Axonal damage correlates with disability in patients with relapsing–remitting multiple sclerosis
Results of a longitudinal magnetic resonance spectroscopy study

Nicola De Stefano,1,2 Paul M. Matthews,1,3 Liqun Fu,1 Sridar Narayanan,1 Jeff Stanley,1 Gordon S. Francis,1 Jack P. Antel1 and Douglas L. Arnold1

1Department of Neurology and Neurosurgery, Montreal Neurological Institute and Hospital, Montreal, Quebec, Canada; 2Institute of Neurological Sciences, Neurometabolic Unit, University of Siena, Italy and 3Department of Clinical Neurology, University of Oxford, Radcliffe Infirmary, Oxford, UK

Summary
It has been difficult to establish a strong correlation between total brain T2-weighted lesion volume on MRI and clinical disability in multiple sclerosis, in part because of the lack of pathological specificity of T2-weighted MRI signal changes. Proton magnetic resonance spectroscopy studies have shown that measurements of the resonance intensity of N-acetylaspartate (which is localized exclusively in neurons and neuronal processes in the mature brain) can provide a specific index of axonal damage or dysfunction. Here we report a 30-month longitudinal study of 29 patients with multiple sclerosis who had either a relapsing or a secondary progressive clinical course. Conventional brain MRI and single-voxel proton magnetic resonance spectroscopy examinations were obtained at intervals of 6–8 months with concurrent clinical evaluation. At the onset of the study, the brain N-acetylaspartate : creatine resonance intensity ratio was abnormally low for the whole group of patients (control mean = 2.93 ± 0.2, patient mean = 2.56 ± 0.4, P < 0.005). There were no significant differences between the relapsing and secondary progressive subgroups. Over the follow-up period, there was a trend towards a decrease (8%) in the brain N-acetylaspartate : creatine ratio for the 11 relapsing patients and a significant (P < 0.001) correlation between changes in the brain N-acetylaspartate : creatine ratio and expanded disability scale scores for the patients in this group. This correlation was even more evident for the patients who had clinically relevant relapses during the 30 months of follow-up (seven of 11 patients). Increases in T2-weighted lesion volumes (35% in 30 months for the group as a whole, P < 0.0001, without differences between the subgroups) did not correlate with disability either in the group of patients as a whole or in the different subgroups. We conclude that indices of axonal damage or loss such as brain N-acetylaspartate may provide a specific measure of pathological changes relevant to disability. Total T2-weighted lesion volumes, although more sensitive to changes with time than brain N-acetylaspartate, may be less relevant to understanding the progression of disability.

Keywords: multiple sclerosis; axons; disability; magnetic resonance spectroscopy; N-acetylaspartate

Abbreviations: AC = anterior commissure; EDSS = expanded disability status scale; MR = magnetic resonance; MRS = magnetic resonance spectroscopy; NAA = N-acetylaspartate; PC = posterior commissure; RRMS = relapsing–remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; TE = echo time; TR = repetition time

Introduction
The use of MRI in clinical studies of patients with multiple sclerosis has contributed greatly to the understanding of the clinical–pathological manifestations of this disease. Measurements of total T2-weighted MRI brain lesion volume have been used in a number of cross-sectional and longitudinal studies, and provide what is becoming a generally accepted index for monitoring the burden of disease in multiple sclerosis patients (Paty and Li, 1993; Miller, 1994; Paty...
et al., 1994; Miller et al., 1996). However, only rather weak correlations have been reported between T2-weighted MRI lesion volume and clinical disability in established multiple sclerosis (Paty and Li, 1993; Khoury et al., 1994; Miller, 1994, 1995; Paty et al., 1994; Filippi et al., 1995). The explanation for this is probably related in part to the pathological heterogeneity of the multiple sclerosis lesions visualized on conventional T2-weighted MRI (which includes a spectrum ranging from acute inflammation and oedema to demyelination, gliosis and axonal damage) and in part to the spatial distribution of lesions in the CNS (Miller, 1994, 1995). There is therefore a reasonable concern that pathological changes reflected in the total T2-weighted lesion volume measurements may be poorly predictive of the outcome and progression of disability. Recent studies suggest that other imaging techniques [e.g. magnetic resonance spectroscopy (MRS), MR measurements of brain and spinal cord atrophy (Losseff et al., 1996a, b; Arnold et al., 1997)] may provide indices that are more closely related to neurological impairment in patients with multiple sclerosis.

In the last few years proton MRS has demonstrated decreases in N-acetylaspartate (NAA) in the brains of patients with multiple sclerosis (Arnold et al., 1990, 1992, 1994; Wolinsky et al., 1990; Matthews et al., 1991; Miller et al., 1991; Van Hecke et al., 1991; Grossman et al., 1992; Davie et al., 1994; Husted et al., 1994). Although the presence of NAA has been demonstrated in 0–2A progenitor cells in vitro (Urenjak et al., 1992), this metabolite is basically localized exclusively in neurons and neuronal processes in the mature brain in vivo (Moffett et al., 1991; Simmons et al., 1991). Decreases in NAA have been used as an index of the axonal damage or loss that occurs presumably secondarily to inflammation and demyelination (Barnes et al., 1991; McDonald, 1994). In recent studies we have demonstrated a highly significant negative correlation between changes in NAA and in clinical disability in patients with isolated, acute demyelinating lesions (De Stefano et al., 1995) and in a patient with established multiple sclerosis followed through periods of relapse and remission (De Stefano et al., 1998). This and related work suggests that axonal damage may be a proximate mechanism of functional impairment in multiple sclerosis (Raine and Cross, 1989; McDonald, 1994; Davie et al., 1995). Relative brain NAA concentration therefore may be a useful marker of disease progression.

In order to estimate the sensitivity of MRS for monitoring disease progression in multiple sclerosis and to test for a possible relationship between changes in brain NAA and changes in clinical disability in a group of patients with established disease, we performed a longitudinal study of multiple sclerosis patients in which changes in central brain NAA were measured using single-voxel MRS and clinical disability was measured concurrently.

**Method**

**Patient population**

Twenty-nine patients with clinically definite multiple sclerosis who had been referred to the Montreal Neurological Hospital Multiple Sclerosis Clinic underwent five MRI/MRS examinations at intervals of 6–8 months over a period of 30 months. Each of these patients had moderate to severe clinical disability with Expanded Disability Status Scale (EDSS) scores (Kurtzke, 1983) of 3–7. Patients were divided into two groups according to recognized criteria (Poser et al., 1983). One group consisted of patients having recurrent relapses and partial remissions [relapsing–remitting multiple sclerosis (RRMS), n = 11]. The other group had developed disease that progressed between attacks [secondary progressive multiple sclerosis (SPMS), n = 18]. An effort was made to approximately match the two patient groups for disability (EDSS scores: median for RRMS = 5.0, range 3–6.5; median for SPMS = 6.0, range 4–7) in order to limit clinical variables (potentially affecting comparisons) between the subgroups. This had the effect of creating groups with a large difference in duration of disease (median for RRMS = 7 years, range 3–23; median for SPMS = 21 years, range 9–33, P < 0.0001). All patients had a clinical evaluation by the same neurologist prior to each MR examination. Two patients withdrew from the study after 18 months of follow-up. In addition, some MR data were not of adequate quality for quantitation and were therefore omitted. Over the follow-up period, complete MR data were obtained for 25 of 29 patients on the first MR examination, for 23 of 29 on the second, for 26 of 29 on the third, for 25 of 27 on the fourth and for 23 of 27 on the fifth.

MR examinations were performed also in a group of 20 healthy control subjects (recruited from hospital and laboratory workers) for statistical comparison. Ten of them were re-examined after 1 year in order to test examination reproducibility (NAA : Cr ratio baseline, 3.00 ± 0.2; NAA : Cr (creatinine) ratio after 1 year, 3.05 ± 0.2). The study was approved by the Montreal Neurological Institute Ethics Committee and informed consent was obtained from all subjects studied.

**MRI/MRS examinations**

Conventional proton MRI and MRS examinations of the brain were obtained in a single session for each examination using a Philips Gyroscan operating at 1.5 T (Philips Medical Systems, Best, The Netherlands). A sagittal survey image was used to identify the anterior commissure (AC) and posterior commissure (PC). MRI data were acquired using a double spin-echo sequence with TR (repetition time) = 2116 ms and TE (echo times) = 30, 78 ms, yielding a set of proton density images and a set of T2-weighted images respectively. Twenty transverse slices 5.5 mm thick, with a 0.5 mm interslice gap, were collected parallel to the line joining the AC and PC for each echo time. These images were used to select an intracranial volume of interest for spectroscopy. Volumes of interest measured 50 mm (left–right) × 70 mm (anterior–posterior) × 20 mm (cranial–caudal). They were oriented parallel to the AC–PC line, centred on the corpus callosum, and included the superior
Axonal changes and disability in multiple sclerosis

Proton spectra were acquired using a 90°, 180°, 180° sequence for volume selection (Ordidge et al., 1987). Magnetic field homogeneity was optimized to a line width of ~5 Hz over the volume of interest using the proton signal from water. Water suppression was achieved by selective inversion of the water resonance prior to volume selection using an adiabatic inversion pulse and adjustment of the waiting time so that data were accumulated when the water signal passed through zero (Patt and Sykes, 1972). Spectra of 256 averages (TR = 2000 ms, TE = 272 ms) were obtained in each study (Fig. 2).

Data analysis
MRI. These images were registered in stereotaxic space using manual homologous landmark matching between the MRI volume and an average (n > 300) 3D MRI brain volume that is coextensive with the Talairach atlas (Talairach and Tournoux, 1988; Evans et al., 1992). Classification of lesion volume was performed by a single observer (S.N.). Using locally developed software on a lesion-by-lesion basis employing an edge-following algorithm, results of representative serial studies were confirmed by three neurologists (N.D.S., P.M.M. and D.L.A.). The lesion outlines were manually edited when necessary. The software offered the ability to toggle between the proton density and T2-weighted images, providing the operator with convenient access to the information in both data sets while defining lesions and facilitating the discrimination of CSF from periventricular plaques (Kamber, 1991) (Fig. 3). The method showed good inter-operator (~6%) and intra-operator (~2%) reproducibility. Outlined data from each MRI examination were transformed into stereotaxic space using the transformation parameters obtained from the registration of the MRI images to the average MRI brain. This transformation allowed a more meaningful comparison of morphometric data from different subjects, who generally varied in absolute brain dimensions.

MRS. Post-processing of the raw data was done on a SUN/SPARC system using XUNspec1 software (Philips Medical Systems). Residual water signal was removed by applying the linear Hankel singular-value decomposition fitting method (de Beer et al., 1992). Resonance intensity areas were then determined directly from time domain data (de Beer et al., 1988, 1992). Each post-processing step was fully automated. Results are expressed as the ratio NAA : Cr, which assumes that Cr is relatively constant in the volume studied. Chemical shifts were calculated relative to NAA at 2.0 p.p.m.

Statistical analysis
For analysis of the data from the first time point, a non-parametric independent test (Kruskal–Wallis) was used to compare indices between normal controls and the two subgroups.

In longitudinal studies, important issues of data analysis such as within-subject correlation and missing data cannot be dealt with properly using a standard analysis of variance approach. We therefore used unbalanced repeated measures models (Jennrich and Schluchter, 1986) for the evaluation of conventional MRI and MRS changes during the course of the study. Using this method the within-subject correlation can be taken into account, missing data can be accounted for.
Fig. 2 Single-voxel proton MR spectrum from the volume of interest illustrated in Fig. 1 (right) and a spectrum from a homologous volume of interest relative to a normal control (left). Note the significant decrease in the NAA : Cr ratio in the patient’s spectrum. The prominent lactate (Lac) resonance at 1.2 p.p.m. arises from lactate in the CSF, a common finding associated with ventriculomegaly. Cho = choline.

Fig. 3 MRI of a patient with multiple sclerosis, illustrating results of tissue segmentation. The total brain ventricular and lesion volumes are outlined on the basis of operator-defined contrast thresholds. The outlined data are then transformed into standard space based on the Talairach atlas (Talairach and Tournoux, 1988; Evans et al., 1992) for a more meaningful comparison of morphometric data from different subjects.

For and the time trend can be assessed whilst adjusting simultaneously for the other covariates (Fu et al., 1996). The general linear model used was of the form

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_n X_n + \varepsilon$$

where $Y$ is a vector of dependent variables measured from one patient over time, $X_1$ ... $X_n$ are vectors of independent variables collected over time from the same patient, $\beta_1$ ... $\beta_n$ are coefficients used to quantify the relationship between dependent and independent variables (e.g. $Y$ and $X$), and $\varepsilon$ is a vector of temporally correlated random errors. The value of $\beta$ indicates how much the dependent variable will change if the independent variable associated with $\beta$ changes by one unit (e.g. a change in NAA : Cr from 2.0 to 3.0). Based on the model, we can conclude that the dependent variable is (not) correlated with an independent variable if the value of $\beta$ associated with the independent variable is (not) significantly different from zero.

For example, considering the NAA : Cr ratio as the dependent variable, different models were used to determine the rate of change of the NAA : Cr ratio with respect to time for the group as a whole and for the two clinical subgroups, RRMS and SPMS:

(i) NAA : Cr = $\beta_0 + \beta_1$ time + $\varepsilon$

(ii) NAA : Cr = $\beta_{RRMS} + \beta_{SPMS} + \beta_{1RRMS}$ time$_{RRMS}$ + $\beta_{1SPMS}$ time$_{SPMS}$ + $\varepsilon$.

The following models were used to estimate the correlation between the NAA : Cr ratio, lesion volume and EDSS in the whole group of patients (iii) and in the clinical subgroups (iv).

(iii) NAA : Cr = $\beta_0 + \beta_1$ EDSS + $\beta_2$ LV + $\varepsilon$

(iv) NAA : Cr = $\beta_{RRMS} + \beta_{SPMS} + \beta_{1RRMS}$ EDSS$_{RRMS}$ + $\beta_{1SPMS}$ EDSS$_{SPMS}$ + $\beta_{2RRMS}$ LV$_{RRMS}$ + $\beta_{2SPMS}$ LV$_{SPMS}$ + $\varepsilon$

where LV is lesion volume.
Axonal changes and disability in multiple sclerosis

Table 1 Multimodal correlations of NAA : Cr and lesion volume in the group as a whole and in the two multiple sclerosis subgroups as determined from linear models (see Method)

<table>
<thead>
<tr>
<th></th>
<th>Whole group</th>
<th>RRMS</th>
<th>SPMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependent variable: NAA : Cr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Independent variables</td>
<td>NAA : Cr</td>
<td>Lesion volume</td>
<td>EDSS</td>
</tr>
<tr>
<td>β (SE)</td>
<td>−0.007 (0.002)</td>
<td>&lt;0.001*</td>
<td>−0.008 (0.002)</td>
</tr>
<tr>
<td>β (SE)</td>
<td>−0.075 (0.04)</td>
<td>&lt;0.1*</td>
<td>−0.11 (0.036)</td>
</tr>
</tbody>
</table>

Dependent variable: lesion volume

| Independent variables | NAA : Cr | EDSS |
| β (SE) | −25 (12.9) | >0.5‡ |
| β (SE) | −3.17 (3.35) | >0.5‡ |

Statistically significant values (P < 0.05) are in bold. NAA : Cr = N-acetylaspartate : creatine ratio; lesion volume = T2-weighted lesion volume; RRMS = relapsing remitting multiple sclerosis group; SPMS = secondary progressive multiple sclerosis group; EDSS = Expanded Disability Status Scale.

Models:

NAA : Cr = β0 + β1EDSS + β2LV + ε

NAA : Cr = β0 + β1EDSS + β2NAA/Cr + ε

NAA : Cr = β0 + β1EDSS + β2NAA/Cr + ε

NAA : Cr = β0 + β1EDSS + β2NAA/Cr + ε

Table 2 Percentage change over the study period of NAA : Cr ratio and lesion volume in the group as a whole and in the two multiple sclerosis subgroups as determined from linear models (see Method)

<table>
<thead>
<tr>
<th></th>
<th>Whole group</th>
<th>RRMS</th>
<th>SPMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA : Cr</td>
<td>&gt;0.1*</td>
<td>−3</td>
<td></td>
</tr>
<tr>
<td>Lesion volume</td>
<td>&lt;0.0001‡</td>
<td>+35</td>
<td></td>
</tr>
</tbody>
</table>

Statistically significant values (P < 0.05) are in bold. NAA : Cr = N-acetylaspartate : creatine ratio; RRMS = relapsing group; SPMS = secondary progressive group.

Models:

NAA : Cr = β0 + β1 time + ε

NAA : Cr = β0 + β1 time + ε

NAA : Cr = β0 + β1 time + ε

NAA : Cr = β0 + β1 time + ε

Using lesion volume as the dependent variable, analogous models were used to assess its rate of change over time [as in models (i) and (ii)] and to determine its relationship with NAA : Cr and EDSS [as in models (iii) and (iv)] (Tables 1 and 2).

Each of the above models was solved by fitting the collected data to the model. For each parameter, the value of β and the associated standard errors were obtained after model fitting. They were used to evaluate the significance of changes over time and the correlation between different indices. A P value of 0.05 was considered to be the threshold for significance.

Results

At the beginning of the study, the mean lesion volume was greater in patients with RRMS (42.9 ± 25 cm³) than in those with SPMS (20.7 ± 10 cm³, P < 0.02). Single-voxel proton MRS of the brain showed low NAA : Cr resonance intensities in the group of patients (mean NAA : Cr = 2.56 ± 0.4) relative to controls (NAA : Cr = 2.93 ± 0.2, P < 0.005), without any significant difference between the two subgroups (RRMS = 2.64 ± 0.3; SPMS = 2.58 ± 0.4).

The primary goal was to determine the relationship between imaging parameters and clinically relevant functional measures. Results of modelling correlations between NAA : Cr, lesion volume and EDSS using either NAA : Cr or lesion volume as the dependent variable are shown in Table 1. Over the period of study, decreases in NAA : Cr were correlated with increases in lesion volume both for the group as a whole (P < 0.001) and for the two clinical subgroups (RRMS, P < 0.001; SPMS, P < 0.01) (Table 1).

A significant negative correlation between NAA : Cr and lesion volume was not observed for the group as a whole or for the SPMS subgroup.

We believe that the failure to find a simple reciprocal
correlation between NAA : Cr and lesion volume is a consequence of an interaction between NAA : Cr and EDSS. When using NAA : Cr as the dependent variable, the precision of model fitting improved by including EDSS in the model because of the close relationship between NAA : Cr and EDSS (see below and Table 1). In contrast, when using lesion volume as the dependent variable the precision of model fitting decreased because of the lack of correlation between lesion volume and EDSS (see below and Table 1). A significant correlation between the small changes in NAA : Cr and in EDSS score was found in the RRMS subgroup ($P < 0.001$) (Fig. 4). This relationship was evident in patients who had clinically relevant relapses over the whole period of study (seven of 11 patients, range of relapses for each subject of 1–12 with a median of 2) and therefore had greater changes in EDSS and NAA : Cr (Fig. 5). In contrast, a similar relationship was not found for the SPMS patients, who showed fewer relapses and smaller changes in EDSS. No significant correlation between lesion volume and EDSS scores was found in either subgroup or in the group as a whole.

Although the total period of study was short relative to the expected rate of progression of patients with EDSS scores in the range of 3–6 (Weinshenker et al., 1989), we also considered the potential sensitivity of MR spectroscopic measures of disease progression. Rates of change over time of NAA : Cr and lesion volume, as determined from linear models, are summarized in Table 2. In this table, the percentage change over the study period is shown as the product of the rate of change obtained from the model fit and the length of the study period. During the 30 months of study no overall change in disability was found. The mean lesion volume increased progressively over time in the whole group (~35% in 30 months ($P < 0.001$)) without any significant difference between the subgroups. No significant changes were found in brain NAA : Cr for the group over the follow-up period, but in the RRMS subgroup there was a trend for a decrease in NAA : Cr over time (~8% at the end of the study, $P < 0.1$).

### Discussion
Quantitative clinical assessments of disease progression in multiple sclerosis are insensitive. Therefore, there has been great interest in developing surrogate markers of disease progression based on MR lesion volume or lesion frequency measurements. However, the correlations between changes in conventional neuroimaging markers (such as the lesion volume and the number of contrast-enhancing lesions) and progression of disability have not been found to be very strong (Paty and Li, 1993; Khoury et al., 1994; Miller, 1994, 1995; Paty et al., 1994; Filippi et al., 1995). The NAA : Cr ratio, as detected by MRS, can provide a specific index of axonal pathology (Arnold et al., 1990, 1992, 1994; Wolinsky et al., 1990; Matthews et al., 1991; Miller et al., 1991; Van Hecke et al., 1991; Grossman et al., 1992; Davie et al., 1994; Husted et al., 1994). In a recent study, we showed that changes in relative NAA concentrations correlate strongly with changes in disability over time in patients presenting with large acute plaques (De Stefano et al., 1995) and in a long follow-up of a patient during periods of relapse and successive remission (De Stefano et al., 1998). In both studies, the lesion volume at presentation was not significantly correlated with the severity of the disability. In the present study, we confirmed that a similar relationship holds in a group of patients with established disease by showing a significant negative correlation between changes in functional
status as measured by EDSS and changes in brain NAA : Cr in RRMS patients.

Our observations and others previously reported support the view that axonal damage may be an important proximate mechanism of neurological impairment in multiple sclerosis (Arnold et al., 1994; De Stefano et al., 1995; Miller, 1995; Matthews et al., 1996). Changes in the brain NAA : Cr ratio were assessed here in a large interest centred on the corpus callosum that included brain regions where axonal projections converge. Since damaged axons undergo anterograde shrinkage and Wallerian degeneration, decreases in NAA : Cr in the volume of interest should reflect damage to the brain outside as well as inside the volume of interest. Moreover, it has been shown that decreases in NAA : Cr can be seen not only in lesions but also in white matter that appears normal on MRI in patients with established multiple sclerosis (Husted et al., 1994; Arnold et al., 1996). Since we used large volumes of interest that were positioned in the central, periventricular region of the brain and that included a relatively small proportion of lesions, it is likely that our Measurements reflect widespread changes in the NAA : Cr ratio.

We have recently reported a single-voxel MRS study of a patient with RRMS followed for 6 years (De Stefano et al., 1998). In that study (as in the present study), partially reversible decreases in NAA : Cr were found diffusely in the white matter, and changes in this measure correlated strongly with disability. The strong correlation between NAA : Cr and disability found in the present study for the RRMS patients who experienced clinically significant relapses during the follow-up period further supports the hypothesis that generalized axonal damage is a major cause of neurological impairment in multiple sclerosis. This is also suggested indirectly by studies relating cerebral and spinal cord atrophy to disability (Losseff et al., 1996a, b).

The failure to observe a significant correlation between changes in NAA : Cr ratio and disability in SPMS patients in this and in previous studies may be due to the generally lower disease activity and smaller changes in disability of patients without clinical relapses (Grossman et al., 1988; Willoughby et al., 1989; Smith et al., 1993; Arnold et al., 1994; Losseff et al., 1996a, b; Matthews et al., 1996; Miller et al., 1996). Alternatively, it may reflect differences in the pathological characteristics of lesions in RRMS and SPMS related either to the underlying biology or to differences in the mean duration of disease. Pathological changes occurring in the early stages of multiple sclerosis in brain tissue (i.e. acute inflammation, axonal shrinkage or loss) may diminish with time and could become too small to be followed in a relatively short longitudinal clinical study of patients with disease of long duration.

Results of this study were expressed as NAA : Cr resonance intensity ratios. Although Cr is evenly distributed throughout the brain and is relatively refractory to change, decreases in Cr in acute multiple sclerosis lesions (Davies et al., 1995) and increases in Cr in or around chronic lesions (Husted et al., 1994; Pan et al., 1996) have been reported. Our experience has been that the relative Cr concentration (expressed as the ratio to Cr in the contralateral, normal hemisphere) decreases in acute lesions, but rapidly normalizes over a few days and remains unchanged for long periods (De Stefano et al., 1995). In that study, as in a previous post-mortem study performed with high-resolution proton NMR spectroscopy in vitro (Davies et al., 1995), decreases in Cr were limited to multiple sclerosis lesions, and no significant changes were seen in the normal-appearing white matter. In the present study, MR signals originated predominantly from normal-appearing brain (lesions accounted for only ~8% of the volume of interest in the whole group of patients, range 1–18%; data not shown), so significant changes in Cr resonance intensities are unlikely. Consistent with this, ratios of choline to Cr, which were within the normal limits at baseline in the whole group of patients, did not show changes during the period of follow-up (data not shown). Thus, changes seen in NAA : Cr ratios in the brains of our group of patients can reasonably be attributed to changes in NAA.

Over the period of study, there were large changes in T2-weighted MRI lesion volume, but a significant correlation between changes in lesion volume and disability was not seen. The failure to find a clear correlation between total T2-weighted MRI lesion volume and EDSS in a series as small as that used in this study is consistent with previous reports (Khoury et al., 1994; Paty et al., 1994; Miller, 1994; Filippi et al., 1995; Matthews et al., 1996). The data suggest to us that caution should be used when total brain lesion volume (as measured from conventional T2-weighted MRI) is used as a marker of disease progression in multiple sclerosis, as this index may not be an accurate measure of the specific pathological changes relevant to clinical disability.

In conclusion, we have shown that clinical disability correlates with brain NAA : Cr in a group of patients with RRMS. The correlation was very evident in patients having relapses during the period of study. This provides a further indication that axonal damage is relevant to understanding chronic disability in multiple sclerosis and suggests that MRS may provide an additional index of disease progression, at least for patients with the relapsing form of the disease. As it may be possible to obtain MRS and conventional quantitative MRI measurements (i.e. cortical extraction, spinal cord measurements, magnetization transfer (Filippi et al., 1994; Gass et al., 1994; Stone et al., 1995; van Walderveen et al., 1995) in the same scanning session, we believe that a multimodal approach to disease characterization could provide a more complete description of the dynamics of multiple sclerosis pathology relevant to understanding disability in multiple sclerosis and may be able to increase the specificity for clinically important changes in multiple sclerosis therapeutic trials.

Acknowledgements
We wish to thank Ms Arlene Cohen for co-ordinating the study and Mr Gilles Leroux and Mr Andre Cormier for...
providing excellent technical support. The study was supported by grants from the Multiple Sclerosis Society of Canada and the Medical Research Council of Canada, and a pilot grant from the National Multiple Sclerosis Society. D.L.A. was supported in part by the Killam Foundation and N.D.S. by a grant from the Progetto Sclerosi Multipla, Istituto Superiore di Sanità, Roma.

References


Matthews PM, Francis G, Antel J, Arnold DL. Proton magnetic...
Axonal changes and disability in multiple sclerosis


