Evidence of reversible axonal dysfunction in systemic lupus erythematous: a proton MRS study

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Our objective was to investigate axonal dysfunction in patients with systemic lupus erythematous (SLE) using proton magnetic resonance spectroscopy (1H-MRS). We studied prospectively 90 SLE patients (mean age of 32.5 years) and 23 normal volunteers (mean age of 33.8 years). We performed single voxel proton MRS using point resolved spectroscopy sequence over the superior–posterior region of the corpus callosum. We measured signals from N-acetyl compounds [N-acetylaspartate (NAA)] at 2.01 p.p.m., choline-based compounds (Cho) at 3.2 p.p.m. and creatine and phosphocreatine containing compounds (Cr) at 3.0 p.p.m. and determined NAA/Cr ratios. After 12 months, MRI and MRS were repeated in 50 patients and 9 volunteers. Patients were divided according to disease activity (measured by SLE disease activity index) during initial and follow-up MRS. We performed paired t-test and ANOVA with Tukey’s post hoc comparisons to evaluate group differences. At study entry, 29 patients had active SLE with involvement of central nervous system (CNS) and 28 patients had active SLE without CNS manifestations. A total of 14 patients had inactive SLE with past CNS presentation, and 19 had inactive SLE without history of CNS involvement. NAA/Cr ratios were significant lower in patients with active SLE, independently of CNS involvement, when compared with patients with inactive SLE (P = 0.005) and controls (P = 0.01). We observed a significant increase in NAA/Cr ratio in 15 patients who had active SLE at initial MRS and inactive SLE at follow-up (P = 0.04). In 10 patients with active SLE both at initial and at follow-up MRS we observed a reduction in NAA/Cr ratio (P = 0.02). By contrast, there was a significant reduction of NAA/Cr ratio in 15 patients who had inactive SLE at initial MRS and active SLE at follow-up (P = 0.001). In 10 patients with inactive SLE both at initial and at follow-up MRS NAA/Cr ratio did not change (P = 0.2). This study shows evidence of axonal dysfunction in patients with active SLE, independently of CNS manifestations that may be reversible, at least in part, during periods of inactivity of disease.

Keywords: axonal dysfunction; magnetic resonance spectroscopy; N-acetylaspartate; systemic lupus erythematous

Abbreviations: ACR = American College of Rheumatology; Cho = choline-based compounds; CNS = central nervous system; Cr = creatine and phosphocreatine containing compounds; LA = lupus anticoagulant; MRS = magnetic resonance spectroscopy; NAA = N-acetylaspartate; PRESS = point resolved spectroscopy; 1H-MRS = proton magnetic spectroscopy; ROI = region of interest; SLE = systemic lupus erythematosus; SLEDAI = SLE disease activity index

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Introduction

Systemic lupus erythematous (SLE) is an autoimmune disease that is frequently manifested by involvement of the central nervous system (CNS) (Omdal et al., 1988; Adelmann et al., 1986). The neuropsychiatric symptoms vary from overt neurological and psychiatric disorders to more subtle signs such as headache, mood disorders and defects in cognitive function (Adelmann et al., 1986; Omdal et al., 1988; Carbotte et al., 1992; West, 1994; Chinn et al., 1997; Sanna et al., 2003; Sibbitt et al., 2003). Although clinical assessment is still the cornerstone in the diagnosis of neuropsychiatric SLE, the diagnosis is often difficult and remains presumptive in some patients.

Proton magnetic spectroscopy (1H-MRS) of human brain in vivo allows non-invasive quantification of biological compounds. It contains a large signal from N-acetyl groups that originates largely from N-acetyl aspartate (NAA), a compound localized exclusively in neurons and neuronal...
processes (Moffett et al., 1991; Simmons et al., 1991). The neuronal marker NAA is reduced in certain diseases with neuronal loss or dysfunction, including cerebrovascular and neurodegenerative diseases, tumours, multiple sclerosis and epilepsy (Miller et al., 1993; Sibbitt and Sibbitt, 1993; Lanfermann et al., 1995; Tien et al., 1996; Wang et al., 1996; Cendes et al., 1997a, 2002). In addition, NAA abnormalities may be reversible in certain conditions (De Stefano et al., 1995; Cendes et al., 1997b).

In SLE, 1H-MRS has been performed in an attempt to detect early CNS involvement (Sibbitt and Sibbitt, 1993; Sibbitt et al., 1994, 1997; Davie et al., 1995; Passe et al., 1995; Brooks et al., 1997; Colamussi et al., 1997) or to demonstrate abnormalities in some patients with neuropsychiatric SLE in whom structural MRI failed to show any focal changes (Sibbitt et al., 1994, 1997; Davie et al., 1995; Friedman et al., 1998).

The purpose of this study was to determine the presence of axonal dysfunction in SLE patients with and without evidence of CNS involvement. We also performed follow-up studies in these patients in order to determine if these abnormalities are transient or permanent.

Subjects and methods

Subjects

In this prospective study, we evaluated 150 consecutive patients (138 women) with four or more criteria for SLE (Tan et al., 1982) seen regularly at our Rheumatology Unit. We excluded patients who were not able to undergo MRI, such as patients with claustrophobia, pacemaker and prosthetic valves, and patients with previous clinical conditions that could influence cerebral atrophy, such as stroke, arterial hypertension, diabetes mellitus, alcohol and drug abuse, and malignancy. Patients satisfying the American College of Rheumatology (ACR) criteria for rheumatoid arthritis, systemic sclerosis, Sjögren syndrome (primary or secondary) or other connective tissue disease and with drug-induced SLE were also excluded. After initial evaluation a total of 10 patients have been excluded. We used the classification proposed by the ACR to analyse neuropsychiatric involvement (ACR, Ad HoC Committee on Neuropsychiatric Lupus, 1999). We considered only primary involvement of the CNS by SLE.

The control group consisted of 23 healthy volunteers with similar age and gender distribution. The study was approved by the Ethical Committee of our institution and informed written consent was obtained from each subject.

Clinical, serologic and treatment features of SLE patients

Data on age at disease onset and disease duration were collected for each patient. Disease duration was defined as the initial manifestation clearly attributable to SLE until the day of magnetic resonance spectroscopy (MRS) acquisition. Disease activity was measured through SLE disease activity index (SLEDAI) (Bombardier et al., 1992) and considered active if scores were >8 points.

All clinical manifestations and laboratory test findings were obtained at baseline visit by careful chart review. Data are systematically recorded in special database on quarterly basis visits by the same investigators using a structured questionnaire (SA and LLTC). The following clinical manifestations were analysed: malar rash, discoid lesions, subcutaneous lesions, photosensitivity, oral ulcers, arthritis, serositis, nephritis, neurological and psychiatric involvement, thrombocytopenia, haemolytic anaemia, Raynaud’s phenomenon, thrombosis, myositis, lung involvement and lymphadenopathy.

Nephritis was diagnosed on the basis of proteinuria exceeding 0.5 g/l with abnormal urinary sediment and/or histological findings. Nephrotic syndrome was defined as proteinuria in excess of 3.5 g/day. Haematological alterations were ascribed to lupus only in the absence of bone marrow suppression (leukopenia <4000 cells/mm³; thrombocytopenia <100 000/mm³; haemolytic anaemia with positive Coombs test). Antinuclear antibodies (ANA) were determined by indirect immunofluorescence using Hep 2 as the substrate and regarded as positive if >1:40. Anti-double-stranded DNA (antiDNA) antibodies were determined by indirect immunofluorescence using Chritidia as substrate and considered positive if >1:10. Precipitating antibodies to extractable nuclear antigens (ENA), including Ro (SSA), La (SSB) and Sm were detected by immunodiffusion and/or microhaemagglutination. Anticardiolipin antibodies (aCL) of the IgG and IgM isotypes were measured by the enzyme-linked immunosorbent assay (ELISA) method as described (Brandt et al., 1995). Lupus anticoagulant (LA) activity was detected by coagulation assays in platelet free plasma obtained by double centrifugation, following the recommendation of the subcommittee on LA of the Scientific and Standardization Committee of the International Society of Thrombosis and Homeostasis (Harris et al., 1987).

CNS manifestations were recorded following ACR case definitions (ACR Ad HoC Committee on Neuropsychiatric Lupus, 1999) and considered active when present at the day of MRI/MRS acquisition.

Patients had clinical and laboratory evaluation at the time of their first MRI/MRS examination and were divided in groups, according to their disease activity, as follows: Group A, active SLE and CNS involvement; Group B, active SLE without evidence of CNS involvement; Group C, inactive SLE and history of CNS manifestations; and Group D, inactive SLE without previous history of CNS involvement. Group E consisted of normal volunteers. At the time of the second MRI/MRS patients were also divided into groups, considering their disease activity at study entry and at follow-up: Group F, active SLE at initial MRS and inactive SLE at follow-up; Group G, inactive SLE at initial MRS and active SLE at follow-up; Group H, inactive SLE both at initial and at follow-up; and Group I, active SLE both at initial and at follow-up MRS. A subgroup of nine normal volunteers had repeated MRS with an interval of 21 months on average (Group J).

Group A had SLEDAI scores indicating active SLE disease even after excluding CNS manifestations from the SLEDAI.

Total doses of corticosteroids and other immunosuppressant medications used since the onset of disease were calculated by careful review of the medical charts. A total of 10 patients with incomplete charts were excluded from this analysis. Doses of oral and parenteral corticosteroids were analysed and converted to the equivalent doses of prednisone. The cumulative dose of corticosteroids used was calculated by the sum of daily dosages versus time (days) of treatment.

MRI and MRS protocol

All subjects had MRI and MRS examination for the purpose of this study, using an Escint 2Tesla scanner (Prestige, Haifa, Israel).
Our MRI protocol consisted of:

(i) Sagittal T1 spin echo = 6 mm thick, flip angle = 180°, repetition time (TR) = 430, echo time (TE) = 12, matrix 200 × 350, field of view (FOV) = 25 × 25 cm;

(ii) Coronal images, perpendicular to long axis of hippocampus, defined by the sagittal images:
   (a) T2-weighted ‘fast-spin echo’ (FSE) = 3 mm thick, flip angle = 120°, TR = 4800, TE = 129, matrix 252 × 320, FOV = 18 × 18 cm;
   (b) T1-weighted inversion recovery (IR) = 3 mm thick, flip angle = 200°, TR = 2800–3000, TE = 14, inversion time (TI) = 840, matrix 130 × 256, FOV = 16 × 18 cm;

(iii) Axial images parallel to the long axis of the hippocampi:
   (a) T1-weighted gradient echo = 3 mm thick, flip angle = 70°, TR = 200, TE = 5, matrix 180 × 322, FOV = 22 × 22 cm;
   (b) Fluid attenuated inversion recovery (FLAIR) = 4 mm thick, flip angle = 120°, TR = 6800, TE = 129, matrix 252 × 328, FOV = 21 × 23 cm;

(iv) T1-weighted 3D gradient echo, acquired in the sagittal plane for multiplanar and reconstruction: 1 mm thick, flip angle = 35°, TR = 22, TE = 9, matrix 256 × 220, FOV = 23 × 25 cm.

Single voxel 1H-MRS was acquired using point resolved spectroscopy (PRESS) sequence (Bottomley, 1987 ) (TR = 1500 ms, TE = 135 ms, NEX = 200) over the superior–posterior region of the left hemisphere at the level of corpus callosum. This area was previously analysed using T1-weighted, T2-weighted and FLAIR sequences. Patients with white matter lesions in this region were not included (n = 30). Therefore, all patients evaluated in this study had normal appearing white matter within the MRS region of interest (ROI).

After the acquisition of scout anatomical images in sagittal planes for localization of corpus callosum, one single voxel (2 × 5 × 1 cm) was placed over the ROI (Fig. 1). Prior to the acquisition, a localized shimming at the ROI was performed to ensure adequate field homogeneity followed by water suppression adjustment.

The spectra were post-processed using software supplied by the machine manufacturer (Elscint 2T Prestige, Haifa, Israel). After zero-filling and baseline correction we determined peak areas by integration of the corresponding signals from N-acetyl compounds (NAA) at 2.01 p.p.m., choline-base compounds (Cho) at 3.2 p.p.m. and creatine and phosphocreatine containing compounds (Cr) at 3.0 p.p.m.. The spectra were scaled in relation to creatine values. Ratios of NAA/Cr were used for analyses.

Spectral acquisition, quantification and analysis were performed by one investigator (S.A.). The evaluation was cross-checked by two spectroscopists (L.M.L. and F.C.), blinded to the name and clinical data of patients and volunteers. The quality of the spectral analysis was judged independently by these two investigators from the parameters linewidth and signal-to-noise ratio (Fig. 2) and spectra with broad peaks and poor separation of individual peaks were excluded from analysis. Values <2 SD from the mean of controls were considered abnormal. A total of 10 patients were excluded because of bad quality spectra.

Therefore, 90 patients (88 women) with mean age of 32.5 years (range 18–59 years, SD = 13.1) were available for evaluation for this study. We repeated MRI and MRS exams in 50 of these patients (48 women) and in 9 volunteers after a minimum interval of 1 year.

**Statistics**

We performed analysis of variance (ANOVA) to test differences among the groups, followed by post hoc Tukey’s HSD for pairwise comparison if necessary. Follow-up MRS results were analysed using paired t-test with Bonferroni’s correction for multiple comparisons. Statistical significance was considered to be present for $P < 0.05$.

**Results**

**Demographic data**

We analysed MRS data of 90 SLE patients. In relation to the different patients groups at study entry, we observed...
the following age and gender distribution: Group A: 29 patients (28 women) with mean age of 32.2 (range 18–56; SD = 12.9); Group B: 28 patients (27 women) with mean age of 33.0 (range 18–59; SD = 13.1); Group C: 14 patients (14 women) with mean age of 31.9 (range 18–50; SD = 13.7); Group D: 19 patients (19 women) with mean age of 33.5 (range 18–56; SD = 12.9).

The control group (Group E) consisted of 23 healthy volunteers (19 women) with mean age of 33.8 years (range 20–60, SD = 13.7 years)

MRI and 1H-MRS studies were repeated in 50 SLE patients (48 women) with mean age of 33.3 years (ranging from 18 to 57 years; SD = 12.2) at study entry. In relation to the different patients groups at follow-up, we observed the following gender and age distribution: Group F, 15 patients (14 women) with mean age of 32.5 (range 18–55; SD = 12); Group G, 15 patients (14 women) with mean age of 33.0 (range 18–57; SD = 13.1); Group H, 10 patients (10 women) with mean age of 32.3 (range 18–50; SD = 13); Group I, 10 patients (10 women) with mean age of 33.6 (range 18–50; SD = 13).

The mean interval between the two 1H-MRS of SLE patients was 19 months (range 12–24 months; SD = 2.3)

MRS studies were repeated in 9 volunteers (7 women) of Group J with mean age of 32.1 (range 20–60; SD = 13.1) at study entry. The mean interval between MRS examinations was 21 months (range 18–24 months; SD = 1.8).

There was no statistical difference between the age and gender distribution among the different groups of patients (Groups A–D and F–I) and volunteers (Groups E and J).

**Clinical, laboratory and treatment features**

The mean disease duration was 64.5 months (range 1–362 months, SD = 48.50) at study entry and 93 months (range 12–421 months, SD = 45.45) at follow-up MRS. At baseline, 65 episodes of CNS manifestations had occurred in 43 patients (29 patients with active and 14 with inactive CNS manifestations) (Table 1). At the time of MRS scans, 57 patients had active SLE, with SLEDAI scores ranging between 10 and 20 (mean 14.56, SD = 6.52). Active CNS manifestations at the first MRS scan were observed in 29 of 57 patients with active SLE. Number of patients who had inactive SLE at the time of first MRS was 33 and 14 of them had past history of CNS involvement and 19 did not. All patients were on steroid use on the day of MRS study, with doses ranging from 5 to 80 mg/day (mean 43 mg/day). IgG antiphospholipid antibodies were positive in 32 patients.

**Disease activity and MRS**

Median NAA/Cr values for each group of patients were: (A), active SLE and CNS involvement, 1.65 (SD = 0.25); (B), active SLE without evidence of CNS involvement, 1.67 (SD = 0.27); (C), inactive SLE and history of CNS manifestations, 1.82 (SD = 0.23); (D), inactive SLE without previous history of CNS involvement, 1.98 (SD = 0.21); (E), volunteers, 1.86 (SD = 0.15) (Table 2).

Median NAA/Cr ratios were significantly lower in patients with active SLE (Groups A and B), when compared with patients with inactive SLE (Groups C and D) (P = 0.005) and volunteers (Group E) (P = 0.001) (Fig. 3).

We did not find a correlation between daily corticosteroid dose or cumulative corticosteroid dose and median NAA/Cr value (r = 0.4).

**Follow-up study**

Patients were divided, according to their disease activity at study entry and at follow-up MRS, into four groups. Group F (n = 15) with active SLE at initial MRS (median NAA/Cr = 1.6; SD = 0.36) and inactive SLE at follow-up (median NAA/Cr = 2.1; SD = 0.31); P = 0.04. Group G (n = 15) with inactive SLE at initial MRS (median NAA/Cr = 1.9; SD = 0.12) and active SLE at follow-up (median NAA/Cr = 1.3; SD = 0.21); P = 0.001. Group H (n = 10) with inactive SLE both at initial (median NAA/Cr = 2.0; SD = 0.48) and at follow-up (median NAA/Cr = 2.1; SD = 0.42) MRS; P = 0.2. Group I (n = 10) with active SLE both at initial (median NAA/Cr = 1.7; SD = 0.21) and at follow-up (median NAA/Cr = 1.38; SD = 0.48) MRS; P = 0.02 (Fig. 4). The NAA/Cr ratios remained constant in

**Table 1 Summary of cumulative neuropsychiatric manifestations that occurred in 43 patients at study entry**

<table>
<thead>
<tr>
<th>Neuropsychiatric manifestations</th>
<th>Number of CNS events (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>18 (27.7)</td>
</tr>
<tr>
<td>Cognitive impairment</td>
<td>20 (30.8)</td>
</tr>
<tr>
<td>Seizures</td>
<td>10 (15.4)</td>
</tr>
<tr>
<td>Acute confusional state</td>
<td>7 (10.8)</td>
</tr>
<tr>
<td>Psychosis</td>
<td>5 (7.7)</td>
</tr>
<tr>
<td>Mononeuropathy</td>
<td>2 (3.1)</td>
</tr>
<tr>
<td>Cranial neuropathy</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Aseptic meningitis</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Total number of events</td>
<td>65</td>
</tr>
</tbody>
</table>

**Table 2 Median NAA/Cr and Cho/Cr values**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Median NAA/Cr at initial MRS (±SD)</th>
<th>Median Cho/Cr at initial MRS (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.65 (0.25)</td>
<td>1.03 (0.3)</td>
</tr>
<tr>
<td>B</td>
<td>1.67 (0.27)</td>
<td>0.98 (0.4)</td>
</tr>
<tr>
<td>C</td>
<td>1.82 (0.23)</td>
<td>1.1 (0.2)</td>
</tr>
<tr>
<td>D</td>
<td>1.98 (0.21)</td>
<td>1.0 (0.3)</td>
</tr>
<tr>
<td>E</td>
<td>1.86 (0.15)</td>
<td>0.96 (0.2)</td>
</tr>
</tbody>
</table>

Values in 90 patients and 23 normal volunteers at study entry. Groups: (A), active SLE and CNS involvement; (B), active SLE without evidence of CNS involvement; (C), inactive SLE and history of CNS manifestations; (D), inactive SLE without previous history of CNS involvement; and (E), normal volunteers. ↓: Significantly lower than normal volunteers.
Outliers are represented by an asterisk (*).

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**Fig. 3** Box-and-whiskers plot showing median NAA/Cr ratio in SLE patients with active SLE and CNS manifestations. No. 1 represents Group A, patients with active SLE without CNS manifestations; No. 2 represents Group B, inactive SLE patients with past history of CNS involvement; No. 3 represents Group C, inactive patients without history of CNS involvement; No. 4 represents Group D and No. 5 represents volunteers, Group E. The box extends from the 25th percentile to the 75th percentile, with a horizontal line at the median (50th percentile). Whiskers extend down to the smallest value and up to the largest. Outliers are represented by an asterisk (*).

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**MRI findings and MRS**

Subtle abnormal MRI findings, in areas outside and far from the MRS ROI, (hyperintense areas suggestive of cerebral microinfarcts) in cortical and subcortical regions were observed in 53 patients at baseline study. These MRI abnormalities were more frequently observed in patients with antiphospholipid antibodies (*P* = 0.04). The number of lesions was counted in all MRI scans. Visual analysis of MRI did not demonstrate significant increase in the number of these lesions during the follow-up study as compared with baseline study.

NAA/Cr ratios were lower in SLE patients with MRI abnormalities when compared with patients with normal MRI (*P* = 0.028). No difference in Cho/Cr values between patients with and without MRI abnormalities was observed.

**Antiphospholipid antibodies and MRS**

SLE patients with positive antiphospholipid antibodies had lower NAA/Cr (*P* = 0.021) values when compared with patients without antiphospholipid antibodies. The frequency of antiphospholipid antibodies was distributed equally among these groups.

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**Discussion**

Although several studies (Sibbitt *et al.*, 1994, 1997; Davie *et al.*, 1995; Friedman *et al.*, 1998, Castellino *et al.*, 2005) showed the usefulness of 1H-MRS in CNS manifestations in SLE, only one previous study (Castellino *et al.*, 2005) analysed SLE patients without CNS manifestations. In our study we observed that SLE patients with active disease had low relative NAA signal intensity, indicating axonal dysfunction, when compared with SLE patients with inactive disease and volunteers. This occurred independently of clinical CNS involvement as defined by the ACR criteria (ACR Ad Hoc Committee on Neuropsychiatric Lupus, 1999).

Cerebrovascular abnormalities may be the basis of diffuse cerebral injury in SLE. Small-vessel injury is primarily associated with decreased NAA/Cr ratio, while medium-vessel injury is primarily associated with increased Cho/Cr ratio (Davie *et al.*, 1995). In our study we observed that patients with white matter abnormalities in regions outside the MRS ROI and patients with antiphospholipid antibodies had more pronounced decreased NAA/Cr ratios, supporting the theory of small vessel involvement in SLE. We did not observe differences in Cho/Cr ratio among the subgroups of SLE patients and normal volunteers.

The ROI for MRS examination in patients with SLE should best reflect the area where the metabolic changes might precede the morphological changes. MRS studies have already been performed in the supraventricular and subcortical white matter (Sibbitt and Sibbitt, 1993; Sibbitt *et al.*, 1994; Friedman *et al.*, 1998; Lim *et al.*, 2000) and in the basal ganglia (Lim *et al.*, 2000). In this study the normal appearing white matter was chosen, despite the presence of small hyperintense lesions in other areas of the brain, because most of the time these MRI abnormalities are referred to as non-specific findings. MRS abnormalities in these regions would support the idea that MRS could precede the appearance of hyperintense lesions in T2 or FLAIR sequences due to CNS involvement of SLE (Castellino *et al.*, 2005). Other studies (Sanna *et al.*, 2003; Sibbitt *et al.*, 2003) have shown the association of non-specific white matter abnormalities and signs and symptoms of CNS manifestations in SLE. The specific susceptibility of the white matter to small vascular lesions is thought to be due to unique vascularization of this tissue. Blood is supplied to the white matter by means of single sources, rendering the deep white matter more vulnerable to vascular insults. In SLE the precise biochemical mechanism that explains the basis of CNS involvement is still unknown (Lim *et al.*, 2000). We therefore choose the supraventricular region, in order to test the hypothesis that early NAA changes might occur in this area in patients with overt CNS manifestations. Our results confirm previous findings (Sibbitt and Sibbitt, 1993; Sibbitt *et al.*, 1994, Castellino *et al.*, 2005) and support this hypothesis. However, in the present study, after 19 months of follow-up, we did not observe that low NAA/Cr ratio predisposed to the appearance of structural lesions detectable by MRI. Perhaps longer periods of observation are necessary to
detect the appearance of these lesions as suggested by a previous study (Castellino et al., 2005).

$^1$H-MRS may be more sensitive in the detection of early CNS involvement in SLE patients. A decrease in NAA level, as shown in this study, indicates not only loss of neurons or neuronal activity, but also neuronal dysfunction secondary to myelin breakdown (Sibbitt and Sibbitt, 1993; Sibbitt et al., 1994; Lim et al., 2000). Decreased NAA/Cr ratio in supratentorial region suggests axonal dysfunction due to extensive small-vessel injury in normal appearing white matter. In our study we also observed that patients with active SLE, independently of CNS manifestations, also had decreased NAA/Cr ratio. We also demonstrated for the first time that the relative NAA reduction in SLE is transient and it is

![Graphs](http://brain.oxfordjournals.org/)

**Table 3** Median NAA/Cr and Cho/Cr values

<table>
<thead>
<tr>
<th>Groups</th>
<th>Median NAA/Cr at initial MRS (±SD)</th>
<th>Median NAA/Cr at follow-up MRS (±SD)</th>
<th>P-value</th>
<th>Median Cho/Cr at initial MRS (±SD)</th>
<th>Median Cho/Cr at follow-up MRS (±SD)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>1.6 (0.36)</td>
<td>2.1 (0.31)</td>
<td>0.04</td>
<td>1.09 (0.4)</td>
<td>0.98 (0.37)</td>
<td>0.4</td>
</tr>
<tr>
<td>G</td>
<td>1.9 (0.12)</td>
<td>1.3 (0.21)</td>
<td>0.001</td>
<td>1.1 (0.3)</td>
<td>1.0 (0.3)</td>
<td>0.7</td>
</tr>
<tr>
<td>H</td>
<td>2.0 (0.48)</td>
<td>2.1 (0.42)</td>
<td>0.2</td>
<td>0.93 (0.2)</td>
<td>0.93 (0.2)</td>
<td>0.9</td>
</tr>
<tr>
<td>I</td>
<td>1.76 (0.17)</td>
<td>1.38 (0.48)</td>
<td>0.02</td>
<td>0.96 (0.2)</td>
<td>0.91 (0.26)</td>
<td>0.4</td>
</tr>
<tr>
<td>J</td>
<td>1.86 (0.17)</td>
<td>1.89 (0.18)</td>
<td>0.12</td>
<td>0.95 (0.16)</td>
<td>0.94 (0.17)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Values in 50 patients and 9 normal volunteers at follow-up. Groups: (F), with active SLE at initial MRS and inactive SLE at follow-up; (G), with inactive SLE at initial MRS and active SLE at follow-up; (H), with inactive SLE both at initial and at follow-up; (I), with active SLE both at initial and at follow-up MRS; and (J), follow-up volunteers.

**Fig. 4** Paired t-test comparing MRS finding at study entry and at follow-up. Panel (A), 15 patients (Group F) with active SLE at initial MRS and inactive SLE at follow-up ($P = 0.04$). Panel (B), 15 patients (Group G) with inactive disease at initial MRS and active disease at follow-up ($P = 0.001$). Panel (C), 10 patients (Group H) with inactive SLE both at initial MRS and at follow-up ($P = 0.2$). Panel (D), 10 patients (Group I) with active disease both at initial MRS and at follow-up ($P = 0.02$).
probably dependent on disease activity. We observed that SLE patients with active disease had low relative NAA signal intensity that returned to normal range after disease remission. In the group of patients with inactive disease both at baseline and follow-up MRS, there was a progressive decrease in NAA values. These findings suggest that axonal dysfunction in SLE patients may be transient and related to disease activity, independently of the presence of CNS involvement or corticosteroid use.

In the follow-up graphs (Fig. 4) we observed that not all patients had the same pattern of NAA/Cr increase or reduction. Further studies are necessary to determine the factors associated with the intensity and rate of NAA/Cr loss or recovery. These factors may help to explain the outliers in Fig. 4. It is possible that the NAA reduction precedes the clinical signs and symptoms of SLE disease activity, among other factors. This could explain the drop of relative NAA/Cr values in the three patients who had inactive SLE disease at both initial and follow-up MRS. However, it is difficult to explain the two outlier patients in Group H who had a more pronounced increase in NAA/Cr values.

The fall of NAA reflects neuronal loss or dysfunction (Passe et al., 1995; Tsai and Coyle, 1995). Previous studies have correlated NAA loss with cerebral atrophy (Sibbitt et al., 1994), cognitive dysfunction and damage index (Brooks et al., 1999). NAA recovery, after initial reduction, has not been reported in SLE before, but was observed in multiple sclerosis (Wolinsky and Narayana, 2002), stroke (Saunders et al., 1995), schizophrenia (Haussinger et al., 1994) and epilepsy (Cendes et al., 1997b).

Cho concentrations, on the other hand, are reported to rise in SLE patients with CNS involvement (Brooks et al., 1997, 1999; Sibbitt et al., 1997; Castellino et al., 2005), but the cause has not been determined, although it may be due to myelin breakdown secondary to neuronal loss or due to inflammatory process (Brooks et al., 1997; Brooks et al., 1999).

In this study we used Cr values as an internal reference, although it has not been demonstrated that Cr is stable in SLE. The facts that we performed MRS in normal appearing white matter and that Cho/Cr ratios were not different among groups give support to the assumption that eventual changes in Cr were minimal and did not produce a great influence in our results.

In conclusion, our findings suggest that SLE activity, independently of CNS involvement, is associated with white matter insult, often not evident by structural MRI or by clinical manifestations. The relative NAA decrease may be a surrogate marker for disease activity in SLE patients and may be useful for follow-up of disease activity. These findings are limited to the area in the brain studied here, and further studies are necessary to confirm these findings.