proton magnetic resonance spectroscopy in normal-appearing white matter of multiple sclerosis patients

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Summary
The aim of this research was to obtain an absolute quantification of the N-acetyl-aspartate, choline, creatine and phosphocreatine levels in normal-appearing white matter by means of 1H magnetic resonance spectroscopy in a group of multiple sclerosis patients (27 with the relapsing–remitting form and 13 with the secondary progressive form). These values were compared with those of a group of 12 age-matched healthy control subjects. A significant decrease in the N-acetyl-aspartate concentration was found in normal-appearing white matter of frontal and parietal brain areas in multiple sclerosis patients compared with the same areas in control subjects. This reduction was more evident in progressive patients. The decrease in the N-acetyl-aspartate concentration in normal-appearing white matter significantly correlated with the Expanded Disability Status and the lesional load. No significant change was found in the concentration of creatine or choline. This finding concurs with previous evidence of heterogeneity in the multiple sclerosis pathological process which is not confined to the lesions and involves not only myelin, but also axons, even in white matter which appears normal on MRI.

Keywords: proton magnetic resonance spectroscopy; normal-appearing white matter; multiple sclerosis; N-acetyl-aspartate; creatine; choline

Abbreviations: ANOVA = analysis of variance; Ch = choline; Cr = creatine + phosphocreatine; EDSS = Expanded disability status scale; FOV = field of view; In = inositol; 1H-MRS = proton magnetic resonance spectroscopy; NAA = N-acetyl-aspartate; TE = echo time; TR = repetition time; VOI = volume of interest

Introduction
Proton magnetic resonance spectroscopy (1H-MRS) permits the in vivo study of certain cerebral metabolites, typically N-acetyl-aspartate (NAA), creatine plus phosphocreatine (Cr), choline (Ch) and inositol (In), in diseases of the CNS, including multiple sclerosis.

Evaluation of changes in cerebral metabolites in lesional areas of multiple sclerosis patients has been obtained with this technique using localized volumes of interest. The most consistent observation with localized 1H-MRS in demyelinating lesions of multiple sclerosis patients is a decrease in the NAA/Cr peak area ratio, whereas an increase in the In/Cr peak area ratio and a rise in the Cho/Cr peak area ratio were usually, but not always, revealed in active lesions (Matthews et al., 1991, 1996; Miller et al., 1991; Miller, 1995; Arnold et al., 1990, 1992, 1994; Larsson et al., 1991; Confort-Gouny et al., 1993; Davie et al., 1994, 1995; Husted et al., 1994; Davies et al., 1995). There are also some reports of the detection of myelin breakdown products (Wolinski et al., 1990; Davies et al., 1993; Koopmans et al., 1993) and a reverse in the NAA/Cr signal intensity ratio in acute multiple sclerosis lesions (De Stefano et al., 1993; Davie et al., 1994).

Recent evidence demonstrated that even normal-appearing white matter is involved in the pathological process of multiple sclerosis, showing a decrease in the NAA/Cr ratio, which is indicative of a reduction of axonal density (Husted...
Table 1 Clinical details of multiple sclerosis patients

<table>
<thead>
<tr>
<th></th>
<th>Relapsing-remitting patients</th>
<th>Secondary progressive patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>27</td>
<td>13</td>
</tr>
<tr>
<td>Males</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Females</td>
<td>22</td>
<td>8</td>
</tr>
<tr>
<td>Age (years)</td>
<td>32.7 ± 6.2</td>
<td>37.4 ± 5.8</td>
</tr>
<tr>
<td>Duration of the disease (years)</td>
<td>4.5 ± 2.0</td>
<td>8.2 ± 5.7</td>
</tr>
<tr>
<td>Expanded disability status scale</td>
<td>Mean ± SD</td>
<td>2.2 ± 0.7</td>
</tr>
<tr>
<td>Range</td>
<td>1.5–3.5</td>
<td>2.0–6.0</td>
</tr>
<tr>
<td>Lesional load score (mean SD)</td>
<td>30.5 ± 7.0</td>
<td>43.7 ± 7.0</td>
</tr>
<tr>
<td>Patients with Gd-enhancing lesions</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Gd-enhancing lesions (mean SD)</td>
<td>3 ± 1</td>
<td>0</td>
</tr>
</tbody>
</table>

et al., 1994; Roser et al., 1995; Tourbah et al., 1996; Schiepers et al., 1997; Fu et al., 1998).

In research conducted on the chemical changes in the brain of multiple sclerosis patients, results are usually expressed as ratios between cerebral metabolites. In particular, signal intensities are expressed relative to total creatine, the concentration of which is assumed not to vary from that of the brain of normal individuals. In fact there are reports of changes in brain creatine levels from studies performed both in vivo and on extracts from post-mortem multiple sclerosis brain specimens, but there are inconsistencies between the results (Husted et al., 1994; Davies et al., 1995; Rooney et al., 1995; Pan et al., 1996).

To confirm previous findings of biochemical abnormalities which are not detected by standard spin echo MRI in normal-appearing white matter of multiple sclerosis patients, we used 1H-MRS to ascertain the absolute in vivo metabolite concentrations of NAA, Ch and Cr. This avoids the use of metabolite ratios which could lead to a misinterpretation of the spectroscopic results.

Methods

Patients

The 1H-MRS analysis was performed on 40 patients with definite multiple sclerosis diagnosed according to the Poser Criteria (Poser et al., 1983). Twenty-seven of the patients were affected by the relapsing–remitting form and 13 by the secondary progressive form. The Expanded Disability Status Scale (EDSS) of each patient was calculated according to Kurtzke (1983). Details of these patients are given in Table 1. None of the patients had undergone immunosuppressant therapy within the 6 months prior to the beginning of the study, nor had any received adrenocorticotropic hormone or corticosteroid treatment within 2 months prior to the study.

1H-MRS was also performed on 12 healthy, age-matched control subjects (mean age = 33.8 ± 5.2 years) with no systemic or neurological diseases.

The study was approved by the scientific ethical committee of the Perugia Municipality (Region of Umbria). Informed written consent was obtained from all the patients and the controls.

MRI

To quantify the lesional load and number of active lesions, an MRI examination was performed in a separate session preceding 1H-MRS using a 1.5 Tesla whole body MRI system (Signa Advantage, GE Medical System). The standard head coil was used for imaging, which was performed by a fast spin echo sequence with an echo train of 8, repetition time (TR) = 4000 ms, echo time (TE) = 18 ms and 100 ms, 5 mm section thickness and a 1 mm gap between sections for complete coverage of the brain, field of view (FOV) = 24×24 cm and acquisition matrix = 256×256. Moreover, T1-weighted (TR/TE 650/15 ms), proton density (TR/TE 2000/15 ms) and T2-weighted (TR/TE 2000/70 ms) images were obtained in the axial plane. Gd-DTPA (gadolinium-diethylenetriaminepentaacetic acid) was administered intravenously in a dose of 0.2 ml/kg body weight (0.1 mmol/kg) followed by a post-injection flush with 10 ml saline. T1-weighted sequences were performed starting 5–10 min after Gd-DTPA administration.

The quantification of MRI abnormalities was obtained as previously described (Ormerod et al., 1987). The assessment of lesions was performed on 15 anatomically defined brain sites (seven periventricular and eight separated from the ventricles). An arbitrary scoring system weighted for lesion size was used to estimate the regional T2-weighted lesonal load: one point was given for each lesion with diameter 5 mm; two points for lesions with a 6–10 mm diameter and three points for those larger than 10 mm. Confluent lesions were given one extra point. The scores of the 15 sites were added up to determine a cumulative lesional load score. Areas of markedly increased signal intensity, unrelated to a physiologically enhancing structure and consisting of at least three pixels, were considered Gd-enhancing lesions.

1H-MRS data acquisition and processing

Using the same magnetic resonance system, spectroscopy assessments were carried out, after a short imaging session which was used to define the volumes of interest for spectra recording. An axial fast spin echo sequence with the echo train = 8, TR = 4000 ms, TE = 18 ms and 100 ms was used, with a 5 mm section thickness, 1 mm gap between sections and the FOV = 24×24 cm.

For each patient, the spectra acquisition was performed in one selected area in the frontal normal-appearing white matter if possible (27 patients: 19 relapsing–remitting, eight secondary progressive) or, alternatively, in the parietal normal-appearing white matter (13 patients: eight relapsing–remitting, five secondary progressive). For the control subjects (12), spectra acquisition was performed in the frontal white matter area in seven cases and in the parietal white matter...
area in five cases. Figure 1 shows the typical VOI locations for spectra acquisition. The VOI could be also contralateral to that shown if needed.

The typical VOI size ranged from 4.5 to 6.5 cc. The STEAM (stimulate echo acquisition mode) sequence was used for spectra acquisition, with the CHESS (chemical shift selective) pre-sequence for water peak suppression (if requested) and after local shimming for magnetic field homogeneity optimization.

Common parameters for all acquisitions were: bandwidth = 2500 Hz, number of points acquired = 4096, middle time ($T_M$) = 13.7 ms and number of averages for the unsuppressed water peak = 1.

Absolute quantification of the signal intensities of NAA, Ch and Cr was obtained using a method based on water compartmentation (Ernst et al., 1993; Kreis et al., 1993) derived from the EEC Concerted Action BIOMED I programme (Keevil et al., 1996; Podo et al., 1996), using a vial of pure water, placed at the side of the patient’s head, as the external standard.

Water content ($S$) from the selected VOI in the normal-appearing white matter was obtained with 10 acquisitions with TR = 10 s and TE = 10, 20, 68, 136, 1000 and 2000 ms. The water peak area was calculated for each free induction decay with fast Fourier transform and Lorentzian fit (Levenberg–Marquardt method) (Gans, 1992). Data were elaborated with a bi-exponential fit:

$$S(TE) = S_{bw}(0) \exp(-TE/T_{2bw}) + S_{csf}(0) \exp(-TE/T_{2csf})$$

in order to separate the contribution of CSF ($S_{csf}$, $T_{2csf} \approx 1$ s) from that of cerebral water ($S_{bw}$, $T_{2bw} \approx 80$ ms) and to obtain the value of cerebral water signal intensity $S_{bw}$ for TE = 0.

We started with a bi-exponential fit with the aim of verifying the exact position of the VOI in the white matter and to exclude the presence of atrophy in the cerebral tissue. CSF is normally absent in the white matter, but we found detectable levels of CSF in three cases; however, they were below 3% of the total water signal and were probably due to a small contamination by the surrounding grey matter.

For the external standard water, five acquisitions were carried out with TR = 10 s and TE = 10, 20, 40, 68 and 136 ms. The water peak area was calculated for each free induction decay with fast Fourier transform and Lorentzian fit. Data were processed with a mono-exponential fit to obtain the value of signal intensity of pure water ($S_0$) for TE = 0:

$$S_0(TE) = S_0(0) \exp(-TE/T_{2bw}).$$

For cerebral metabolites, four acquisitions (number of averages = 128; phase cycle = 8) were carried out with TR = 4 s and TE = 68, 136, 204 and 272 ms. The acquisition of the unsuppressed water peak (number of averages = 8, phase cycle = 8) was also performed for eddy current corrections. The metabolite peak areas were calculated for each free induction decay using Lorentzian–Gaussian apodization, fast Fourier transform and Gaussian fit. No correction was made for the baseline. Data for each peak were processed with a mono-exponential fit to obtain the metabolite signal intensity ($S_{met}$) for TE = 0:

$$S_{met}(TE) = S_{met}(0) \exp(-TE/T_{2met}).$$

The effect of resonance of macromolecules with a short $T_2$ was negligible due to the long echo times used.

Metabolite concentration was obtained by:

$$n_{met}/m_b = S_{met}/S_{bw} \times R r_d/r_b \times 10^3/M \times 2\nu_{met} \text{ (mmol/kg wet weight)},$$

where $n_{met}$ = number of the metabolite moles, $m_b$ = brain mass, $R = S_{bw}/(S_{bw} - S_{csf})$, $p_w$ and $p_b$ = water and brain density ($p_b = 1.047$ g/cm$^3$), $M = $ molecular weight of water and $\nu_{met} = $ number of protons per molecule which contribute to the resonance signal ($\nu_{met} = 3$ for NAA and Cr, $\nu_{met} = 9$ for Ch).

When calculating the brain water fraction $R$, the different localization of the VOI in the acquisition of cerebral water and the external standard pure water was taken into account, using previous measurements on phantoms.

The VOI for the acquisition of spectra in multiple sclerosis patients was chosen so that it was clearly separated from the plaques. In a few cases (one relapsing–remitting patient and two secondary progressive patients), there could have been a small signal contamination from demyelinating lesions due to the great lesional load, making the VOI positioning difficult.

We preferred not to quantify the In resonance, because In features a strongly coupled complex spectrum which could...
lead to questionable results with this method. Neither lactate nor lipids were consistently present in our spectra.

To verify the reproducibility of the method, four control subjects underwent a second $^1$H-MRS 1 month after the first examination. We found that the maximum variation in the metabolite concentrations was >3.2%.

To be sure that the $T_1$ relaxation did not influence the values of metabolite concentrations obtained with $TR = 4\, s$, we repeated the spectroscopic acquisitions in two control subjects and in four multiple sclerosis patients (two with relapsing–remitting form and two with secondary progressive form) using a $TR = 6\, s$ in the same session. In all cases, the maximum variation found in the metabolite concentrations was lower than 2.1%. We consider therefore that the use of a $TR = 4\, s$ is quite sufficient to make the effects of the $T_1$ relaxation time negligible in determining the metabolite concentrations, both in patients and control subjects, while at the same time making it possible to reduce drastically the length of the $^1$H-MRS session.

**Statistics**

ANOVA (analysis of variance) was used to compare the values of the cerebral metabolites of the control group with those of both patient groups. Fisher’s least significant difference was also used to compare the main effect means in the ANOVA. Five per cent was chosen as the level of statistical significance.

The Spearman correlation coefficient ($r$) was also calculated between the brain metabolite concentrations and the duration of the disease, the EDSS and the lesional load in both patient groups.

**Results**

No significant difference was found for the brain water fraction $R$ (corrected for the ratio between the density of pure water and brain tissue) between controls ($R = 0.614 \pm 0.015$, mean $\pm$ SD) and multiple sclerosis patients ($R = 0.61 \pm 0.025$). The overall mean $R$ value for all subjects that we obtained was $R = 0.612 \pm 0.02$, very similar to that calculated by Ernst *et al.* (1993). In contrast, biochemical methods revealed a value of $R = 0.73$ (Lentner, 1981; Ernst *et al.*, 1993), which is considerably larger than our values. A possible explanation for this is the presence of a water compartment at very short $T_2$ (13 ms), which is due to water molecules trapped between myelin layers very slowly exchanging with cellular water (MacKay *et al.*, 1991), which cannot be assessed with our method. The presence of this component, together with a possible uncorrected measurement of the blood water content in our normal-appearing white matter voxels and the presence of eddy current effects in the signal acquisition, could account for the discrepancy between in vivo spectroscopic and biochemical $R$ values (Ernst *et al.*, 1993).

Table 2 shows values of NAA, Cr and Ch detected by absolute quantification with $^1$H-MRS in both patients and control subjects. NAA peak values in the frontal and parietal normal-appearing white matter were significantly lower in multiple sclerosis patients than in control subjects. A more accentuated and significant reduction in the NAA values was observed in progressive multiple sclerosis patients compared with relapsing–remitting multiple sclerosis patients, both in the frontal and in the parietal areas. On the other hand, no significant differences were found in the Cr and Ch peak values between multiple sclerosis patients and control subjects and, within the group of multiple sclerosis patients, between those with the relapsing–remitting form and those with the progressive form. Moreover, the values of all metabolite concentrations did not differ significantly between the two normal-appearing white matter areas chosen for the spectra acquisition (frontal and parietal) in either control subjects and multiple sclerosis patients.

For the brain water $T_2$, no significant difference emerged between controls ($T_2 = 79.8 \pm 1.1\, ms$, mean SE) and multiple sclerosis patients ($T_2 = 80.8 \pm 0.6\, ms$). In addition, no significant difference was found for the metabolites $T_2$ estimation between controls ($T_{2\text{NAA}} = 434 \pm 26\, ms$, $T_{2\text{C}} = 212 \pm 10\, ms$, $T_{2\text{Ch}} = 300 \pm 30\, ms$) and patients ($T_{2\text{NAA}} = 462 \pm 19\, ms$, $T_{2\text{C}} = 220 \pm 9\, ms$, $T_{2\text{Ch}} = 320 \pm 23\, ms$), although it should be noted that relaxation times always tended to be longer for multiple sclerosis patients than for control subjects.

Figure 2A–C displays the magnetic resonance spectra obtained from a VOI including frontal normal-appearing white matter in a control subject, in a multiple sclerosis patient with the relapsing–remitting form of disease and in a multiple sclerosis patient with the secondary progressive form of disease.

A statistically negative correlation emerged between EDSS scores and NAA values ($r = -0.79$, $P < 0.01$) in the normal-appearing white matter of the entire multiple sclerosis group (Fig. 3A). At the same time a similar negative correlation ($r = -0.78$, $P < 0.01$) was evident in multiple sclerosis patients between the lesional load measured according to Ormerod *et al.* (1987) and the absolute measure of NAA in normal-appearing white matter (Fig. 3B). No significant correlation was found between the duration of disease and NAA values in either patient group.

**Discussion**

Quantitative measurements of MRI-defined brain lesions can provide an index of the extent and activity of disease in multiple sclerosis patients. However, the relationship between these quantitative indices of disease activity and severity and the clinical features of multiple sclerosis patients are not well established (McFarland *et al.*, 1992). Heterogeneity of the pathological changes in multiple sclerosis could explain the contrasting results concerning the correlation between lesional
Fig. 2 MRS spectra from the frontal normal-appearing white matter volumes of interest of a control subject (A), a relapsing–remitting multiple sclerosis patient (B) and a secondary progressive multiple sclerosis patient (C). Ch = Choline; Cr = Creatine; NAA = N-acetyl-aspartate. The NAA peak in both multiple sclerosis patients is lower than that in the control subject; this reduction is more evident in the progressive form patient. There is no difference in the Ch and Cr peaks of multiple sclerosis patients and controls.

Table 2 Metabolite concentrations (mM/kg wet weight, mean ± SD) in frontal and parietal normal-appearing white matter of multiple sclerosis patients and controls

<table>
<thead>
<tr>
<th></th>
<th>NAA (mean ± SD)</th>
<th>Ch (mean SD)</th>
<th>Cr (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Relapsing–remitting multiple sclerosis patients</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal-appearing white matter frontal area</td>
<td>9.84 ± 0.95*</td>
<td>2.39 ± 0.37</td>
<td>7.30 ± 0.70</td>
</tr>
<tr>
<td>Normal-appearing white matter parietal area</td>
<td>10.21 ± 0.73*</td>
<td>2.27 ± 0.36</td>
<td>7.82 ± 0.70</td>
</tr>
<tr>
<td>Mean</td>
<td>9.95 ± 0.93*</td>
<td>2.36 ± 0.37</td>
<td>7.45 ± 0.73</td>
</tr>
<tr>
<td><strong>Secondary progressive multiple sclerosis patients</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal-appearing white matter frontal area</td>
<td>8.78 ± 0.73**</td>
<td>2.09 ± 0.29</td>
<td>7.71 ± 0.63</td>
</tr>
<tr>
<td>Normal-appearing white matter parietal area</td>
<td>8.98 ± 0.45**</td>
<td>2.11 ± 0.20</td>
<td>7.74 ± 0.93</td>
</tr>
<tr>
<td>Mean</td>
<td>8.86 ± 0.65**</td>
<td>2.10 ± 0.25</td>
<td>7.72 ± 0.72</td>
</tr>
<tr>
<td><strong>Control subjects</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White matter frontal area</td>
<td>10.75 ± 0.76</td>
<td>2.28 ± 0.39</td>
<td>7.58 ± 0.58</td>
</tr>
<tr>
<td>White matter parietal area</td>
<td>11.00 ± 0.60</td>
<td>2.35 ± 0.29</td>
<td>7.62 ± 0.49</td>
</tr>
<tr>
<td>Mean</td>
<td>10.85 ± 0.69</td>
<td>2.31 ± 0.34</td>
<td>7.60 ± 0.52</td>
</tr>
</tbody>
</table>

*P < 0.01 versus control subjects; **P < 0.01 versus control subjects and versus relapsing–remitting patients.
load and clinical disability in patients affected by multiple sclerosis (McDonald et al., 1994).

$^1$H-MRS has been proposed as an additional method to conventional MRI for studying the progression and natural history of multiple sclerosis, as well as the short- and long-term effects of immunomodulatory treatment.

In this study we measured the absolute concentration of the principal brain metabolites in the proton spectrum of a group of control subjects and multiple sclerosis patients.

Regarding the methodological approach used in this study, the choice of a long TR (4 s) for the acquisition of spectra should be emphasized as this makes the effects of longitudinal relaxation times ($T_1$) of brain metabolites negligible. Acquisition of spectra at different echo times allows correction of the effects of the transverse relaxation times ($T_2$), and the compartmental analysis applied makes it possible to distinguish between cerebral water and CSF. Moreover, the use of an external reference (pure water), placed at the side of the patient’s head, provides a standard reference value in a short time and without variation in the coil load.

Values obtained in control subjects are comparable with those of the literature (Kreis et al., 1993; Cady, 1996) with some slight differences which could result, at least in part, from the different methodological approaches. The difficulties encountered in the quantitation methods for brain metabolites (baseline distortion, poor water suppression and field homogeneity, $T_1$ and $T_2$ effects, peak overlapping, etc.) are well known (Cady, 1996). Moreover, the lack of a consistent method for absolute metabolite quantitation could account for the discrepancies in the values obtained by different groups of workers, especially for values of brain metabolites with lower signal/noise ratios.

Some recent results on metabolite concentrations obtained by $^1$H-MRS in healthy individuals are given in Table 3. The NAA values found in our control subjects in both parietal and frontal white matter differ, at most, by 15% from the values obtained in these other studies and there are no significant variations between values detected in parietal and frontal areas. Our results for Ch were comparable with those of previous studies, with the exception of the values detected by Pauwels and Frahm (1998) which are 30% lower. The Cr values detected in control brains are similar to those obtained by Soher et al. (1996) and Duc et al. (1998), but are 30% higher than those found by Keevil et al. (1996) and Pauwels and Frahm (1998), confirming the great difficulty in the quantitation of metabolites with a lower signal/noise ratio.

Our findings confirm previous reports of a reduction in the concentration of N-acetyl moieties in white matter areas which are apparently not affected by the demyelinating process in multiple sclerosis patients when compared with control subjects and which is not accompanied by significant changes in Ch and Cr absolute concentrations.

For creatine in particular, our results did not concur with previous $^1$H-MRS evidence of increases or decreases in creatine in normal-appearing white matter either in vivo or in post-mortem brain tissue of multiple sclerosis patients (Husted et al., 1994; Davie et al., 1995; Rooney et al., 1995; Pan et al., 1996).

The decrease in NAA content detected in normal-appearing white matter in our study could be interpreted as a consequence of axonal damage in lesions adjacent to but not located in the spectroscopic volumes of interest examined, which are responsible for the secondary degeneration of axons and nerve cell bodies in these VOI. A similar result was obtained by Davie et al. (1995) in the cerebellar white matter of patients affected by multiple sclerosis with cerebellar deficit, which did not appear to be affected by the demyelinating process. These authors suggested that axonal loss was implicated in the development of persistent clinical disability in multiple sclerosis.

Narayanan et al. (1997) also observed a reduction in NAA

### Table 3: Some recent results* from the literature on absolute MRS quantification of cerebral metabolites in white matter of healthy subjects.

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of subjects</th>
<th>Methods</th>
<th>Parietal white matter</th>
<th>Frontal white matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keevil et al.</td>
<td>(1996)</td>
<td>Multicentre study</td>
<td>Internal water standard</td>
<td>9.7 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>(10 centres: up to five subjects for each centre)</td>
<td></td>
<td>2.2 ± 1.3</td>
<td>–</td>
</tr>
<tr>
<td>Soher et al.</td>
<td>(1996)</td>
<td>Multicentre study</td>
<td>Internal water standard</td>
<td>13.4 ± 1.84</td>
</tr>
<tr>
<td></td>
<td>(100 subjects)</td>
<td>Simulation phantom</td>
<td></td>
<td>2.1 ± 0.52</td>
</tr>
<tr>
<td>Duc et al.</td>
<td>(1998)</td>
<td>28 subjects</td>
<td>Simulation phantom</td>
<td>12.6 ± 1.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.4 ± 0.51</td>
</tr>
<tr>
<td>Pauwels et al.</td>
<td>(1998)</td>
<td>22 subjects in frontal white matter</td>
<td>LC model</td>
<td>10.6 ± 0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 subjects in parietal white matter</td>
<td></td>
<td>1.68 ± 0.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Present work</td>
<td>12 subjects</td>
<td>External water standard</td>
<td>11.5 ± 0.63</td>
<td>8.0 ± 0.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.4 ± 0.30</td>
</tr>
</tbody>
</table>

*For comparison, all concentrations are given as mM/litre, mean ± SD.
both in regions with high and low lesion probability in multiple sclerosis brains, suggesting that diffuse axonal loss extends beyond the inflammatory lesions, due to Wallerian degeneration along projection pathways which have been disconnected from their origin as a result of axonal transection.

Axonal transection was evidenced histopathologically in a recent study by Trapp et al. (1998) at the edges of active demyelinating regions, and by Ferguson et al. (1997) who used amyloid precursor protein as a histopathological marker of damaged axons, revealing axonal injury throughout the acute lesions and at the margin of active chronic lesions in multiple sclerosis brain specimens. Although these authors associated axonal damage and degeneration with inflammation, it cannot be excluded that it also extends to normal-appearing white matter, as evidenced in this study, in which, at each acquisition, we avoid contamination of surrounding voxels containing lesions. On the other hand, it cannot be ruled out that the modifications in NAA levels observed in normal-appearing white matter could reflect inflammation and lesions below the spatial resolution of MRI, as suggested by Husted et al. (1994).

Many biochemical and immunological studies have demonstrated macroscopic abnormalities in normal-appearing white matter in multiple sclerosis, such as astrocytosis (even in regions devoid of perivascular inflammation), reduction in phospholipids, differences in myelin lipid bilayer organization, alteration in myelin membrane and glial proteins, perivascular presence of T cells, B cells and macrophages and elevated Fos expression (Einstein et al., 1972; Winterfeld and Debuch, 1977; Prineas et al., 1978; Allen et al., 1979, 1981; Newcombe et al., 1980, 1986; Chia et al., 1984; Wood and Moscatello, 1984; Yu et al., 1991).

Evidence for subtle changes in normal-appearing white matter from T2-weighted images was also seen in studies of magnetization transfer, demonstrating reduced magnetization transfer ratios in multiple sclerosis patients compared with control subjects (Loevner et al., 1995) and their relevance to multiple sclerosis disability was emphasized by Filippi et al. (1995).

Further proof of microscopic disease in normal-appearing white matter of multiple sclerosis patients derives from the reports of prolonged T1 and T2 water relaxation times compared with control subjects. These have been attributed either to diffuse abnormalities or to small lesions undetectable by visual inspection of conventional MR images (Barbosa et al., 1994). If microscopical lesions are present in normal-appearing white matter, as can be seen from previous histological studies, and/or if a degeneration or persistent dysfunctioning of axons occurs, then either of these situations could account for the reduced NAA content found in our study and in previous studies. This reduction could contribute to the persistent neuronal deficit and disability of multiple sclerosis patients together with the extent of permanent myelin loss (irreversible disability) and inflammation confined to active lesions (partially or totally reversible disability).

We found a strong negative correlation between disability and NAA values in normal-appearing white matter of the entire multiple sclerosis patient group. The more accentuated decrease in NAA values observed in our progressive multiple sclerosis patients could be the expression of a greater axonal loss and/or damage in the same patients who had greater lesional load and higher EDSS values; this was true in the absence of evidence of active inflammation underlying demyelinating lesions in the secondary progressive form.

The relationship between normal-appearing white matter abnormalities and neurological deficit in multiple sclerosis patients was also demonstrated in a recent study based on spectroscopic imaging carried out by Fu et al. (1998), who detected changes in the NAA/Cr ratio in areas not affected by demyelinating lesions. In the same study, most of the 15.7% decrease in the NAA/Cr observed in patients examined longitudinally over a 30-month period occurred in normal-appearing white matter. The decrease in the NAA/Cr ratio was significantly correlated with changes in disability in the
relapsing–remitting subgroup and was interpreted as the accumulation of secondary axonal damage responsible for functional impairment. In the opinion of the authors, this is of particular relevance for understanding chronic disability in this disease.

The present research therefore supports the usefulness of 1H-MRS for detecting biochemical changes underlying the pathologic process of multiple sclerosis including changes in normal-appearing white matter. These biochemical changes were also suggested by other magnetic resonance parameters, such as low magnetization transfer ratio and \( T_1 \)-hypointensity (Miller et al., 1998). The absolute quantification of N-acetyl moieties appears to be more promising than normal-appearing white matter \( T_2 \) and texture in reflecting clinical parameters, particularly disability (Gasperini et al., 1996). A significant improvement in understanding 1H-MRS abnormalities in multiple sclerosis would nevertheless come from a direct correlation of MRS findings with histopathological data, but until now few attempts have been made in this direction.

References


